

Polycyclic aromatic hydrocarbons: Sources, effects and biodegradation

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Abstract: *Polycyclic aromatic hydrocarbons (PAHs) are a class of hazardous organic compounds consisting of three or more fused benzene rings in linear, angular, and cluster arrangements. PAHs occur in the environment as mixture of two or more compounds resulting from the incomplete combustion of organic materials. They are widely distributed and effect the environment as well as human health. Most of the PAHs are proved to be carcinogenic compounds. There are several microbial strains able to biodegrade those persistent pollutants. White-Rot Fungi with their lignin-degrading enzyme complex are the most perspective microorganisms in biodegradation of PAHs.*

Key words: *Polycyclic aromatic hydrocarbons, PAHs, carcinogens, white-rot fungi, lignin-degrading enzymes, laccase.*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 different chemicals that are formed during the incomplete burning of coal, oil and gas, garbage, or other organic substances like tobacco or charbroiled meat. PAHs are usually found as mixture containing two or more of these compounds. They are a class of hazardous organic chemicals consisting of three or more fused benzene rings which can be in linear, angular, and cluster arrangements. Polycyclic aromatic hydrocarbons can be separated in two groups based on the number of the benzene rings in their molecule. Low molecular weight (LMW) PAHs has three or less aromatic rings, while high molecular weight (HMW) PAHs has four or more rings.

The primary source of PAHs contamination is human activity, including fossil fuel combustion and industrial processing. Polycyclic aromatic hydrocarbons enter the environment from a multiplicity of sources which include: direct aerial fallout, use and disposal of petroleum products or from natural sources such as oil seeps and forest fires. Even though PAHs are not synthesized chemically for industrial purposes, there are a few commercial uses, as intermediaries in pharmaceuticals, agricultural products, photographic products, thermosetting plastics, lubricating materials, and other chemical industries, for many PAHs.

The persistence of PAHs in the environment is due mainly to their low water solubility which rapidly leads to their association with sediments. Recent research has shown that there are several microorganisms that can biodegrade PAHs. This process results in the decontamination of sediments and surface soils. PAHs can be totally degraded or partially transformed by either a group of microorganisms or by a single microorganism [1].

CHEMICAL CHARACTERISTICS, SOURCES AND USES OF PAHs

Polycyclic aromatic hydrocarbons are a class of over 100 different compounds that contain three or more fused benzene rings. Some PAHs may contain ring structures with fewer than six carbon atoms, when in others one of the carbon atoms in the benzene ring could be substituted by nitrogen, sulphur or oxygen atoms which results in the formation of heterocyclic aromatic compounds. Hydrogen atoms in the PAH molecule could also be substituted by alkyl groups. Their position in the structure of the PAH is very important for the determination of its carcinogenicity.

Based on their molecular structure polycyclic aromatic hydrocarbons can be classified as low molecular weight (LMW) PAHs or high molecular weight (HMW) PAHs. LMW PAHs contain three or less aromatic rings while HMW PAHs have four or more fused benzene rings. Physical and chemical properties of these substances depend on the differences in the structure and size of individual PAH. As pure chemicals PAHs generally exist as colorless, white or pale yellow-green solids. They have a faint pleasant odor.

PAHs enter the environment in variable pathways and usually are found as mixture

containing two or more of these compounds. Sources of PAHs can be divided to natural and anthropogenic. Natural sources include forest and grass fires, oil seeps, volcanoes, chlorophyllous plants, fungi and bacteria. Anthropogenic sources of PAHs include petroleum, electric power generation, refuse incineration, home heating, production of coke, carbon black, coal tar, and asphalt, internal combustion engines.

The major source of PAHs is the incomplete combustion of organic material. However, there are few compounds which are used for commercial needs. Anthracene is used as an intermediate in dye production, in the manufacture of synthetic fibers and as a diluent for wood preservatives. In medicine anthracene is used for the synthesis of the chemotherapeutic agent Amsarcine [2]. Acenaphthene is used as a dye intermediate, in the manufacture of pharmaceuticals and plastics and as an insecticide and fungicide. Fluorene is used as a chemical intermediate in many processes, in the formation of polyradicals for resins, and in manufacturing of dyestuffs. Phenanthrene is also used in the production of dyestuffs and in the manufacturing of explosives. Fluoranthrene is used as a lining material to protect the interior of steel and ductile-iron drinking water pipes and storage tanks [3].

