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Microbial Remediation of High Molecular Weight PAHs from Environment: An Overview

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Abstract: Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants generated from both natural and anthropogenic processes. They are found in various matrices such as air, water, soil and also in processed food products (highly cooked meats) and are mainly classified based on their molecular weight into two groups, Low Molecular Weight (LMW) and High Molecular Weight (HMW) compounds. Out of these two, HMW PAHs are known to be persistent for longer periods and found to show mutagenic and carcinogenic effect in many organisms. Bioremediation is an effective and safe method compared to many other processes. Various researchers have studied and reported on bioremediation of PAH compounds by bacteria, algae, fungi, yeasts and plants till date. The remediation organisms are found to convert into highly toxic PAH compounds to less harmful compounds. Microbial consortium degradation studies on inter-generic and inter-species microorganisms on PAH degradation has shown to improve the degradation rate when compared with individual organisms. Major enzymes involved in the bioremediation are manganese peroxidase (MnP), lignin peroxidise, laccaseetc. This review paper focuses on the degradation of HMW PAHs by different microorganisms.
Keywords : Carcinogenic, Enzymes, Microorganisms, HMW PAHs.

Introduction:

Polycyclic aromatic hydrocarbons (PAHs) are non-specific[1,2],neutral, non-polar organic compounds[3], and are produced generally when insufficient oxygen or other factors result in incomplete combustion of organic matter (e.g., in engines and incinerators, when biomass burns in forest fires). They also occur in oil, coal, and tar deposits, and are recognized as a common pollutant in air, water and soil[4,5,6].PAHs are a major component of creosote used for technical wood preservative preparation of the wood[5].Incomplete combustion that leads to formation of PAHs might also happen in industrial operations, cooking on barbecues, in flames and in cigarette smoke. They are also present in food. The highest intake is shown to come from food grains, oils and fats. Smaller amounts come from vegetables and cooked meat[7,8].They are also formed during the thermal decomposition of organic molecules and their subsequent recombination[9].PAHs are also commonly found as a major source in the processing of food (such as drying and smoking) and cooking of foods at high temperatures (grilling, roasting and frying)[7,8].Incomplete combustion at high temperature (500–800°C) or subjection of organic material at low temperatures (100–300°C) for long periods result in PAH production. With an increase in molecular weight, their solubility in water decreases; melting and boiling point increase and vapour pressure decreases[9].Many of the researchers demonstrated the bioaccumulation experiments using aquatic organisms which had a deleterious effect on test organisms[1,2].Because of their wide distribution, it is thus important to monitor these compounds. PAHs are aromatic hydrocarbons with two or more fused benzene

rings[3,6,10].Amongst the PAHs, few of them are known to be carcinogenic, mutagenic and teratogenic (linked to birth defects)[11,12,13].The compounds may be classified as low molecular weights (LMW) or high molecular weights (HMW). LMW PAHs are those containing two or three benzene rings, while those with four or more benzene rings are called HMW PAHs[12,13]. LMW PAHs are relatively water soluble, but those containing four or more rings are quite hydrophobic and insoluble. However, the higher the molecular weight of a PAH, the more likely it is to absorb to soil organic matter. This tendency to strongly adsorb to particulate matter renders the HMW PAHs less available and thus less susceptible to remediation. HMWPAHs have high resonance energies due to the dense clouds of pi-electrons surrounding the aromatic rings, making them persistent in the environment and recalcitrant to degradation. Another reason for the compounds to exhibit the recalcitrant nature is their low aqueous solubility and high soil sorption[6].LMW PAHs were more susceptible to ROS oxidation as compared to HMW PAHs, resulting in a higher production of fluorene(FLU) and phenanthrene (PHE) intermediates, similarto those by photo-degradation. This type of degradation, driven by ROS, occurred in the algal cells when under unbearable stress[14]. Many HMW PAHs (e.g.Benzo(a)pyrene and benzo(b)fluoranthene, benzo(e)pyrene and benzo(j)fluoranthene) are structural isomers with the same molecular weight, but different structural formulae. Purified HMW PAHs (five benzene rings and above) are solids and generally do not volatilize because their melting points are greater than 100°C. HMW PAHs also has large resonance energies which make them thermodynamically stable, though they can be photo-oxidized at various aromatic positions to form quinines[15].Brief classification of HMW PAHs is given below in Table 1, with their physical characteristics. Dermal exposure can represent a significant health risk in settings involving potential contact with soil contaminated with PAHs. However, there is limited work on the ability of PAHs in contaminated soil to reach the skin surface via desorption from the soil[16].

