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RESEARCH ARTICLE

BIOREMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOILS: A REVIEW

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ABSTRACT

Fishes are susceptible to spoilage which occurred as a results of different factors including high level of Toxins known as polycyclic aromatic hydrocarbons (PAHs) build up in soils because they are not soluble in water and do not fluctuate. The primary causes of their production are human and natural resulting from incomplete combustion of both liquid and solid fuels. The possible mutagenic and carcinogenic effects on the environment could pose a concern to human and animal health. One method for breaking down and mineralizing PAHs into carbon dioxide, water, and secondary metabolites is bioremediation, which uses bacteria and fungi. The sources, distribution environmental impact, and current bioremediation approaches for detoxifying PAHs-polluted soils are all included in this present review. These techniques do not only benefit the environment but also lessen the hazards and negative effects of PAHs on humans, animal's agriculture and ecosystem.

KEYWORDS

Polycyclic Aromatic Hydrocarbons, Bioremediation, Soil, Microorganisms

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are carbon- and hydrogen-containing chemical compounds made up of several benzene rings (Smith, 1990). They are released into the environment as a result of incomplete combustion of fossil fuels or inadvertent discharge during the transportation, use, and disposal of petroleum products (Smith, 1990). PAHs are poisonous, carcinogenic, and mutagenic; thus, their presence in the environment is a major worry and has a negative impact on human health. The United States Environmental Protection Agency (USEPA) has identified 16 PAHs as priority pollutants, and their levels in industrial effluents are routinely monitored. Anthropogenic activities, including oil spills, engine oil spills, residential combustion of wood and coal, and bush burning, are major contributors of polycyclic aromatic hydrocarbons released into the environment. PAHs enter the environment through the air as the sooty part of smoke or ash, then absorbed to particulate matters and get deposited into the lithosphere and hydrosphere as a result soil concentration tended to increase particularly in urban and industrialized areas (Wilson and Rose, 2007). Human exposure to PAHs is via inhalation, ingestion, or dermal contact with high risks to public health. Exposure to PAHs can also occur if the skin touches contaminated soil or product like heavy oils,

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coal tar, roofing tar or creosote. Once the PAHs enter human body, they can spread and target fat tissues, organs which include kidneys, lungs and liver causing cancer (Zhao *et al.,* 2009).

Bioremediation is a microbial-mediated process that removes polycyclic aromatic hydrocarbons by degradation, biosorption, and biotransformation (Rabani *et al.,* 2020). It is an appropriate remedial measure because it is cost-effective and environmental sustainable. Furthermore, the use of microorganisms may provide advantages for a variety of reasons, including the fact that they are adapted to the contaminated environment, allowing the inoculum to survive (Kazuga *et al.,* 2001), and microorganisms can spread through the soil via propagation, as bacteria can access xenobiotics (Eniola *et al.,* 2014).

Table 1: Hazardous effects of polycyclic aromatic hydrocarbons

Sector	Hazardous effects
Agriculture	Soil fertility reduces, physiological properties and have adverse effect on seed germination
Human	It causes skin cancer, sinonasal cancer, lungs cancer and adverse effects on central nervous system which leads to eye irritation, nausea, vomiting and diarrhea
Ecosystem	Imbalance of the eco system and food chain, loss of biodiversity
Plant and animal	Loss of chlorophyll and disease such as cancer and central nervous system disorders

Source: (Cocker *et al.,* 2006).