Environmental fate, routes of exposure and effect on human health

Polycyclic aromatic hydrocarbons are formed as result of incomplete combustion of organic materials. PAHs are widespread in the environment and those which are emitted in air can be transported over long distances. PAHs in the ambient air are vapors or adsorbed into airborne particle matter. Gas-to-particle partition of PAHs depends on their molecular weight, temperature, humidity and precipitation. In general HMW PAHs which have high temperature of condensation are adsorbed on the airborne particles, while LMW PAHs that have low temperature of condensation mostly appear in the gas phase [4]. Research shows that air contributes up to 20% of total human exposure to PAHs and comes in second position (after food) as a source of these pollutants for humans.

The fate of PAHs in aquatic systems depends on various factors and processes. Because of their low water solubility and high affinity for organic carbon, two-thirds of PAHs in aquatic systems are primarily found sorbed to particles that either have settled to the bottom, or are suspended in the water column. Only one-third of them are found in dissolved form. While some of the processes in the aquatic system like photooxidation, hydrolysis, biotransformation, biodegradation and mineralization lead to transformation of PAHs to other substances, other such as bioaccumulation, adsorption, desorption and resuspension are responsible for the cycling of these compounds throughout the aquatic environment.

Particularly 90% of the total PAHs are located in soil. They are usually present in the environment as complex mixtures. In 1989 Jones et.al. reported that concentration of PAHs and benzo(a)pyrene in particular, has increased over 20 fold for 4 years between 1983 and 1987 [5]. The concentration of PAHs in contaminated soils from industrial sites can vary depending on the industrial activity associated with the site. Other potential sources of PAHs in soil include sludge disposal from public sewage treatment plants, automobile exhaust, irrigation with cake oven effluent, use of soil compost and fertilizers. Sorption of PAHs to soil and sediments increases with increasing organic carbon content and with increasing surface area of the sorbent particles.

PAH exposure occurs on a regular basis for most humans. The major exposure pathway to PAHs in the population is from breathing ambient and indoor air, smoking, eating food containing PAHs. Tobacco smoke contains variety of PAHs such as benzo(a)pyrene and more than 40 known or suspected human carcinogens. Other route of exposure is through foodstuffs. Barbecuing food over charcoal, wood or other type of fire greatly increases the concentration of PAHs. Some crops like wheat, rye and lentils may synthesize or adsorb PAHs via water, air or soil.

The effect on human health depends mainly on the length and route of exposure, the concentration of PAHs one is exposed to and the toxicity of the PAHs. Other factors like pre-existing health and age are also important.

PAHs effect on human health can be divided to acute or short-term effects and chronic or long term effects. Occupational exposures to high levels of mixtures containing PAHs have resulted in symptoms such as eye irritation, nausea, vomiting, diarrhea and confusion. Mixtures of PAHs are also known to cause skin irritation and inflammation. Health effects from chronic or long-term exposure to PAHs may include decreased immune function, cataracts, kidney and liver damage, asthma-like symptoms. Repeated contact with skin may include redness and skin inflammation. Naphtalene for example can cause the breakdown of red blood cells if inhaled or ingested in large amounts [6].

Reactive metabolites of some PAHs, such as epoxides and dihydrodiols, have the ability to bind with cellular proteins and DNA. The resulting biochemical disturbances and cell damage lead to mutations, development of malformations, tumors and cancer. The most common PAH that causes cancer in laboratory animals is benzo(a)pyrene. Ingestion of high levels of the PAH during pregnancy results in birth defects and decreased body weight of the offspring. U.S. Center for Children's Environmental Health report studies that demonstrate that exposure to PAH pollution during pregnancy is related to adverse birth outcomes including low birth weight, premature delivery and heart malformations. Genotoxic effects for some PAHs have been demonstrated both in rodents and in vitro test using mammalian (including human) cell lines. Most of the PAHs are not genotoxins by themselves and they need to be metabolized to the di-epoxides which react with DNA, thus inducing genotoxic damage.

