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Biosurfactants as Key Agents in Microbial Remediation of Polycyclic Aromatic Hydrocarbons: A Review

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Abstract

The growth of population and industrialization has resulted in environmental contamination by organic pollutants, which are less soluble in water. Among these, polycyclic aromatic hydrocarbons (PAHs) occupy a major group, produced from incomplete combustion of petroleum, coal, oil, and wood. These are widely distributed in soil, sediments, water, and air. PAH exposure causes adverse health problems in humans and mammals such as carcinogenic, mutagenic, and teratogenic effects. PAHs are hydrophobic, lipophilic, persistent, and recalcitrant to microbial degradation because of their complex chemical structure and high molecular weight. However, their biodegradation can be enhanced by biosurfactant-producing organisms. The amphiphilic nature of biosurfactants increases the solubility and bioavailability of PAHs, thereby promoting their microbial degradation and making biosurfactants integral to effective bioremediation processes. The biodegradability, non-toxicity, stability, and robust performance of bio-surfactants across variable environmental conditions present them as compelling substitutes for synthetic surfactants in bioremediation applications to overcome environmental problems associated with PAHs pollution. This review emphasizes the role of biosurfactants in the bioremediation of PAHs and their mechanisms, potential organisms producing biosurfactants, selection, production, and the factors influencing their efficacy, and the challenges and future perspectives in this field.

Keywords: Biosurfactants, Bioremediation, Polycyclic aromatic hydrocarbons, Mechanisms, Production

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Introduction

The persistent release of harmful organic contaminants, stemming from natural and anthropogenic activities such as industrial expansion, vehicular exhaust, cigarette smoke, municipal waste drain-offs, forest fires, and volcanic eruptions underscores the urgent need for effective pollution mitigation strategies (Liu *et al.*, 2019). These organic pollutants cover a wide array of xenobiotic chemicals including polychlorinated biphenyls, polycyclic aromatic hydrocarbons, gasoline, paints, plastics, adhesives, pesticides, and toluene (Sánchez, 2022). The frequent usage of petroleum and its derivatives has released a major portion of polycyclic aromatic hydrocarbons into the environment (Silva *et al.*, 2014). Polycyclic aromatic hydrocarbons, a major constituent of petroleum, pose a severe threat to human health and ecosystem (Souza *et al.*, 2014). Polycyclic aromatic hydrocarbons stand out due to their carcinogenic and mutagenic properties, necessitating the development of innovative remediation approaches (Nanca *et al.*, 2018; Mobeen & Dawood, 2022). Polycyclic aromatic hydrocarbons contain two or more fused benzene rings arranged in various positions. PAH properties vary significantly with molecular weight, affecting their behavior and fate in the environment. The hydrophobic nature of PAHs facilitates their cellular uptake, where they induce cytochrome P450 enzyme expression, catalysing their biotransformation into reactive intermediates capable of forming DNA adducts, thereby contributing to carcinogenicity (Honda & Suzuki, 2020). Therefore, it is crucial to eliminate or detoxify PAHs from the environment to alleviate toxic effects on humans, plants, and animals.

Conventional methods for PAHs removal, including soil washing and thermal treatments, often prove costly, ineffective, and environmentally disruptive, creating a demand for eco-friendly alternatives (Patel *et al.*, 2020). This has led to increased interest in bioremediation techniques, which control the natural abilities of microorganisms to degrade pollutants (Koshlaf & Ball, 2017). Bioremediation, which harnesses the metabolic abilities of microorganisms to degrade or transform contaminants, has emerged as a promising strategy (Lipińska *et al.*, 2014). The major challenge for biodegradation of PAHs is their low water solubility. However, certain microbes can produce surface-active compounds known as biosurfactants. Biosurfactants are amphipathic and can be synthesized by bacterial, fungal, and yeast strains (Nie *et al.*,