Structure of Polycyclic Aromatic Hydrocarbons

The molecular and chemical arrangements of polycyclic aromatic hydrocarbons are shown below. PAHs with an angular arrangement are more stable than with a linear arrangement. PAHs not only contain carbon and hydrogen but in a broader way heterocyclic (heteroatom), i.e., PAH containing nitrogen, sulphur, and carbon atoms. PAHs are formed due to the thermolysis of various organic molecules in the environment. PAHs are flat, crystalline solids with high melting and boiling points but with low vapour pressure and water solubility. PAHs range in appearance from colourless to white or pale yellow-green. On the basis of molecular weight, the PAHs are classified as having low molecular weight (LMW), having two or three fused benzene rings such as naphthalene, fluoroene, and phenanthrene, and high molecular weight (HMW) PAHs are those having four fused benzene rings such as Benzo (A) Pyrene, Chrysene and Pyrene. Polycyclic aromatic compounds are also lipophilic which gives them a place in the environment, primarily in soil and sediment. PAHs have characteristics UV Absorbance spectra with many bands each unique for each ring structure thus each isomer has a different UV absorbance spectrum (200nm-400nm) which helps in the identification of PAHs (Cerniglia and Shuttleworth 2002).

Table 2: Molecular and Chemical properties of polycyclic aromatic hydrocarbons

PAHs	Mass (Da)	Vapour pressure (Pa)	Log K_{ow}	Solubility
Naphthalene	128.18	12.0	3.58	30
Acenaphthene	154.20	4.02	3.92	3.6
Phenanthrene	178.24	0.0161	4.46-4.63	1-2
Fluorene	166.23	0.13	4.18	2.00
Anthracene	178.24	0.001	4.45	0.015
Pyrene	202.26	0.0006	5.88-6.7	0.12-0.18
Chysene	228.30	6.08×10^{-7}	5.01-7.10	0.00015
Fluoranthene	202.26	0.001	5.22	0.25
Benz(a)anthracene	228.30	2.0×10^{-5}	5.99	0.01
Acenaphthylene	155.20	3.87	3.90	3.88
Benzo(a)pyrene	252.32	7.0×10^{-7}	5.78-6.5	0.001-0.006

Keys: K_{ow}: Octanol water coefficient; Da=Dalton, Pa=pressure

Sources and Distribution of PAHs in soil

Industrial, agricultural, and anthropogenic activities all contribute to increased environmental contamination in soil. The widespread distribution of PAHs and their negative impact on human health has piqued the interest of numerous resources in the mechanism of degradation and their potential fate in soil. Petroleum-based goods provide the majority of energy for industry as well as daily life in urban and industrialized areas. As a result, spills and leaks occur on a daily basis throughout petroleum product exploration, production, refining, transportation, and storage. Anthropogenic sources of PAHs in the environment include fuel burning, cars, petroleum product spills, and waste incineration (Cerniglia and Shuttleworth 2002)

Tobacco cigarette smoking is a significant source of PAH exposure to smokers and secondary smokers. PAHs released to the atmosphere are subject to short and long range transport and are distributed onto soil. PAHs can photolyse, oxidize, bind to suspended particles, sediments and accumulate in soil (Margesin *et al.,* 2003). In biotransformation process, biological agents like microorganism convert the complex organic contaminants to other simpler organic compound mostly to nontoxic or less toxic substance which can be finally mineralized by other organism to carbon dioxide, water and inorganic compounds in co- metabolism (Medina Bellver *et al.,*2005).

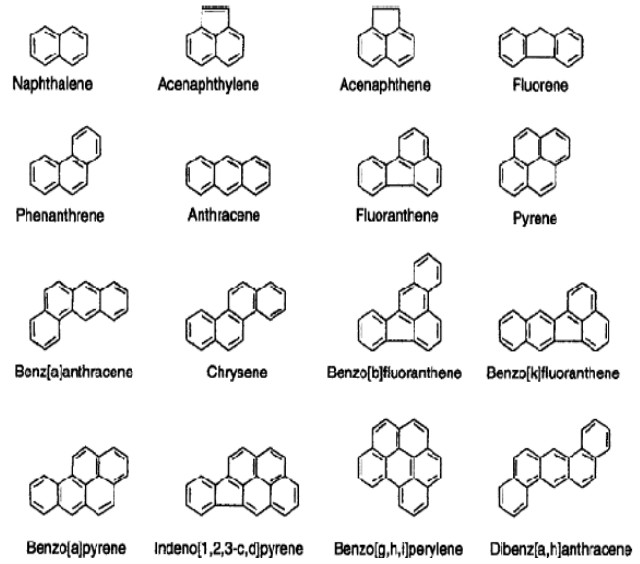

Figure 1: USEPA's Priority PAHs pollutant.

