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Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHs): An Enzymatic Viewpoint

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Abstract

Original Research Article

Polycyclic aromatic hydrocarbons (PAHs) are a class of hazardous organic pollutants that pose significant environmental and health risks due to their persistent nature and carcinogenic properties. Traditional remedial techniques often fall short in effectively reducing PAH contamination in soil and water. Bioremediation, leveraging the metabolic capabilities of microorganisms, has emerged as a promising alternative for the degradation of PAHs. This abstract focuses on the enzymatic mechanisms involved in the bioremediation of PAHs, highlighting the role of microbial enzymes such as dioxygenases, dehydrogenases, and transferases in the enzymatic breakdown of complex PAH structures. These enzymes facilitate the oxidation, ring-cleavage, and mineralization of PAHs into less harmful compounds. Recent advances in molecular biology and bioengineering have enabled the identification and enhancement of specific PAH-degrading enzymes, improving the efficiency of bioremediation processes. Moreover, understanding the enzymatic pathways and interactions among microbial consortia offers insights into developing effective bioremediation strategies. This review underscores the importance of enzymatic approaches in the bioremediation of PAHs and suggests future directions for research in enzyme engineering and microbial ecology to optimize bioremediation processes for contaminated environments.

Keywords: Bioremediation, PAHs, Enzymes, Pollutants.

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INTRODUCTION

The method of bioremediation uses microorganisms or their enzymes to break down pollutants in the environment and restore contaminated areas. The persistent nature and possible toxicity of polycyclic aromatic hydrocarbons (PAHs) make them an important class of environmental pollutants. A broad class of compounds containing two or more fused aromatic rings arranged in linear, angular, or clustered configurations is known as polycyclic aromatic hydrocarbons (PAHs), or polyarenes. Small PAHs are those that have less than six aromatic rings, whereas big PAHs are those that have more than six aromatic rings (Haritash and Kaushik, 2009; Davletgildeeva and Kuznetsov, 2024). They exist as colourless, white or light yellow solids that have high melting and boiling temperatures, low vapour pressure, and poor water solubility. Their solubility in water diminishes as their molecular weight rises, as does their melting and boiling points and vapour pressure (Patnaik, 2007; Kinga *et al.*, 2024).

Because they are produced by the incomplete combustion or pyrolysis of many organic materials, including coal, oil, petroleum gas, and wood, PAHs are found in large quantities. Of all the non-halogenated molecule classes in the biosphere and environment, PAHs have the most range of structural variations. Furthermore, the amounts of these hydrocarbons in water and sediment will continue to rise due to ongoing oil production and transportation (Davletgildeeva and Kuznetsov, 2024) The environmental fate of PAHs includes leaching, adsorption on soil particles, chemical oxidation, photo-oxidation, and volatilisation (Haritash and Kaushik, 2009; Kinga et al., 2024). Their tenacity rises with their molecular weight, and they are challenging to break down in natural matrices. Because of their pervasiveness in the environment, resistance to biodegradation, potential for bioaccumulation, and mutagenic and carcinogenic effects—which can be brought on by breathing in PAH-containing air at work, coming into contact with air, water, or soil close to hazardous waste sites, or consuming tainted milk or water, among other things—these compounds are therefore a serious concern (Lei *et al*., 2007; Wang *et al*., 2014; Zhao *et al*., 2014). They are mostly created by incomplete burning of fossil fuels, industrial emissions, and natural sources like wildfires. They are organic molecules made up of many fused aromatic rings (Davletgildeeva and Kuznetsov, 2024).

Enzymatic Mechanisms in PAH Bioremediation

Many microorganisms, including bacteria, fungus, and algae, can change or break down PAH pollutants; however, the biodegradation process assisted by bacteria and fungi has received the greatest research attention (Ghosal *et al*., 2016). Nonetheless, compared to the usage of bacteria or fungus, enzyme-based bioremediation methods have a number of benefits (such as increased specificity and mobility of the enzymes, as well as the absence of reliance on costly coenzymes or cofactors of enzymatic bioremediation) (Villaverde *et al*., 2019). Furthermore, these technologies can function well across a wider variety of environmental factors, including temperature, ionic strength, and pH (Kinga *et al*., 2024). Redox enzymes, such as mono- and dioxygenases and peroxidases, often catalyse the initial critical steps in the most prevalent microbial degradation pathways, as seen in Fig. 1.