2010) and offer various advantages such as enhanced microbial activity, bioavailability, and solubility. Microbial strategies involve robust enzymatic systems comprising stable enzymes produced under extreme conditions, along with the ability to synthesize natural surfactants that enhance the bioavailability of hydrophobic organic pollutants (Kaczorek *et al*., 2018). Biosurfactants enhance the hydrophobicity of the microbial cell surface, thereby facilitating the access to and utilization of hydrophobic substrates by the producing microorganisms (Perfumo *et al*., 2010; Vischer & Burkard, 2023). For instance, rhamnolipids produced by Bacillus Lz-2 were used to increase the solubilization of PAHs, facilitating enhanced biodegradation (Shudong *et al*., 2015). Hence, the application of biosurfactants in bioremediation has been recognized as a promising approach to mitigating PAH's environmental pollution.

Polycyclic Aromatic Hydrocarbons Properties, Sources and Bioavailability

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of organic compounds/pollutants comprised of multiple fused benzene rings, produced due to incomplete combustion of coal, oil and petroleum, automobile exhaust, forest fires, and cigarette smoke. The increasing demand for processed petroleum and agricultural products has contributed to the contamination of the environment with PAHs (Satyaprakash *et al*., 2017). Due to low water solubility and lipophilic nature, PAHs tend to persist in the environment, accumulating in soils and sediments, and posing long-term risks to the environment and health of humans. PAHs can contaminate various environmental compartments, including soil, water, and air, posing risks to ecosystems and human health. The environmental impact of PAHs is further exacerbated by their ability to undergo long-range transport, facilitating their dispersal across geographical boundaries. PAHs are classified based on chemical structure and molecular weight including low and high molecular weight PAHs. The no. of aromatic rings in low molecular weight is ≤3, whereas in high molecular weight is >3. For example, low molecular weight PAHs include naphthalene, fluorene, acenaphthene, anthracene, and phenanthrene; whereas high molecular weight PAHs comprise chrysene, benz(a)anthracene, pyrene, benzo(a)pyrene, benzo(b)fluoranthene, and indeno(c,d)pyrene. PAHs are neutral and non-polar molecules, their hydrophobicity, vapor pressure, and solubility in water vary with molecular weight as shown in **Table 1**. As molecular weight increases, the melting and boiling points increase, and the vapor pressure and solubility in water decreases. The US Environmental Protection Agency (EPA) recognized that 16 PAHs are highly concerned pollutants. The International Agency for Research on Cancer (IARC) categorized three specific PAHs as probable carcinogens (Group 2A) namely benzo(a)anthracene, dibenz(a,h)anthracene, and benzo(a)pyrene (Nagar *et al*., 2023). Considering PAH's status as carcinogenic, widespread, and persistent chemicals in the environment, raises significant concern on PAHs pollution. PAH toxicity has adverse health effects on humans, animals, aquatic life, and plants. For instance, in humans PAH exposure via inhalation and dermal contact cause cancer, respiratory, neurological, and cardiovascular problems (Barathi *et al*., 2023). Hence, it is critical to plan effective, sustainable, and eco-friendly remediation strategies to eliminate carcinogenic PAHs from water and soil sediments. Among various available bioremediation methods, microbial bioremediation with biosurfactant production is a promising and sustainable approach for the effective handling of PAH environmental pollution.

Table 1. Properties of some polycyclic aromatic hydrocarbons (NCBI, 2025)

