# Microbial Bioremediation Techniques for Polycyclic Aromatic Hydrocarbon (PAHs)—a Review

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Abstract This review summarizes current knowledge of the polycyclic aromatic hydrocarbons (PAHs) contaminant in the environment and the importance of using benzo(a)pyrene in this study. It highlights the removal techniques in eliminating the PAHs and their limitations on the bioremediation method. The factors that affect the remediation were thoroughly explained in this paper, focusing on removing PAHs using bioremediation. The remediation methods using bacteria and fungi have emerged as the potential degrader of PAHs pollutants and are being extensively studied to evaluate the best removal conditions. The PAHs degradation pathways have also been discussed in this paper to provide insight on microbial bioremediation in reducing PAHs.

**Keywords** High molecular weight (HMW) · Polycyclic aromatic hydrocarbons (PAHs) · Microbial bioremediation · Pathways

# 1 Introduction

The high population and the misbehaviour of human activities contribute to environmental conditions that

N. A. Ismail · N. Kasmuri (🖾) · N. Hamzah School of Civil Engineering, College of Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia e-mail: norhafezahkasmuri@uitm.edu.my worsen nowadays. Pollution, which mostly comes from anthropogenic activities, needs to be encountered seriously to avoid harm for the present and the future. Polycyclic aromatic hydrocarbons (PAHs) are contributors to the contaminants. The environment is exposed to the contamination of PAHs as high demand for processed petroleum and agricultural products. These compounds exist with different numbers of benzene rings and eventually affect nature and consumers differently. Their presence creates problems such as triggering malignancy in human cells and abnormal growth of tissue in animals (Abdel-Shafy & Mansour, 2016).

The PAHs' molecular weight has been classified as low molecular weights (LMW) and high molecular weights (HMW). LMW PAHs contain two or three benzene rings, while HMW PAHs are those with four or more benzene rings (Nwaichi & Ntorgbo, 2016). LMW PAHs are relatively water-soluble, but HMW is comparatively hydrophobic and unsolvable in water, disturbing the removal process (Mandal & Das, 2015). Biodegradation is a commonly used method for eradicating pollutants, including PAHs (Kronenberg et al., 2017). It is a natural way of breaking down organic matter into nutrients that can be utilized and reprocessed by different biota, a greater choice for the remediation techniques (Dzionek et al., 2016). Bioremediation can be implemented in the polluted area, and the specific microbes will be used to break down the pollutants. The selection of the limiting factors is essential to promote the microorganism's growth. The



organisms mostly involved in this process are bacteria, yeast, and fungi (Abatenh et al., 2017). The bacteria are commonly used in removing the PAHs, especially for the low molecular weight (LMW) PAHs. As the fungi became as important as bacteria in the bioremediation of PAH-contaminants, their ability for the tasks have been completely recognized (Khatoon et al., 2021). However, the fungi do not use PAHs as their sole carbon and energy sources, like the bacteria, but they convert PAHs co-metabolically to detoxified chemical products (Shah et al., 2019).

# 2 Existence of Polycyclic Aromatic Hydrocarbons in the Environment

Hydrocarbons are one of the impurities that exist in the surrounding environment. These organic composites structures comprise hydrogen and carbon (Mohammadi et al., 2020). Their presence can be seen in several kinds of arrangement: linear linked, branched, or cyclic molecules and has been regarded as aromatic or aliphatic hydrocarbons (Mahgoub, 2019). The examples of these hydrocarbons are the one that consists of benzene  $(C_6H_6)$  in their composition and the structure that can be observed in three forms which are alkanes, alkenes, and alkynes (Abozenadah et al., 2017). Polycyclic aromatic hydrocarbons (PAHs) are hydrocarbon contaminants (Honda & Suzuki, 2020). The main origins of PAH contamination are from industrial fabrication that widely persists globally, which contributes to the PAH existence (Abdel-Shafy & Mansour, 2016). This pollutant has been studied thoroughly because of its toxicity, environmental persistence, and effects on nature. PAHs, which easily can be found in soil, water, and air, became severely dangerous to living organisms (Gupte et al., 2016).