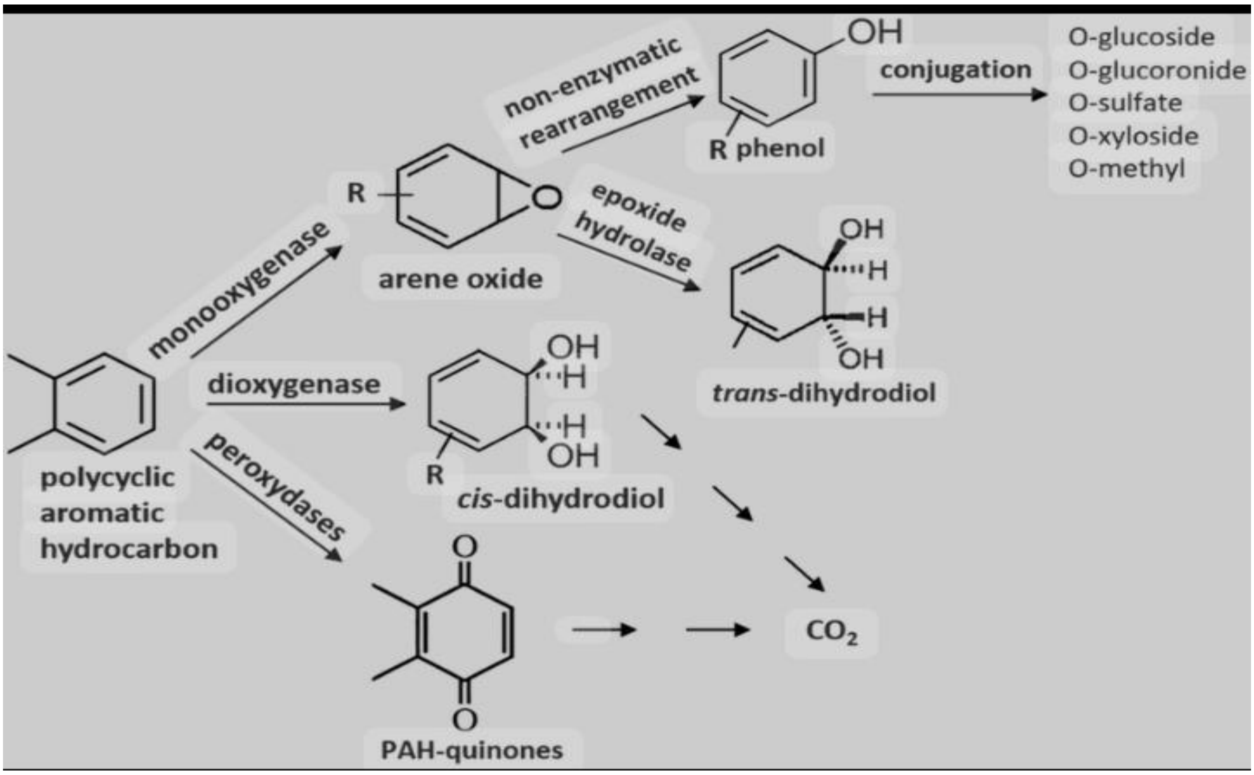

Figure 1: Microbial pathways for PAH degradation. The major enzymatic pathways leading to degradation of different PAH compounds are shown (Kinga *et al*., 2024)

Key Enzymes Involved in the Breakdown of PAHs

Certain characteristics of the enzymes, such as enhanced stability, substrate selectivity, and kinetic properties, are necessary for the use of enzymes in the breakdown of pollutants (Kinga *et al*., 2024). Particular enzymes produced by bacteria and fungi are mostly responsible for the bioremediation of PAHs. By changing PAHs into less hazardous or more easily biodegradable substances, these enzymes aid in their breakdown. An outline of the main enzymatic mechanisms at play is provided below:

1- Oxygenases

(i) Monooxygenases

This involves adding one oxygen atom to the substrate, which results in more breakdown products. Monooxygenase is the primary enzyme responsible for the breakdown of PAHs in fungus. According to Patel *et al*. (2020), there are two primary types of fungus that are utilised in PAH bioremediation: lignolytic fungi, often known as white rot fungi, and non-lignolytic fungi. Lignolytic fungi can oxidise PAHs to biphenolic intermediates, which may then be oxidised to quinones, thanks to a few enzymes that are specific for breaking down lignin, such as laccases, manganese peroxidases, and lignin peroxidases (Aydin *et al.*, 2017). In contrast, cytochrome P450 monooxygenase-like enzymes that may oxidise PAHs are produced by non-lignolytic fungus (Ghosal *et al.*, 2016).

(ii) Dioxygenases

These enzymes split the rings of aromatic compounds by introducing molecular oxygen. Two carbon atoms in the structure of PAHs can be broken down by these enzymes. There are mostly two kinds:

(a) Naphthalene dioxygenase: Specifically target naphthalene, converting it into 1,2-dihydroxy-naphthalene, which can undergo further transformation.

(b) Biphenyl dioxygenases: Known to degrade larger PAHs by oxidizing the aromatic rings (Ghosal *et al.*, 2016).

2. Ligninolytic Enzymes:

(i) Laccases and Peroxidases: Produced by fungi, these enzymes can oxidize PAHs and other aromatic compounds. They play a critical role in the breakdown of complex aromatic structures via electron transfer mechanisms.

3. Dehydrogenases:

These enzymes catalyze the oxidation of PAHs, facilitating their conversion into intermediate metabolites that can be further degraded by microbial pathways.

4. Hydrolases:

Enzymes like esterases and lipases can catalyze the hydrolysis of PAHs, leading to the formation of less complex and more biodegradable compounds (Kinga *et al.*, 2024).

Ring Cleavage

After the first oxidation, the oxidised PAHs compounds undergo further enzymatic degradation by ring breakage, which is made possible by enzymes like protocatechuate 3,4-dioxygenase or catechol 1,2-dioxygenase. This cleaves the oxidised PAHs compounds into smaller, more water-soluble molecules. In aerobic microorganisms, hydroxylation processes are often used to activate an aromatic substrate, and dioxygenases that cleave the ring perform the crucial de-aromatization phase. It is believed that ring-cleaving dioxygenases catalyse important processes in the aerobic microbial breakdown of aromatic substances. As of right now, ring-cleaving dioxygenases are classified into three major groups. Class I and II dioxygenases are responsible for the cleavage of the aromatic ring of catechol and its derivatives by intradiol- or extradiol-type enzymes, respectively.

These enzymes catalyse an oxidation process that yields cis, cis-muconic acid and its derivatives (class I, ortho cleavage) or, in the case of Meta cleavage (class II), 2-hydroxymuconate semi-aldehyde derivatives (Bugg and Ramaswamy, 2008; Vaillancourt *et al.*, 2006). Gentisate and its derivatives include two hydroxyl groups in a para orientation, which are necessary for class III ring cleaving oxygenases (Adams *et al.*, 2006). 3-maleylpyruvate and its derivatives are the end products of gentisate oxidation by third-class dioxygenases, also known as gentisate dioxygenases (Fetzner, 2012). There is little discernible sequence homology among the various types of ring-cleaving dioxygenases, suggesting that they have developed mostly independently (Chau *et al.*, 2001). However, there are some similarities between class III dioxygenases and class II (extradiol) dioxygenases. For instance, the active sites of both classes include catalytically active Fe(II) ions. In contrast, the active sites of class I intradiol dioxygenases include Fe(III) ions (Kinga *et al.*, 2024).

Environmental Factors Affecting Efficiency of Enzyme Bioremediation

Numerous environmental conditions impact the effectiveness of enzymatic bioremediation. The efficacy of bioremediation has mostly been studied in optimal laboratory settings with ambient mesophilic temperature and a pH that is circum-neutral. However, in practice, bioremediation can only work at locations where the environment supports microbial growth and exhibits related enzyme activity, allowing the microorganisms to attack the contaminants enzymatically and transform them into innocuous chemicals. It appears that a variety of biotic and abiotic factors (including pH, nutrient availability, and the bioavailability of the pollutants) can vary from one location to another. These variations can then affect the bioremediation process in those environments by either slowing down or speeding up the growth of the microorganisms that break down the pollutants (Kinga *et al.*, 2024). The main environmental factors that could affect the rate of biodegradation of PAHs in the environment are summarized below.