PAH (aromatic ring number)	Chemical Formula	Molecular weight (g/mol)	Melting & (ºC) Boiling points (ºC)	Vapor pressure at 25ºC (mm Hg)	Solubility in water at 25ºC (mg/L)
Naphthalene (2 ring)	$C_{10}H_8$	128.17	80.2 & 217.9	0.085	31
Acenaphthene (3 ring)	$C_{12}H_{10}$	154.21	93 & 277.5	0.0022	3.9
Fluorene (3 ring)	$C_{13}H_{10}$	166.22	114.76 & 294	$6x10^{-4}$	1.69
Anthracene (3 rings)	$C_{14}H_{10}$	178.23	216 & 341.3	$6.56x10^{-6}$	0.0434
Pyrene (4 ring)	$C_{16}H_{10}$	202.25	151.2 & 394	$4.5x10^{-6}$	0.135
Chrysene (4 ring)	$C_{18}H_{12}$	228.3	225 & 448	$6.23x10^{-9}$	$2x10^{-3}$
Benzo(a)pyrene (5 rings)	$C_{20}H_{12}$	252.3	179 & 496	$0.1x10^{-7}$	$1.62x10^{-3}$
Indeno(1,2,3-c,d)pyrene (6 ring)	$C_{22}H_{12}$	276.3	164 & 536	$1.01x10^{-10}$	$1.9x10^{-4}$

Biosurfactants in PAHs Bioremediation

Biosurfactants are surface-active compounds produced by microorganisms, possessing both hydrophobic and hydrophilic regions that interact with different polarity molecules allowing them to reduce surface and interfacial tension between liquids, solids, and gases (Sharma *et al*., 2022). Their amphiphilic nature is crucial for enhancing the bioavailability and decreasing surface tension of hydrophobic pollutants like PAHs, thereby facilitating bacterial access. Biosurfactants are highly beneficial compared to synthetic surfactants due to being non-toxic, biodegradable, higher specificity, and effective in extreme environments (Pi *et al*., 2017; Sanchali *et al*., 2024). These substances consist of a hydrophilic region composed of amino acids, mono- or polysaccharides, or peptides, and a hydrophobic region made up of saturated or unsaturated fatty acids (Ludovichetti *et al*., 2024; Sanchali *et al*., 2024). Biosurfactants are classified as glycolipids, phospholipids, polymeric macromolecules, lipopeptides/lipoproteins, and fatty acids (Nie *et al*., 2010). Further biosurfactants sub classified based on chemical structure and molecular weight as low and molecular weight biosurfactants. High molecular-weight surfactants bind strongly to the surface, whereas low molecular-weight surfactants decrease surface and interfacial tension (Vogel *et al*., 2023; Banerjee *et al*., 2024). Among these, glycolipids and lipopeptides are promising biosurfactants in solubilizing PAHs, and facilitate access to microbial enzymes that break them down. Biosurfactants can increase the solubility of PAHs through micelle formation, wherein the hydrophobic tails of the bio-surfactant molecules aggregate to form a core that solubilizes the PAH molecules, while the hydrophilic heads interact with the surrounding water, facilitating their transport and uptake by microorganisms (Lamichhane *et al*., 2017; Mahmood *et al*., 2022). Eventually, results in the degradation of PAHs into less hazardous compounds as shown in **Figure 1**. Furthermore, biosurfactants can also promote the detachment of PAHs from soil particles, facilitating their mobilization and transport to microbial cells. Beyond their solubilization capabilities, biosurfactants can also alter the cell surface hydrophobicity of microorganisms, promoting their adhesion to hydrophobic pollutants and enhancing direct contact between the cells and PAHs.