Table 1: Classification of HMW PAHs

PAH Name	Rings	Mol. Wgt. (g/mole)	Solubility in Water(mg/L)	Vapor Pressure (Pa)	Log (K_{ow})
Fluoranthene	4	202.26	0.26	$1.08x10^{-3}$	5.22
Benz[a]anthracene*	4	228.29	0.011	$2.05x10^{-5}$	5.91
Chrysene	4	228.29	0.0015	1.04x10-6	5.91
Pyrene	4	202.26	0.132	5.67x10-4	5.18
*Benzo[a]pyrene**	5	252.32	0.0038	6.52x10-7	5.91
Benzo[b]fluroranthene*	5	252.32	0.0015	1.07x10-5	5.80
Benzo[k]fluroranthene*	5	252.52	0.0008	1.28x10-8	6.00
Dibenz[a,h]anthracene*	6	278.35	0.0005	2.80x10-9	6.75
Benzo[g,h]perylene*	6	276.34	0.000026	1.33x10-8	6.50
Indeno[1,2,3cd]pyrene*	6	276.34	0.062	1.87x10-8	6.50

*The U.S. EPA has classified PAH as possible human carcinogens

Degradation of HMW PAHs by Bacteria:

Natural attenuation is a technique based on the degradation capacity of indigenous bacteria. By this method, allowing the ecosystem to revert back closes to its original conditions as well as converting the toxic compounds into harmless ones and also avoids habitat damage[17].Reports about bacterial percentage degradation for HMW PAHs are presented in Table 2.

Table 2: Various reports on bacteria for degradation of HMW PAHs:

Name of microorganisms	Compound (Degradation percentage)	References
Bacillus sp., *Brevibacterium* sp., *Delftia s*p., *Dietzia* sp., *Gordonia* sp., *Kocuria* sp., *Naxibacter* sp., *Microbacterium* sp., *Mycobacterium* sp., *Rhizobium* sp., *Rhodococcus* sp., *Stenotrophomonas* sp., *Streptomyces* sp.	Fluoranthene (34.4%) Chrysene (20.5%) Benzo[a]pyrene (23.6%)	[18]
Pycnoporus sanguineus, *Coriolus versicolor*, *Pleurotus ostreatus*, *Fomitopsis palustris*,*Daedalea elegans*	Pyrene 4.4%, 42%, 32% 7.3% 26.1%respectively	[19]
Pedobacter sp.,*Bacillus* sp., *Paenibacillus* sp.,Uncultured Sphingobacterials bacterium clones, Uncultured Proteobacterium clone	Chrysene (27.6%), Benzo[a]pyrene (24.3%) Pyrene (22.5%)	[12]
Mycrobacterium spp.	Benzo[a]pyrene (10.1 %)	[20]