Polycyclic aromatic hydrocarbons (PAHs) are classified as hazardous chemicals in nature and the environment anticipated by many anthropogenic activities (Patel et al., 2020). The formation of the output generated is determined by the nature of the exposed substance and the temperature itself. For example, the quantity of PAHs released after agricultural and forest fires varies with ignition conditions (Faboya et al., 2020). Generally, pollutants will not easily decompose and are present in the ecosystem longer when exposed to the environment. Therefore, these PAHs mostly exist as pollutants in the air, soil, sediments, surface water and groundwater (Srivastava et al., 2019). PAHs come from natural and anthropogenic sources (Ghosal et al., 2016). The forest or bushfire and thermal geologic production naturally form PAHs (Lawal, 2017). However, anthropogenic sources of the PAHs usually came from fuel combustion, domestic heaters, waste incinerators, and petroleum products' spillage in the environment (Nikitha et al., 2017). In addition, manufacturing activities, such as processing, combustion, and disposal of fossil fuels, are typically related to the existence of PAHs in these places, which can be considered extremely polluted (Yan et al., 2016).

As mentioned earlier, most PAHs are originally present and mainly emitted from both anthropogenic activities and natural sources, drive out into the atmosphere, where they get partitioned into the particulate and gaseous phase, before being transported and deposited back to the water bodies, soil, vegetation as the final sinks (Guo et al., 2011). According to Wang et al. (2009), a large fraction of the PAHs in the environment is found in the soils and sediments, their ultimate sinks. However, these PAHs pollutants also became the most significant contaminants in the marine environment, resulting in soil and water contamination and affecting bioaccumulation in the food and animal tissues (Włóka et al., 2014).

#### 2.1 Polycyclic Aromatic Hydrocarbons in Air

The formation of polycyclic aromatic hydrocarbons (PAHs) has become one of the most important air pollution types of research because of the significant environmental impact attributed to these compounds. The PAHs formed in the environment due to the combustion of carbon-based fuel, including fossil fuels, woods, and agricultural biomass (Stogiannidis & Laane, 2015; Tian et al., 2015). They are presented in atmospheric aerosols, volatile particles, soot, and commonly together with other airborne toxic chemicals (Abdel-Shafy and Mansour 2016). Low molecular weight (LMW-PAHs) is mainly present in the gas phase, such as waste incinerators, vehicle engines and coke production plants, while high molecular weight (HMW-PAHs) are commonly associated with atmospheric particulate matter (PM) (Gaurav & Yadav, 2020; Samburova et al., 2017). Moreover, the study by Låg et al. (2020) stated that the incomplete combustion of coal and several organic materials such as fossil fuels and cigarette smoking would produce a mixture of pollutants, including PM. There were many organic chemicals in PM, including the polycyclic aromatic hydrocarbons (PAHs), which have the highest toxicity in cytotoxicity, carcinogenicity, and mutagenicity (Yang et al., 2021). The pyrene and phenanthrene concentration levels from the diesel exhaust and wood smoke particles usually exceed the limit of the carcinogenic indicator (benzo[a]pyrene).

Furthermore, Vari et al. (2020) studied the emission of PAHs in both urban and rural areas. Traffic and vehicular exhaust became the major contributor to the urban areas, while agricultural practices included burning biomass and grassland. Teixeira et al. (2015) also found the increment of air pollution due to increased vehicle fleet and industrial expansion. Their findings prove the influence of PAHs emissions from mobile sources, mostly from gasoline and diesel engines combustion, for all their studied sites. Moreover, the dust may be present in fine particles and usually comes from various sources such as road surface materials, vehicular depletion, oils and fuels, tires particles and coal power plants. Hsu et al. (2015) also supported the PAHs emission with NO, NO<sub>2</sub>, PM2.5 and SO<sub>2</sub>, showing the emissions were from these sources, including industrial stacks, vehicles, and residential heating. Besides, the high temperature through warmer environment deposes the PAHs from the condensed phases such as soil, vegetation, particles, and water and into the atmosphere.

Then, the fates of PAHs in the atmosphere after emission from sources include partitioning between gas and particulate phases, particle size distribution, dry and wet deposition on water bodies, soil, vegetation, and other receptor surfaces (Cheruiyot et al., 2015). During incomplete combustion, the emission of PAHs is primarily caused by unburned PAHs found in fossil fuels, and there is a direct relationship between the PAH content of the fuel and PAH emission and formation from exhaust streams (Lee et al., 2011).