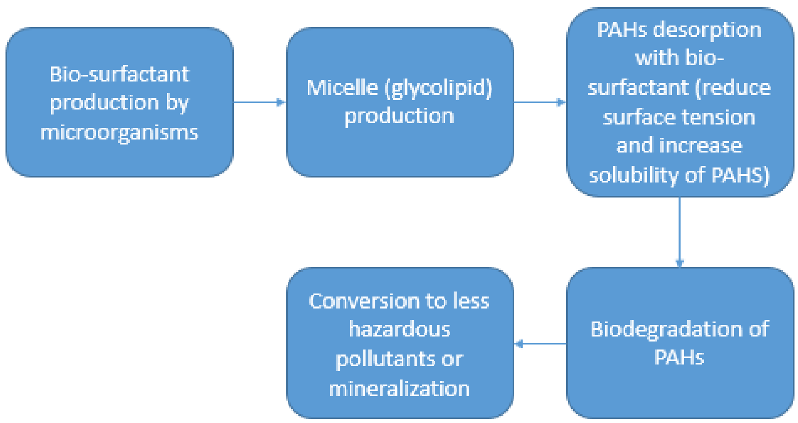

Figure 1. PAHs biodegradation strategy via microbial biosurfactant production

Mechanisms of Biosurfactant Assisted PAHs Bioremediation

Biosurfactants enhance the bioremediation of PAHs through several mechanisms, including increasing the solubility and bioavailability of PAHs, enhancing microbial access to PAHs, and thus promoting the biodegradation of PAHs. By reducing surface tension and interfacial tension, biosurfactants facilitate the emulsification of PAHs and the formation of micelle, increasing the surface area available for microbial attack. Biosurfactants can interact with the cell membranes of microorganisms, increasing their hydrophobicity and promoting their adhesion to PAHs. Biosurfactants can directly interact with PAH molecules, altering their chemical structure and making them more susceptible to enzymatic degradation.

To mitigate oil spill pollution in the marine environment, synthetic surfactants are generally considered in the ocean, however, these are associated with certain drawbacks including toxicity, degradability, and secondary pollution (Liu *et al*., 2022; Sharma *et al*., 2022). Biosurfactants are a major alternative to synthetic surfactants, which have high emulsifying action and surface activity. When biosurfactants are released by microbes into aqueous PAHs suspension, their monomers form sphere-shaping micelles, as a result hydrophobic end of the surfactant turns towards the center, composing the nucleus, whereas the hydrophilic end is turned to the surface and interact with water. Thus, biosurfactants increase the solubility of PAHs, reduce surface tension at the interface, enhance the bioavailability of PAHs to microbes, and facilitate higher degradation rates (Soberon-Chavez & Maier, 2010). Some of the biosurfactants reported in the literature for improving the degradation of PAHs are presented in **Table 2**.

Table 2. Biosurfactants produced by different bacteria enhance the biodegradability of PAHs

Biosurfactant	Bacteria	Type of PAHs degraded with enhanced percentage degradation	Reference
Rhamnolipid	*Pseudomonas aeruginosa* SR17	Enhanced degradation to 100% for floranthene, benz(b)fluorene, and benz(d)anthracene	(Rupshikha *et al*., 2018)
Rhamnolipids and surfactants	*Pseudomonas aeruginosa* S5	Degradation of total PAHs increased to 18.97%.	(Tingting *et al*., 2021)

Lipopeptide	Mixed culture of *Pseudomonas* and *Pannonibacter*	The degradation of pyrene and phenanthrene was enhanced by up to 74.28% and 63.05%, respectively.	(Irfan *et al*., 2024)
Rhamnolipid	External supply	Achieved the highest degradation rate of 95% with the external addition of biosurfactants	(Wang *et al*., 2016)

Biosurfactants mediate the intricate interplay between microorganisms and PAHs, enhancing the overall efficiency of bioremediation processes (Silva *et al*., 2014). Biosurfactant lipopeptides produced by *Pseudomonas* sp. can remove PAHs such as Benzo(a)pyrene, Chrysene, and Benzo (b) fluoranthene from contaminated soil (Xia *et al*., 2014). The exact role of biosurfactants in the dissipation of mixed PAHs, modulation of microbial community structure, and influence on metabolic profiles remains unclear (Irfan *et al*., 2024). This enhanced bioavailability and biodegradability of PAHs with biosurfactants represents a significant advancement in environmental biotechnology, offering a sustainable alternative to traditional chemical methods.

Factors Influencing Biosurfactant Efficacy

Several factors influence the efficacy of biosurfactants in PAH bioremediation, including the type and concentration of biosurfactants, the type and concentration of PAH, the microbial community composition, and environmental conditions such as pH, temperature, and salinity. The optimal biosurfactant concentration is crucial for effective PAH solubilization and biodegradation, as excessive concentrations may exhibit inhibitory effects on microbial activity. The composition of the microbial community plays a significant role in determining the extent of PAH biodegradation, with certain microbial species exhibiting higher PAH degradation capabilities.