(i) Temperature

Since polluted sites are not always at ambient temperatures for the activities of the resident microorganisms, temperature has a significant impact on the biodegradation of PAHs there. An rise in temperature causes PAHs to become more soluble, which raises the compounds' bioavailability. Conversely, when the temperature rises the amount of dissolved oxygen falls, which lowers the metabolic activity of aerobic mesophilic microbes. In contrast to thermophiles, which function best at high temperatures, there are certain bacteria known as psychrophiles that can function at low temperatures. Certain contaminants may change into new compounds at high temperatures, and frequently the daughter product seems more dangerous than the parent compound, which prevents the biodegradation rate of the original chemical (Chan *et al.*, 2006). However, there are reports on PAH biodegradation in severe temperatures. In general, the majority of research have looked at PAH biodegradation under moderate temperatures. For instance, Brakstad and Bonaunet (2006) observed that petroleum hydrocarbons, including PAHs, biodegraded in saltwater at moderate temperatures (0–5◦C), but Thermus and *Bacillus spp*. showed biodegradation of PAHs and long chain alkanes at extremely high temperatures (60–70◦C) (Feitkenhauer *et al*., 2003).

(ii) pH

Additionally, pH is important for biodegradation activities, including PAH biodegradation. Microorganisms are often sensitive to pH, and most of them prefer settings that are close to neutral (6.5–7.5) for normal functioning. Nonetheless, it has been observed that the pH is far from neutral in many PAH-contaminated areas. For instance, a significant amount of demolition debris, such as brick and concrete, is commonly found at certain abandoned gasworks sites. The pH of the native soil may rise as a result of these materials leaching. Additionally, the oxidation of sulphides caused by the leaching of some materials, such as coal waste, can create an acidic environment (Ghosal *et al*., 2016). These alkaline or acidic environments may hinder microbial activity, which in turn slows down the biodegradation of PAHs in certain areas. In order to create an environment that is conducive to efficient biodegradation, it is therefore advised to modify the pH at those locations by adding chemicals. Basic soils can be treated with ammonium sulphate or ammonium nitrate, while acidic soils can be treated by liming with calcium or magnesium carbonate (Chan *et al*., 2006).

(iii) Oxygen

Although organic pollutants, such as PAHs, can biodegrade in both aerobic and anaerobic environments, the majority of research is on PAH bioremediation in aerobic environments, where oxygen serves as a co-substrate and a rate-limiting component. Oxygen is necessary for the activity of mono- and dioxygenase enzymes in the early oxidation of the aromatic rings during the aerobic biodegradation of PAHs. Oxygen is occasionally provided from an outside source for the in situ bioremediation of pollutants. External oxygen addition can be accomplished by air injection into the polluted location, drainage, tilling, and the use of oxygen-releasing compounds (Chua *et al*., 2001; Bewley and Webb, 2001). Magnesium peroxide (ORC R), an oxygen-releasing molecule, was used in a research to improve the intrinsic bioremediation of BTEX chemicals in an aquifer (Chau *et al*., 2001, Ghosal *et al*., 2016). In a different research, it was reported that an aquifer polluted with phenols, BTEX chemicals, and PAHs may be bioremediated in situ using a sequence of injection and abstraction wells to circulate sodium nitrate as the oxygen source (Bewley and Webb, 2001).

(iv) Nutrients

Another rate-limiting element for the effective bioremediation of habitats affected by PAHs is the availability of nutrients. Microorganisms need a variety of minerals, including iron, phosphorus, potassium, and nitrogen, in addition to an easily digested carbon source, for proper development and metabolism. Therefore, to improve bioremediation of pollutants, nutrient supplementation is necessary at pollutant-contaminated areas with low nutrient content in order to promote the development of autochthonous microorganisms (Atagana *et al*., 2003). Low levels of phosphorus and nitrogen in marine settings cause poor petroleum hydrocarbon biodegradation. However, too much nutrition availability can potentially prevent contaminants from being bioremediated (Chaillan *et al*., 2006). Numerous studies have documented how excessive nutrient levels hinder the biodegradation of organic contaminants, particularly PAHs (Chaîneau *et al*., 2005).