# 2.2 Polycyclic Aromatic Hydrocarbons in Wastewater

Wastewater has resulted from the human influence on the aquatic environment, which originated from the effluent of several day-to-day deeds involving industrial activity, domestic usage, commercial and agronomic sectors (Fufă et al., 2017). Since the wastewater comes from numerous sources, many contaminants exist in the sewage. In addition, the toxins such as heavy metals, hydrocarbons and other pollutants have harmful effects on the sensitive receptors, including humans, animals, and the environment (Masindi & Muedi, 2018; Srivastava et al., 2019). The wastewater from all domestic and industrial sources has been collected at the major reservoir called the wastewater treatment plant (WWTP). Therefore, WWTP also became one of the sources of PAH in environments. Here, PAHs are emitted into WWTP, and due to the wastewater treatment's efficiency, PAHs have been discharged through the river as effluent. PAHs were absorbed in the sludge and later degraded or volatilized into the air during the process. Industrial water is more likely to have a higher risk of contamination than the municipal WWTP since wastewater is directly coming from a source such as coke production plants, including PAHs (Zango et al., 2020).

A high temperature, for example, could result in a large amount of low molecular weight PAHs evaporating into the atmosphere. HMW PAHs are more likely to adsorb on sludge containing high organic matter (Sun et al., 2018). PAHs with a low ring can easily release to the atmosphere, while PAHs with a large ring tend to adsorb onto particles near the source. Although PAH migration and transformation in WWTPs are complex, it can be noticed that the influent concentration has a significant impact on PAH concentration in the effluent. A large amount of sediment caused by automobile exhaust emissions and particle adsorption on the surface raises the concentration of PAHs in WWTPs via surface runoff during rainy days (Sun et al., 2018). As a result, rainfall may be a significant contributor to increasing the PAHs contamination. Besides home cooking and gas combustion, there may be higher PAH levels in enclosed indoor environments, introducing more PAHs into WWTPs through cleaning and other activities (Bai & Li, 2022). The major anthropogenic contributions of PAHs were considered from the effluent of WWTP (Edokpayi et al., 2016).

Additionally, Qamar et al. (2017) also studied the sources of PAHs in vehicle-wash wastewater. This type of wastewater came from the vehicle wash and maintenance, which involved the removal of oil,

dirt, traffic grime and particles using surfactants and degreasing solvents. The process involves the large volume of clean water altered into the wastewater, consisting of petroleum products and toxic chemicals such as diesel, engine oil, gasoline, greases and lubricants, which were washed away from the surface and engine of the vehicle (Gowthaman et al., 2017). As the number of vehicles increases, vehicle-wash wastewater also rises. These vehicles may bring the PAHs attached to its body in the form of dust, smoke, rainy mud, and other atmospheric deposition. This wastewater with a high PAH concentration gets into the adjacent sewers, irrigation, and municipality channels without any prior treatment and affects the PAH concentration in WWTP (Qamar et al., 2017).

# 2.3 Polycyclic Aromatic Hydrocarbons on Soil

Several studies point out that plants can absorb and translocate PAHs to their above-ground parts (Marchal et al., 2014; Yu et al., 2013). In addition, PAHs may also be introduced to the soils via sewage fertilizers, coal and petroleum, atmospheric deposition, and other exposure sources for PAHs (Chou et al., 2015; Wołejko et al. 2017). Li and Ma (2016) stated that the accumulation of sludge would increase the content of PAHs in the soils. They also found that 3-ring of PAHs dominate the control soil whereas 4-6 ring of PAHs prevails in the sewage sludge. It can be denoted that landfilling process is not desirable for sewage sludge due to the limited availability of sites for landfills, and the sludge was used as the farmland fertilizer to avoid other pollution problems (Kwon et al., 2018). However, this prevention method has become inadequate due to organic contaminants and pathogenic bacteria, including PAHs in the sewage sludge. Batistella et al. (2015) stated that the presence of PAH in sewage sludge can be attributed to the direct disposal of these pollutants in the effluent and the transport of PAH from the atmosphere via rainwater.

Additionally, agricultural practices also increase the PAHs concentration in soils as many activities, including irrigation involved throughout the process (Haddaoui et al., 2015). One of HMW PAH, which is pyrene showed the highest concentration, indicating the presence of light petroleum products and biomass burning. According to Zhou et al. (2015), soils in urban areas have a higher concentration of PAHs than rural areas as the dust consists of toxic substances. These substances would be transferred to the other parts of the environment, including soils by municipal sewage system and urban and street runoff, re-deposition, and resuspension (Jiang et al., 2016). Moreover, those processes would also transfer the PAHs from the soils to the water bodies.