BIODEGRADATION

Polycyclic aromatic hydrocarbons can be biodegraded by several microorganisms. There are some bacterial strains known to degrade those compounds. However, bacteria are known to degrade only low molecular weight PAHs. Therefore fungi and white-rot fungi in particular, due to their ability to degrade LMW PAHs as well as PAHs with four or more fused benzene rings, represent great interest for the research teams. White-Rot fungi are the most perspective class of microorganisms in that matter. Their non-specific extracellular enzymes are able to degrade lignin as well as wide range of environmental pollutants, which is why they are object of many studies in the past few years.

Lignin-peroxidase (LiP), manganese-peroxidase (MnP) and laccase (Lac) are the lignin-degrading enzymes which are produced by white-rot fungi. Those enzymes are extracellular and have low substrate specificity. LP and MnP are heme-containing enzymes and they require the presence of hydrogen peroxide to express their enzymatic activity towards lignin and lignin-related compounds. Laccases are multicopper phenol oxidases which can oxidize large range of phenolic compounds and aromatic amines.

One-electron oxidation, leading to generation of cation radicals of the pollutants, is performed by the ligninolytic enzymes. Those radicals can initiate chemical reactions like cleavage of C-C bonds or hydroxylation, which can result in more hydrophilic derivatives. Those products are cometabolized by the fungal strain in the presence of appropriate carbon source or carbon dioxide [7]. However, the mechanism of oxidation of lignin and lignin-related compounds is not quite clear, because those reactions include many low molecular weight cofactors, which also may act as redox mediators.

The most analyzed fungal strain until now is *Phanerochaete chrysosporium*, which is the producer of LiP and MnP, involved in the production of anthraquinone from anthracene [8]. The other group of enzymes, produced by white-rot fungi is laccases. They catalyze the reduction of one dioxygen molecule to two molecules of water and oxidizing aromatic substrates at the same time. Laccases have low substrate specificity, which makes them objects of several investigations regarding biodegradation of xenobiotics and aromatic

pollutants. The reactivity of laccases can be expanded towards nonphenolic lignin subunits by using aromatic mediators as ABTS, HBT and others.

The oxidation of 14 PAHs was tested with laccase, produced by *Trametes versicolor*. The removal rate of acenaphthylene was 37% and it was the highest to be measure, those for anthracene and benzo(a)pyrene were 18 and 19%, respectively, and the removal rate for the rest of the PAHs were under 10%. The addition of HBT to the reaction mixture induced the oxidation of PAHs resulting in almost complete removal of acenaphthylene, acenaphthene, fluorene, anthracene, benzo(a)pyrene, and perylene. The mediator addition increased the oxidation of pyrene and benzo(a)anthracene from 6 and 8% to 48 and 53% respectively [9]. Phenanthrene is one of the most abundant PAH in the environment and it is often used as indicator for PAH-contaminated wastes. It's been proved that static cultivating conditions for white-rot fungi are more efficient in biodegradation as well as enzyme production. The degradation of phenanthrene by laccase produced by *Trametes versicolor* doesn't show much a difference from this observation. In static conditions degradation rate was 65% while the rate in shaking culture was 46% [10]. In comparison with other PAHs which biodegradation was enhanced with the addition of HBT in the reactive mixture, phenanthrene shows no such enhancement in the presence of the mediator [9].

The most carcinogenic, mutagenic and teratogenic PAHs is benzo(a)pyrene (BaP). Since it has five fused benzene rings BaP belongs to HMW PAHs which are difficult to biodegrade due to their low water solubility. However, BaP can be biodegraded by *Trametes versicolor* and its laccase in certain reaction conditions. The best degradation rate of almost 100% can be obtained at pH 4; 40°C, 10% acetonitrile and ABTS used as mediator [11].

Even though a lot of microorganisms can degrade single PAHs the degradation of mixtures of them is more important since that is the way they occur in the environment. Due to their ligninolytic enzymatic system white-rot fungi are most likely to possess the ability of biodegradation of such mixtures. Recent studies show that biodegradation of poorly available polycyclic aromatic hydrocarbons can be enhanced by the addition of easily available ones in the reaction mixture [12].

CONCLUSION

The pollution of the environment is result from the everyday human activities. Polycyclic aromatic hydrocarbons are not only the most persistent pollutants but also they are one of the most dangerous, proved to be carcinogenic, compounds that could affect human health. An effective new method for their biodegradation needs to be developed to avoid future environmental catastrophes. The perfect choice for such method are white-rot fungi with their lignin-degrading enzyme system.

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