(v) Bioavailability

The percentage of a chemical that may be absorbed or changed by living things during an experiment is known as bioavailability in the context of a biological system. This proportion can change depending on the mass transfer parameters, which include biological processes like absorption and metabolism, hydrological activities like mixing, and physicochemical processes controlling dissolution, desorption, and diffusion (Semple *et al*., 2003). One of the most important elements in the bioremediation of contaminants is thought to be bioavailability. Because of their low water solubility and high propensity to sorb to organic materials (such as coal tar humic acids and black carbon) and mineral surfaces (such as clays) in the soil matrix, PAH biodegradation in the environment is frequently restricted. Because PAHs are absorbed in coal-tar and black carbon, which reduces their bioavailability, there are many accounts of inadequate or ineffective rehabilitation of PAH-contaminated sites (Northcott and Jones, 2001; Hong *et al*., 2003; Bucheli *et al*., 2004; Cornelissen *et al*., 2005; Liu *et al*., 2009; Benhabib *et al*., 2010; Rein *et al*., 2016). Once more, when molecular weight increases, PAHs become less soluble in water, which lowers their bioavailability. A phenomenon known as "ageing" of the contaminant has been observed: the longer hydrophobic organic pollutants (such as PAHs) are in contact with soil, the more irreversible the sorption and the worse the contaminants' chemical and biological extractability (Alexander, 2000; Luo *et al*., 2012). According to reports, PAHs' extractability and bioavailability drastically decline with age, which might be a major rate-limiting factor for in situ bioremediation (Alexander, 2000; Megharaj *et al*., 2002; Luo *et al*., 2012; Abdel-Shafy and Mansour, 2016).

(vi) Inhibition Due to Excess Substrate or End Product

The elimination or detoxification of pollutants from a particular environment is the main objective of bioremediation. On the other hand, the pollutant may occasionally change into a more hazardous byproduct. Verifying that the pollutant has fully mineralised at the conclusion of treatment is crucial for this reason (Mendonca and Picado, 2002; Lundstedt *et al*., 2003). The elimination of PAHs and the buildup of oxy-PAHs, such as PAH-ketones, quinines, and coumarins, as dead end products were both seen in a research that used a bioreactor to remediate PAH-contaminated gasworks soil utilising in situ bioremediation (Lundstedt *et al*., 2003). Because oxy-

PAHs are more hazardous than their parent PAHs, this study emphasises how important it is to monitor the metabolites during bioremediation, especially for harmful dead-end products, and assess the metabolites' toxicity both prior to and during treatment (Lundstedt *et al*., 2003). The impact of substrate interaction during biodegradation is essential in deciding the destiny of PAHs in nature since they are found in the environment as mixtures. According to reports, the bacterial population first breaks down the high molecular weight PAHs in a combination before moving on to the low molecular weight PAHs (Ghosal *et al*., 2016). Additionally, it has been noted that a specified bacterial coculture's ability to degrade other PAHs is inhibited by high naphthalene concentrations (Chau *et al*., 2001; Ghosal *et al*., 2016). Similarly, using two pure cultures, it was observed that naphthalene, methylnaphthalene, and fluorene in binary mixes competitively inhibited the degradation of phenanthrene (Ghosal *et al*., 2016). Therefore, for in situ bioremediation of PAHs to be effective, the concentration of PAHs in the polluted locations as well as the possibility of the creation of the harmful dead end product or products must be taken into account.

CONCLUSION

Understanding the enzymatic mechanisms in the bioremediation of polycyclic aromatic hydrocarbons provides valuable insights into how to effectively address environmental contamination. Through the application of microbial enzymes, strategies can be developed to enhance the degradation of these stubborn compounds, contributing to cleaner soils and water resources, and mitigating the impact of PAHs on ecosystems and human health. Future advancements in molecular biology and biochemistry are expected to further enhance these bioremediation efforts.

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