# 2.4 Polycyclic Aromatic Hydrocarbons in Water Bodies

According to the US Environmental Protection Agency (USEPA) (1993), about one third of entire water pollution due to the various toxic chemicals, including PAHs, were discharged into the rivers and other water bodies (Bargiel & Zabochnicka-Swiatek., 2018). The PAHs could end up in the ground and surface water due to the runoff, resulting in environmental problems. Furthermore, coal fire plants that contribute to high electricity generation are worrisome as PAHs have existed in the plant.

PAHs also has been classified as priority pollutants in water and sediments. Some of the PAH characteristics, which are semi-volatile and susceptible to dispersion, made them pass between the atmosphere and the earth's surface in recurrent, temperature-driven cycles of deposition and volatilization under environmental conditions (USEPA, 1993). Zhang et al. (2019a and b), studied the source of PAHs in the coastal water and found that they mainly come from coal combustion, the oil spill from ships and petroleum combustion. Ya et al. (2014) stated that the source of PAHs to the coastal water involves both direct and indirect processes. The direct inputs of PAHs in the coastal water include river discharge, traffic emission, sewage outflow, and oil spill, while indirect inputs are atmospheric deposition and air-water gas exchange. Meanwhile, the semi-volatile PAHs and their characteristics in both gas and particle phases proved that they could be transferred into coastal water bodies by both dry and wet depositions (Kim & Chae, 2016).

## 3 Classes of PAHs

The PAH composition can be categorized as low molecular weights (LMW) or high molecular weights (HMW), depending on the number of benzene rings. The LMW PAHs contain two or three benzene rings, while HMW PAHs for those with four or more benzene rings (Edward et al., 2020). Figure 1 shows LMW and HMW PAHs with different benzene rings. Their difference makes the remediation action is peculiar in both substances. Each PAH class needs its remediation to be eliminated or reduced in wastewater or any media that PAHs exist. It is only applicable for the soil and differs from wastewater, which comes from many sources (Manasa & Mehta, 2020). The characteristic of HMW PAH is more likely to adsorb in soil organic matter when they emerge in the soil. The inclination of these substances to intensely adsorb on the particulate matter makes the HMW PAHs less obtainable as a free compound and thus less susceptible to remediation (Lasota & Błońska, 2018). In chemical terms, the HMW PAHs have a high resonance of energies due to the dense clouds of pi-electrons, which surround the aromatic rings of the compound. The energy resonance measures the additional steadiness of the conjugated system in contrast with the equivalent number of isolated double bonds. The high resonance energies make them more persevering in the ecosystem and have less reaction towards degradation, making the HMW PAHs not preferable for biodegradation compared to the LMW PAHs (Dushyant & Bharti, 2018).

Polycyclic aromatic hydrocarbons (PAHs) are hydrocarbons containing two or more fused benzene rings in linear, angular, or clustered arrangements (Mahgoub, 2019). The PAHs are highly environmental contaminants that consist of fused aromatic rings. PAH compositions include two to seven-membered (fused) benzene rings. They are hydrophobic, which mean that this substance can mix easily in oil compared to water, and the compounds with aqueous solubility lessen to almost linear with a greater amount in molecular mass (Lamichhane, 2017). Generally, the increase of the number of fused benzene rings will decrease the PAHs solubility and increase the hydrophobicity of PAHs (Hussain et al., 2018a, b). These characteristics eventually affect the removal, so HMW PAH becomes harder to remove from the wastewater. It also affects the number of studies being conducted on this PAH removal. Although HMW PAHs were discovered to present more towards total PAHs than LMW PAHs, many studies were conducted on LMW PAHs compared to HMW PAHs because of their limitations (Jian et al., 2020). Investigating PAHs' microbial degradation has resulted in the isolation of numerous bacteria, fungi, and algae that are competent in degrading PAHs (Sakshi & Haritash, 2020).

Apart from biodegradation, PAH's fate in nature differs depending on the surrounding atmosphere. For example, PAH can go through photo-oxidation in air, whereas this compound can undertake photo-oxidation and chemical oxidation (Abdel-Shafy & Mansour, 2016). In addition, certain PAHs like naphthalene and alkyl naphthalene are moderately eliminated via volatilization (Ghosal et al., 2016). Naphthalene is the simplest polycyclic aromatic hydrocarbon (PAH) because of the smallest number of rings, which is two. PAHs has been considered a threat to human health as some of these pollutants have been labelled as carcinogenic, mutagenic, and teratogenic (Patel et al., 2020).