Table 2: Selected bacterial strains effective for bioremediation of PAHs contaminated soil.

PAHs degrading microorganisms	References
Proteus mirabilis strain 10c	Obayori *et al.,* 2017
Sphingomonas sp HS362	Hwa *et al.,* 2005
Pseudomonas sp ARP26	Coral *et al.,* 2005
Staphylococcus sp PN/Y	Malick *et al.,* 2007
Bacillus subtilis MI6K and M19F	Oyetibo *et al.,* 2017
Arthrobacter sulphureus RKJ4	Samanta *et al.,* 1999
Mycobacterium sp PYR1	Kelly *et al.,* 2007
Burkholderia cepacia BU3	Kim *et al .,* 2003
Pseudomonas putida P16	Seo *et al.,* 2007
Sphingomonas paucimobilis EPA 505	Story *et al.,* 2001
Pseudomonas aeruginosa	Weissentels *et al.,* 1990
Acidovoras delafiedii P41	Samanta *et al.,* 1999
Brevibacterium sp HL4	Samanta *et al.,* 1999
Mycobacterium vanbaalenii PY9	Kim *et al.,* 2003

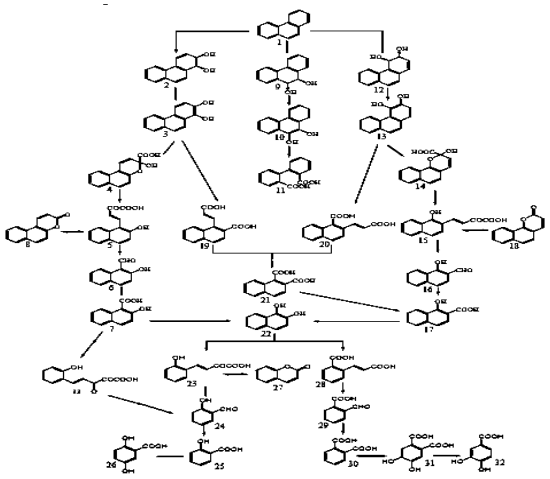

Figure 2: Metabolic pathways for the degradation of PAHs by bacteria (Samanta *et al*., 1999).

Keys: Compound designations: 1, phenanthrene; 2, cis-1,2-dihydroxy-1,2-dihydrophenanthrene; 3, 1,2- dihydroxyphenanthrene; 4, 3-hydroxy-3H-benzo[f]chromene-3-carboxylic acid; 5, 4-(2-hydroxy-naphthalen-1- yl)-2-oxo-but-3-enoic acid; 6, 2-hydroxy-naphthalene-1-carbaldehyde; 7, 2-hydroxy-1-naphthoic acid; 8, 5,6- benzocoumarin; 9, cis-9,10-dihydroxy-9,10-dihydrophenanthrene; 10, 9,10-dihydroxyphenanthrene; 11, 2,2'- diphenic acid; 12, cis-3,4-dihydroxy-3,4-dihydrophenanthrene; 13, 3,4-dihydroxyphenanthrene; 14, 2-hydroxy- 2H-benzo[h]chromene-2-carboxylic acid; 15, 4-(1-hydroxynaphthalen-2-yl)-2-oxo-but-3-enoic acid; 16, 1- hydroxy-naphthalene-2-carbaldehyde; 17, 1-hydroxy-2-naphthoic acid; 18, 7,8-benzocoumarin; 19, 1-(2- carboxy-vinyl)-naphthalene-2-carboxylic acid; 20, 2-(2-carboxy-vinyl)-naphthalene-1-carboxylic acid; 21, naphthalene-1,2-dicarboxylic acid; 22, naphthalene-1,2-diol; 23, 2-hydroxybenzalpyruvic acid; 24, salicylic aldehyde; 25, salicylic acid; 26, gentisic acid; 27, coumarin; 28, 2-carboxycinnamic acid; 29, 2-formylbenzoic acid; 30, phthalic acid; 31, 3,4-dihydroxyphthalic acid; 32, protocatechuic acid; 33, trans-2,3-dioxo-5-(2'- hydroxyphenyl)-pent-4-enoic acid.