Bioremediation is known to potentially break down PAHs to harmless end products such as carbon dioxide. For bioremediation to be applied successfully, microorganisms capable of degrading native pollutants must be present under environmental conducive conditions[21].Some specific PAH were degraded to a greater extent by biostimulation also[17].Microorganisms being a effective remediator posses certain interesting characteristics which include, tolerance to high metal concentrations, efficient capacity of surfactant production and capacity to reproduce in dry and nutrient-deficient conditions. Therefore, they could be successfully applied to bioremediation by bioaugmentation of soil co-contaminated with hydrocarbons, pesticides and even metals in a given the characteristics of the isolated hydrocarbonoclastic strains[18]. *Pseudomonas*, *Flexibacter*, *Sphingomonas, Balneimonas* and *Pantoea* are belonged to active uncultured bacterial genera of which latter two have not been previously reported earlier as PAH degraders. The majority of the species identified belonged to *Pseudomonas* genus during the creosote biodegradation which has been widely studied in bioremediation processes. Some bacteria encompass an inherent ability to degrade the creosote without earlier experience[17]. It also found that incubating in PAH-contaminated soils with a bacterial consortium might be a promising way intended for bioremediation process. Microbial consortia are more competent than unadulterated cultures in biodegradation of PAHs. This is possibly because broader enzymatic capacity is achieved by the selection of these dead end products formed mainly by co-metabolism processes and the formation of toxic intermediate metabolites is counteracted[12]. Removal of HMW PAHs was done by the capacity of native bacteria associated (*Gordonia*, *Mycobacterium*, and *Rhodococcus*) with vermicompost (VC), humic acids (HA), sugar cane bagasse (SCB) and the earthworm *Eisenia andrei* (EaW). A number of complementary biological, chemical, and modelling techniques were used as assessment tools; including the sediment PAH concentrations, bacterial community analysis and the PAH availability[21]. It is also found that, co-cultivation of rice and PAH-degrading bacteria (*Acinetobacter* sp.) may have a great potential to accelerate the bioremediation process of PAH-contaminated soil (spiked phenanthrene and pyrene) under sodden conditions[22].The objective of this study was to evaluate natural attenuation and bioremediation in the form of biostimulation and bioaugmentation as an effective amendment and potential treatment for PAH polluted environments.

Degradation of HMW PAHs by Algae:

Initially the fate of different pollutants, including PAHs was studied by freshwater algae, *Nitella specialis*[1,23].Later on, same author have studied the bioconcentration and the transformation of a priority PAH, benzo[*a*]pyrene (BaP), by *Chara aspera*(Chara algae), *Cladophora glomerata* and *Enteromorpha intestinalis* (greenalgae), *Chorda filum* and *Fucus vesiculosus*(brownalgae) and *Furcellaria lumbricalis*(redalgae)[24].The PAH degradation efficiency of four microalgal species, namely, *Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda*, and *Selenastrum capricornutum* were evaluatedto remove a mixture of fluoranthene and pyrene (each at a concentration of 0.5mg/l), and fluoranthene (1.0 mg/l), pyrene (1.0 mg/l). It was found that the removal efficiency of fluoranthene and pyrene in a mixture was higher or comparable than its respective single compound which can be suggested that the presence of one particular PAH incentivised the removal of

another one PAH[25].*Nitzschia sp.* was higher than those of *S. costatum* in terms of accumulation and degradation abilities of PAHs. These two algal species were found to be slower in terms fluoranthene (FLA) degradation which indicating that FLA was a more recalcitrant PAH compound. Almost similar types of results as earlier case, the microalgal species also showed comparable or higher efficiency in the removal of the PHE–FLA mixture than PHE or FLA singly. Thus, suggesting that the presence of one PAH stimulated the degradation of the other PAH compound[26].The biosorption and biodegradation ability of individual and mixed PAH species were exhibitedby microalgae, including *Chlamydomonas sp*., *Chlorella miniata*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda*, *Selenastrum capricornutum* and *Synechocystis sp.*[25,27,28]. The effects of different metals (cadmium, copper, nickel and zinc in a mixture) on biosorption and biodegradation was investigated of five mixed HMW PAHs, namely fluorene (FLU), phenanthrene (PHE), fluoranthrene (FLA), pyrene (PYR) and benzo[a]pyrene (BaP), by *Selenastrum capricornutum*[14].Some of the algae employed for degradation of HMW PAHs are presented in Table 3.

Table 3: Various reports on algae for degradation of HMW PAHs:

Name of Species	Compound (Degradation percentage)	References
Selenastrum capricornutum	Benzo[a]pyrene (7 %)	[14]
Isochrysis galbana	Benzo[a]pyrene (10 %)	[29]
Chara (Chara aspera,) Enteromorpha intestinalis, Cladophora glomerata	Benzo[a]pyrene (42–49%)	[24]