Fig. 1 Chemical structures of 16 polycyclic aromatic hydrocarbons (Ghosal et al., 2016)



# 4 The Effect of PAHs

Since the PAHs exist in the terrestrial environment. health will generally be subject to the time and route of exposure and the concentration of PAHs. When the time contact is prolonged, the PAHs will affect the health in a very serious condition (Pavanello et al., 2020). Ranjan et al. (2017) had shown the adverse effects on human wellbeing due to exposure to PAHs. Short-term exposure could affect the human condition and wellbeing, including eye irritation, nausea, and vomiting. Nevertheless, long-term exposure became more serious to the health, including kidney damage, cancers, liver impairment, and cataracts. These effects of the PAHs disclosure are supported by Gupte et al. (2016), in which the PAHs such as anthracene, benzo(a), pyrene and naphthalene can give rise to skin inflammation after contact with the substance.

Benzo(a)pyrene (BaP) has been categorized by the USEPA (1993) as a main concern of contaminant because of the chosen compound, which is identified as carcinogenicity, teratogenicity, or acute toxicity (Lawal, 2017). The International Agency for Research on Cancer Classification Monographs Volume (IARC) showed great concern to BaP, classified as Group 1, showing that it is carcinogenic to humans compared to other PAHs (IARC, 2021). However, a huge variety of organisms are competent in degrading the low molecular weight of PAH, such as naphthalene and phenanthrene. The study to degrade the high molecular weight of PAHs, such as BaP, need to be observed and improved as this pollutant brings dangerous impact to the animal, human and nature (Dushyant & Bharti, 2018).

#### **5** Techniques in Removing PAHs

PAHs are affected by several conditions: physicochemical, chemical, and biological processes that would alter their fate and transport in the subsurface ecosystem (Alegbeleye et al., 2016). Some treatment methods for wastewater include physical, chemical, and biological treatment. Some examples of physical treatment are adsorption and filtration. Patel et al. (2020) stated that the physical method could not completely remove the PAHs due to the no structural changes in PAHs when this method simply transfers the PAHs from water and soil. The chemical method was introduced to cover up the physical treatment weakness, such as the inefficient physical methods for PAH removal and timeconsuming physical treatment (Peng et al., 2018). The chemical treatment process requires the use of chemical substances. Many types of chemical materials can be used to treat the contaminants. These compounds' usage usually can change the chemical and physical properties of the contaminants. Examples of chemical treatments are Fenton and zero-valent iron. Then, due to several disadvantages of physical and chemical methods, including cost, procedural complexity and lack of complete degradation, the biological methods have gained much attention for PAH remediation (Ghosal et al., 2016). The environment-friendly is one of the main advantages of biological treatment. Examples of biological methods are phytoremediation, bacteria remediation, fungi remediation and bacteriafungi remediation. However, this biological method would also have a few disadvantages, affecting PAH removal (Mojiri et al., 2019). Table 1 shows the benefits and the drawbacks of some of the removal techniques of PAHs.

#### 5.1 Bioremediation

There are many remediation methods, such as phytoremediation, fungi remediation, and bacteria remediation. Bioremediation refers to the limits related to physicochemical processes by eliminating the organic pollutants at a lowered rate and in suitable environments (Bhatt et al., 2021). Bioremediation is the remediation technique that uses mainly plants and microorganisms such as bacteria and fungi to detoxify contaminants in the soil and other environments. This method depends on the metabolism of the consumption of perilous constituents by plants or microorganisms, leaving by-products in the environment which are no more lethal after the degradation (Loss & Yu, 2018). Apart from that, fungi and bacteria are often used to remove PAHs (Ghosal et al., 2016). Furthermore, this method signifies an ecological resolution to the issue of manufacturing production, which had become a risk to social wellbeing. Thus, this method can be prevalent as a corrective option for toxin eradication involving PAHs.