Bioremediation Techniques of Pahs Contaminated Soil

Bioremediation involves the process of biodegradation and biotransformation where by polycyclic aromatic hydrocarbons are transformed or degraded to an environmentally safe levels in soil. The micro-organisms used for the biodegradation may be native or indigenous (In-situ) to a contaminated area or may be extraneous and brought to the contaminated site (Ex-situ).

Bioventing: This technique involves controlled stimulation of air flow by delivering oxygen to unsaturated zone in the contaminated soil in order to increase bioremediation, by increasing activities of indigenous microbes. In bioventing, amendments are made by adding nutrients and moisture to enhance bioremediation with the ultimate goal to achieve microbial transformation of pollutants to harmless state. Frutos *et al.,* (2010) reported the effectiveness of bioventing treatment in remediation of PAHs contaminated soil and recorded 93 % contaminant removal after seven months.

I. **Bioaugmentation:** It involves the application of autochthonous or allochthonous wild type or genetically modified microorganisms to polluted hazardous waste sites in order to accelerate the removal of undesired compounds. This approach is to enhance the degree or rate of degradation of the complex pollutants by the addition of pollutant-degrading microorganisms. Enhancing the microorganisms of the contaminated site will not only improve the elimination of the pollutants from the particular site but also at the same time increases the genetic capacity of the desired site (Eniola *et al.,* 2014).

II. **Biostimulation**: It is a remediation technique that is highly efficient, cost effective and eco-friendly in nature. Biostimulation refers to the addition of limiting nutrients like phosphorus, nitrogen, oxygen, electron donors to severely polluted sites to stimulate the existing bacteria to degrade the hazardous and toxic contaminants. The addition of limiting nutrients improves the degradation potential of the inhabitant microorganisms (Eniola *et al.,* 2014).

III. **Bioreactor:** is a vessel in which raw materials are converted to specific product(s) following series of biological reactions. There are different operational modes of bioreactors, which include: batch, fed-batch, sequencing batch, continuous and multistage. Bioreactor provides optimal growth conditions for bioremediation. The bioreactor based treatment of polluted soil has several advantages as compared to in-situ bioremediation procedures. Bioreactor-based bioremediation process having excellent control of pH, temperature, agitation and aeration, substrate and inoculum concentrations. The flexible nature of bioreactor designs allows maximum microbial degradation.

IV. **Phytoremediation:** Phytoremediation is an emerging technology that uses various plants to degrade, extract or immobilize polycyclic aromatic hydrocarbons. There are various mechanism such as phytovolatilization, rhizoremediation, phytotransformation, and phytostabilization.

V. **Analytical techniques of Bioremediation in PAHs contaminated soil:** Analytical techniques for bioremediation studies efficiency of PAHs contaminated soil includes

Gas Chromatography Mass Spectrophotometer

Gas chromatography Mass spectrophotometer is at technique which uses gas as the mobile phase while the stationary phase can either be solid or nonvolatile liquid. It is a technique for separating or identifying components in a mixture. Gas chromatography is divided into three parts, the injector, the column, and the detector. All this three components work together to give the GC its analytical efficiency (Chavez *et al.,* 2004). It is mostly used to monitor the degradation rate of the microorganism in PAHs contaminated soil.

High-Performance Liquid Chromatography

It is a specific form of column chromatography generally used to separate, identify, and quantify the active compounds. HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that moves the mobile phase through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvents used. The polluted soil sample to be analyzed is introduced in small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase. The amount of retardation depends on the nature of the analyte and composition of both stationary and mobile phase. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time (Chavez *et al.,* 2004).