It was just observed in nature for easily degradable matter by considering the in situ generation of photosynthetic oxygen by microalgae could directly help the microbial aerobic metabolism of organic pollutants. Indeed, with the help of electron acceptor7 by the aerobic microflora, they produce the molecular oxygen that was used, and in return they will release carbon dioxide during the mineralization process completing the photosynthetic cycle to degrade the organic matter[31].An environmental hazard assessment suggested that some aquatic systems are sufficiently contaminated with PAH due to its photo-induced toxicity that causes a hazard to natural algal communities. Therefore, it can be confirmed that the PAH component of the photo induced toxicity hazard assessment will decrease by any reduction in the bioavailability of PAH in natural waters[10].Under golden and white light irradiation, a freshwater microalga *Selenastrum capricornutum* was studied for the transformation and removal of HMW PAHs, namely benz[a]anthracene, benzo[b] fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-c,d]pyrene and benzo[g,h,i]perylene. The transformation of benzo[a]pyrene, a single PAH compound, by *S. capricornutum* and another green microalgal species, *Chlorella sp*., demonstrated that the transformation in dead cells was similar which be indicated the process was algal-species independently. Most likely dead algal cells acted as an accelerated the photo degrader and photo sensitizer of PAHs[31]. The role of algal concentration in the transfer of organic contaminants in a food chain has been studied *Isochrysis galbana* as the phytoplankton foodsource, and the common mussel (*Mytilus edulis*) as the primary consumer by using the ubiquitous model PAH, benzo[a]pyrene (BaP) as the contaminant[32].

Degradation of HMW PAHs by Fungi:

There have been fewer studies on the degradation of PAHs by fungi in comparison to those of bacteria and algae. Isolation of three fungi strains from soils at the side of a freeway, a gas station, and a lake in Japan which was grow on pyrene as the sole carbon source, was tentatively classified as members of the *Trichoderma/Hypocrea* genus and the *Fusarium* genus respectively. It was found that pyrene-degrading activity was more effective at pH 4 for fungal strains[35].The synergistic effect of a combination of soil microorganisms and white rot fungi has also been observed for pyrene mineralization by *Dichomitus squalens*and *Pleurotus* sp.[36]. Information on fungi for degradation of HMW PAHs are presented in Table 4.

As observed in the *Salmonella typhimurium* revertant test performed with strains TA100 and TA98, fungal oxidation of benzo[*a*]pyrene resulted in rapid and almost complete elimination of PAHs high mutagenic potential. Furthermore, no direct mutagenic metabolite could be detected during oxidation by fungus.The

remaining weak mutagenic activity of fungal cultures containing benzo[*a*]pyrene metabolites towards strain TA98 with indigenous microflora was further decreased by subsequent incubations[35].

Table 4: Various reports on fungi for degradation of HMW PAHs:

Name of Species	Compound	References
Trametes versicolor ATCC 42530	Benzo[a]pyrene (23%)	[5]
Lentinus tigrinus CBS 577.79		
Bjerkandera sp. strain BOS55	Benzo[a]pyrene (8.5%)	[35]
Phanerochaete chrysosporium	Benzo[a]pyrene (Study mechanism only)	[36]

Degradation of HMW PAHs by Yeast:

Six different yeasts species were examined for their ability to metabolize benzo(a)pyrene, biphenyl and naphthalene. Out of the organisms tested, these *C. lipolytica* oxidized benzo(a)pyrene to 3-hydroxybenzo(a) pyrene and 9-hydroxybenzo(a)pyrene[37].Information on yeast for degradation of HMW PAHs are presented in Table 5.

Metabolites were isolated and identified by different techniques like absorption spectrophotometry, mass spectrometry and thin-layer, gas liquid and high-pressure liquid chromatography etc. The structures of these metabolites were confirmed by comparison with authentic compounds[37].

The potential of yeast abundance, isolated from the sediments of 13 coastal sites in Massachusetts was quantified for to biotransformation of PAHs was also demonstrated. Their plate counts of yeasts varied between 10^2 to 10^7 CFU/g (dry weight) in sediment samples. The most abundant genera isolated and identified included *Candida*, *Cryptococcus*, *Rhodotorula*, *Torulopsis* and *Trichosporon*[40].