The effectiveness of biosurfactants in PAH bioremediation is influenced by a complex interplay of factors, requiring careful optimization and adaptation to specific environmental conditions. The type and concentration of biosurfactants play a critical role in determining the extent of PAH solubilization and biodegradation, with different biosurfactants exhibiting varying efficiencies depending on the PAH compound and environmental context. The composition of the microbial community is another important determinant, as certain microbial species possess superior PAH degradation capabilities. Understanding and optimizing these factors are essential for maximizing the effectiveness of biosurfactant-assisted bioremediation strategies.

Biosurfactant Production and Selection

Biosurfactants, amphiphilic molecules produced by living organisms, are gaining increasing attention as sustainable alternatives to synthetic surfactants (Vieira *et al*., 2021). A wide variety of microorganisms, including bacteria, fungi, and yeasts, are capable of synthesizing biosurfactants, utilizing various substrates such as carbohydrates, lipids, and hydrocarbons. Certain microorganisms synthesize biosurfactants exclusively in the presence of hydrophobic substrates, whereas others are capable of producing them during growth on both hydrophobic and hydrophilic carbon sources (Gautam & Tyagi, 2006). The selection of biosurfactant-producing microorganisms and the optimization of fermentation conditions are crucial for maximizing biosurfactant production and reducing production costs. Biosurfactant production can be influenced by factors such as the growth substrate, temperature, pH, and sources of nitrogen and carbon (Sanches *et al*., 2021). The production of biosurfactants by microorganisms involves a complex interplay of metabolic pathways and regulatory mechanisms, requiring careful optimization of fermentation conditions to maximize yields. Marine microorganisms have evolved distinctive metabolic and physiological traits to thrive in diverse habitats characterized by wide variations in temperature, pressure, salinity, pH, and nutrient availability (Maneerat, 2005). Furthermore, the isolation and genetic modification of microorganisms with enhanced biosurfactant production capabilities are promising strategies for improving biosurfactant yields. The selection of appropriate biosurfactant-producing microorganisms and the optimization of fermentation conditions are critical steps in ensuring high yields and cost-effective production.

Challenges and Future Perspectives

Despite the promising potential of biosurfactants in PAH bioremediation, several challenges remain, including the high production costs, the limited availability of suitable biosurfactants for specific PAH compounds, and the potential for biosurfactant degradation under certain environmental conditions (Fenibo *et al*., 2019). Future research directions include the development of more efficient and cost-effective biosurfactant production methods, the discovery and characterization of novel biosurfactants with enhanced PAH degradation capabilities, and the optimization of biosurfactant application strategies for various environmental settings. Biosurfactants offer a promising avenue for sustainable and effective PAH bioremediation. Biosurfactant-amended bioremediation has limitations and potential causes of failure, so investigating the effects of biosurfactants on biodegradation and phytoextraction efficiency must be understood before treatment processes are designed (Ławniczak *et al*., 2013). The strategies for improving natural surfactant production by microbes, cost-effectiveness, and the commercialization of biosurfactants for petroleum hydrocarbons are areas that need attention (Dhanya, 2021).

Conclusion

Biosurfactants offer a promising and sustainable approach to the bioremediation of polycyclic aromatic hydrocarbons. However, several challenges remain, including high production costs, limited availability of suitable biosurfactants for specific PAH compounds, and the potential for biosurfactant degradation under certain environmental conditions. To fully unlock the potential of biosurfactants in PAH bioremediation, future research should focus on emerging methods, discovery and characterizing novel biosurfactants with enhanced PAH degradation capabilities and optimizing biosurfactant application strategies for various environmental settings. Addressing these challenges and further advancing the biotechnology associated with in-situ biosurfactant production can pave the way for the widespread adoption of this environmentally friendly remediation approach.

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