Table 1	Advantages and	disadvantages of	f PAHs removal	techniques

Types of Remediation	Removal Techniques	Advantages	Disadvantages	References
Physical Remediation	Adsorption	<ul> <li>Green and sustainable chemistry</li> <li>Low-cost adsorbent</li> <li>High porosity and efficiency</li> </ul>	<ul> <li>Difficulty in biomass recovery after sorption</li> <li>Complicated regenera- tion</li> <li>Low mechanical strength and density</li> <li>High cost when using activated carbon</li> </ul>	Adeola & Forbes, 2020; Balati et al., 2015; Gautam et al., 2014
	Forward Osmosis Filtra- tion	<ul> <li>Low external pressure demand</li> <li>Less affected by foul- ing</li> </ul>	<ul> <li>It requires an additional chemical to prepare draw solution</li> <li>Fewer membrane options</li> </ul>	Sabir et al., 2015; Guo et al., 2015; Jafari et al., 2015; Korenak et al., 2017
Chemical Remediation	Fenton Reaction	<ul> <li>Performed at room temperature and ordi- nary temperature</li> <li>Possesses high perfor- mance and non-toxicity</li> </ul>	<ul> <li>High costs of reagents</li> <li>The large volume of iron sludge</li> </ul>	Huang et al., 2016
	Zero Valent Iron (ZVI)	<ul> <li>Simple process</li> <li>Low operating costs</li> <li>Wide range of applications</li> </ul>	<ul> <li>Removal effectiveness depends on the size and reactivity of ZVI particles</li> <li>Not fully complete removal of PAHs</li> </ul>	Li & Yang, 2018
Biological Remediation	Phytoremediation	<ul> <li>It is a green technology and environmentally friendly</li> <li>Less expensive</li> <li>It does not require expensive equipment or highly specialized personnel</li> </ul>	<ul> <li>Bioavailability of plant and plant slow growth rate</li> <li>Longer remediation time and cannot treat deep contaminants</li> <li>Toxicity</li> </ul>	Malik et al., 2017; Fasani et al., 2018; Khodaver- diloo et al., 2020; Rao & Babu, 2014
	Fungi	<ul> <li>The resistance to different environmental conditions</li> <li>Able to grow on a variety of media</li> <li>Able to produce enzymes for the degrading of organic pollutants</li> </ul>	• Only can utilize the PAHs as a sole source of carbon and energy	Ghosal et al., 2016
	Bacteria	• They are already adapted to contami- nated sites	<ul> <li>The slow rate of PAHs removal</li> <li>Abiotic factors influential such as pH and temperature</li> <li>Inefficient for the high molecular weight PAHs removal</li> </ul>	Kumari et al., 2018
	Bacteria and fungi	<ul><li> Reduce the toxicity of media</li><li> High removal</li></ul>	• It needs to find the best combination	Deveau et al., 2018

# 5.1.1 Bacteria

Bacteria are proven to eradicate organic contaminants via biodegradation mechanisms with specific degrading enzymes. They can adapt to the PAHs and are extensively exposed to the environment, employed for the bioremediation process. PAHs-degraded bacterial genera are commonly known as *Arthrobacter, Bacillus, Stenotrophomaonas, Vibrio, Corynebacterium, Mycobacterium*, and *Sphingomonas* (Abo-state et al., 2017; Babu et al., 2019). These bacteria are mostly isolated from municipal sludge, PAH-contaminated soil, and petroleum sludge (Kumari et al., 2018). Table 2 shows a variety of bacteria isolated from the different environments for the PAH-degrading process.

Bacteria can be categorized into gram-negative and gram-positive bacteria (Mai-Prochnow et al., 2016) as this classification is based on cell wall structure development. The external cell wall structures distinguished these two groups (Dörr et al., 2019), and Fig. 2 shows the cell wall structure of gram-positive and gram-negative bacteria. The gram-negative bacterium comprises periplasmic space, plasma membrane,

and a thin peptidoglycan cell wall layer. Apart from that, an extra outer membrane exists in the gram-negative, rich with lipopolysaccharide, which envelopes the thin layer of the peptidoglycan cell wall (Bruslind, 2021). Meanwhile, the gram-positive bacterium comprises periplasmic space, plasma membrane, and a thick peptidoglycan cell wall layer (Mai-Prochnow et al., 2016). The examples of gram-positive bacteria, normally used in bacteria bioremediation, are Mycobacterium, Rhodococcus, Corynebacterium, and Actinobacterium (Mohd-Kamil et al., 2016; Hamedi & Poorinmohammad, 2017; Yadav et al., 2018). Then, Sphingobacterium, Pseudomonas, Sphingomonas, and Stenotrophomonas maltophilia are gram-negative bacteria typically being used (Smułek et al., 2020; Spini et al., 2018; Teng et al., 2021).