Table 5: Various reports on yeasts for degradation of HMW PAHs:

Name of Species	Compound(Degradation percentage)	References
Candida, Pichia, Rhodotorula, Sporidiobolus.	Pyrene(75%), Chrysene(66%), Benzo(b)fluoranthene (79%), Benzo(k)fluoranthene(81%), Benzo[a]pyrene(76%),Dibenzo(a,h)anthracene(83%), Perylene(70%), Indeno(1,2,3-cd)pyrene (84%), Benzo(g,h,i)perylene(78%)	[38]
Pichia anomala	Chrysene (75.9%) Benzo (a) pyrene (63.8%)	

Enzymes involvedinbiodegradation study of PAHs:

PAHs degradation was done mainly by three different classes of enzymes such as: oxygenase, dehydrogenase and ligninolytic enzymes.A diverse group of fungi have the ability to degrade wide range of PAHs nonspecially by ligninolytic (lignin) and non-ligninolytic enzymes[40].With the help of their extracellular ligninolytic enzyme systems, white rot fungi can oxidize PAH rapidly has therefore raised interest in the use of these organisms for bioremediation of PAH polluted soils[41,42]. Typically white rot fungi possess ligninolytic enzymes which are known to degrade PAHs and to detoxify HMW PAH (BaP)-polluted sediments and soils[40]. In *P. chrysosporium*(a lignin-degrading basidiomycete),ligninase is responsible for the initial steps in the metabolism of BaP. By this study it was identified as a main organic soluble products resulting from the oxidation of B(a)P by different ligninase preparations[43].Benzo(a)pyrene was oxidized with crude and purified extracellular ligninase preparations.Both form of enzymes, the crude and the purified fractions oxidized the substrate to three organic soluble products, namely benzo(a)pyrene 1,6-, 3,6-, and 6,12-quinones respectively. Ligninase attacks different types of aromatic compounds by creating cation radical ions[36].The term lignin-modifying enzymes (LMEs) should be used instead of the term ligninase, since these enzymes are not

hydrolytic but oxidative (electron withdrawing) by their mechanisms. Fungal ligninolytic enzymes (ligninase) are lignin peroxidase, laccase and manganese peroxidise (MnP). Transformed pyrene into 4,5-dihydropyrene was studied using immobilized MnP enzyme on nano-clay efficiently. Free MnPwas generally half as efficient as immobilised MnP. With the help of in vitro studies, laccase isoforms confirmed their ability to transform benzo(a)pyrene, though naphthalene and phenanthrene were not degraded[19].Lignin peroxidases are believed to be involved in the degradation of PAHs in white rot fungi. In addition to these enzymes, P450 monooxygenases in some fungi were implicated in the PAHs degradation[44].Only if the pollutants are found dissolved in organic solvents such as acetonitrile, tetrahydrofuran or dimethyl formamide, soyabean peroxidase (SBP) it catalyses the oxidation of a variety of PAHs. Another shortcoming of SBP is that they canfunction efficiently in the limited pH range (2.0–2.5). Despite its limitations, this enzyme is a good choice because of its efficiency even at this limited pH range and their high conversion rate (>90%)[19].By six different macroalgal species, the species-specific biotransformation of benzo(a)pyrene, a priority PAH, was dependent on the unique enzyme system for BaP detoxification of each species, including cytochrome P450, *o*-diphenol oxidase and peroxidase was studied[24,25].In preliminary investigations, the catalytic activities of some oxidoreductases in the transformation of PAHs were studied, where BaP was exposed to some enzymes and enzyme preparations. The activity of two representatives of the proposed active enzymes was experimentally analyzed in a water solution of BaP[1,23].In contrast to bacteria, fungi oxidizes aromatic hydrocarbons with the help of a cytochrome P-450 monoxygenase catalysed reaction[49,50,51]. Such type reaction appears to be similar to that reported for mammalian enzyme systems. Furthermore, studies revealed that the determining factor for oxidation was the ionisation potential (IP); indeed, when the molecules with an IP < 7.55 eV were degraded only[47].

Conclusion:

Biodegradation of HMW-PAHs has been observed to occur during the bioremediation process with an associated change in the relative abundances of organisms constituting the microbial community. From the various studies discussed in the review, the consortium of different microorganism might be a promising solution for degradation of HMW PAHs. The present review study will thus serve as an important reference in planning future for HMW PAHs experimental studies as well as developing an important technique of bioremediation rationales and approaches.

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