Molecular Methods of Bioremediation in PAHs Contaminated Soil

Proteomics Genomic and metabolomics have been recently employed in studies of environmental microbiology and have shown their high impact on the field of biodegradation and bioremediation (Chavez *et al.,* 2004). Proteomics is an effective technique to identify proteins and their functions involved in the biodegradation of aromatics while metabolomics can be used to profile degradation products of PAHs and primary metabolites in response to PAH exposures. A good number of genomic sequences or expressed sequence tags (ESTs) of bacteria are used for effective bioremediation. Genomic is the technique used to study the genetic makeup of microorganism. These include several PAH degrading bacteria in the genus of *Mycobacterium, Acinetobacter, Arthrobacter, and Burkholderia* (Gratia *et al.,* 2006). Polymerase Chain Reaction detection of genes encoding for microbial monooxygenases and dioxygenases such as *nah*Ac, *phn*Ac, NIDA, and NARB are useful for the detection of PAHs degrading microbial populations. However, one of the most commonly used approaches for the detection and identification of microorganisms is the PCR amplification of microbial ribosomal RNA (rRNA) genes (e.g., 16S, 18S, 23S rRNA). The rRNA genes are the basis for microbial phylogenetic analyses, as several million sequences have been published in the GenBank database. During bioaugmentation treatments, rRNA of introduced microorganisms can be easily amplified by PCR and detected by gel electrophoresis. In most cases it is necessary to analyze the rRNA amplification products by additional techniques, such as terminal-restriction fragment length polymorphism (T-RFLP), or fully sequencing the amplified product, to increase the specificity of detection and identification. On the opposite end, quantitative PCR (qPCR) or real-time PCR (RT-PCR) has been also used to quantify microorganisms after introduction to different environmental matrices (Kikuchi *et al.,* 2002). One of the most specific and popular ways to perform qPCR is with the use of Taqman probes. In this technology, Taq polymerase cleaves a fluorogenic. Taqman probe that binds to an internal site within the sequence being amplified during the extension step, which releases a fluorescent molecule (fluorophore), resulting in fluorescence. The cycle threshold value (Ct) is determined at the point where a significant increase in the fluorescence emission occurs, as compared with a background baseline. A larger initial concentration of target DNA results in a lower Ct value. qPCR eliminates the use of gels and allows the sample to be analyzed in real time, in less time than conventional PCR. Quantitative PCR has been used in bioremediation studies to calculate the copy number of the benzyl succinate synthase gene (BSSA) and naphthalene dioxygenase (*nah*Ac) in PAHs contaminated soils bioaugmentated with degrading microbial consortia.

Factors Influencing PAHs Degradation in Soil

The bioremediation of polycyclic aromatic hydrocarbons depends on the amount and nature of the rings present as well as on the diversity of microbes or plants been used. It depends upon various physical and chemical factors such as

- ✓ Soil texture
- ✓ Pressure
- ✓ Moisture content
- ✓ Available oxygen
- ✓ Availability of nutrients
- ✓ Bio surfactants
- ✓ Microbial diversity and consortium
- ✓ pH
- ✓ Salinity
- ✓ Nature of PAHs content
- ✓ Temperature
- ✓ Genetic composition of the microbes

Conclusion

Microbial bioremediation is an effective and inexpensive approach to degrade and remove polycyclic aromatic hydrocarbons from contaminated soils as long as the population of microorganisms or plants is conducive to the biodegradation of the contaminants. Furthermore, with recent developments and applications of molecular techniques to biodegrade PAHs polluted soil, the processes of polycyclic aromatic hydrocarbons catabolism and gene detection in bacteria have advanced substantially. Following this, many novel catalytic mechanisms have been understood and characterized by different bacteria. Also the application of genetically engineered and enhanced microbes for bioremediation can also been developed for environmental sustainability.

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