According to De Carvalho and Caramujo (2018), gram-negative bacteria are repeatedly recognized as efficient PAH degraders. However, when employed in a mixed consortium of bacteria cultures, the grampositive bacteria can degrade the PAHs (Olowomofe et al., 2019). It is due to high fatty acid in the lipopolysaccharide membrane of gram-negative. Moreover, the research found that gram-positive bacteria have

Table 2 Variety of bacteria isolated from different environment for PAH-degrading

Bacteria degrader	Isolated location	Country isolation	References
Stenotrophomonas sp. Microbacterium sp. Arthrobacter sp.	rhizosphere soils	Australia	Sivaram et al. (2019)
Stenotrophomonas maltophilia, Ochrobactrum anthropi, Pseudomonas mendocina, Microbacterium steraromaticum Pseudomonas aeruginosa	contaminated soil of Mathura oil refinery, Mathura, India tyre wastes dump site (Qaiser Bagh, Lucknow, India)	India	Kumari et al. (2018)
Pseudomonas aeruginosa Serratia marcescens	Arak petrochemical wastewater	Iran	Fathi and Ebrahimipour (2018)
Bordetella avium	petroleum refinery wastewater	Egypt	Abo-state et al. (2017)
Pseudomonas aeruginosa	petrochemical industrial waste	India	Fulekar (2017)
Ochrobactrum halosaudia AJH1 Ochrobactrum halosaudia AJH2 Pseudomonas aeruginosa AJH3	SWCC (Saline Water Conversion Company), Jeddah, Saudi Arabia	Saudi Arabia	Arulazhagan et al. (2016)
Raoultellaornithinolytica, Serratia marcescens, Bacillus megaterium Aeromonas hydrophila	Diep and Plankenburg river systems	South Africa	Alegbeleye et al. (2016)
Cycloclasticus sp. 78-ME	petroleum deposits of the sunken tanker Amoco Milford Haven, Mediterranean Sea	Italy	Messina et al. (2016)
Corynebacterium uroalyticum	domestic, municipal sludge	Malaysia	Othman et al. (2016)



been frequently studied concerning PAHs degradation. In the study conducted by Dushyant and Bharti (2018), *Mycobacterium sphingomonas*, a gram-positive bacterium, completely mineralized the PAHs, making the research on gram-positive bacteria PAH degraders become more appealing to be investigated.

From the previous report, even though these grampositive and negative bacteria have different cell wall structures, both effectively reduce the PAHs. The capacity of bacteria to decompose the PAHs may not rely on the cellular cell structure but may be attributed to the certain multi-component enzymes system acquired (Lawal, 2017; Mohapatra & Phale, 2021). Bacteria owning the PAHs degrading enzymes can consume the PAHs. Nevertheless, solubility that correlates to the bioavailability of PAH to degraders plays a significant part in the effectiveness of PAH degradation.

According to previous studies, different effects occur when the PAHs are mixed in bacteria's bioremediation. For example, Ghosh and Mukherji (2017) found that the removal of pyrene, which is the high molecular weight of PAH, showed significant improvement with a high concentration of phenanthrene or fluoranthene. However, the existence of high acenaphthene revealed a minimal adverse effect. Apart from that, pyrene degradation exceeded 95% when all the three PAHs were present at 25 mg/L concentration. This pyrene removal study was conducted using the bacteria Pseudomonas Aeruginosa. However, there is research done by Goswami et al. (2018), which showed that the PAHs in a single condition was found to be substantially higher than the interaction between the substrates on PAH deprivation. Therefore, this removal analysis is conducted, which employed the bacteria of Rhodococcus opacus DSM 43,205. By comparing those studies, they proved that the different types of bacteria would show different removal results. Table 3 shows the performance of PAH-degrading bacteria to degrade specific PAH.

Other than that, Othman et al. (2016) has conducted a study using *Corynebacterium uroalyticum* bacteria that showed 88% degradation. This rate

Table 3	Performance of	PAH-degrading	bacteria to	degrade specific PAH
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Bacteria	PAHs	Initial concen- tration (mg/L)	Removal (%)	References
Rhodococcus opacus	naphthalene	50	91.6	Goswami et al., (2018)
	phenanthrene		82.3	
	fluoranthene		80.7	
Ochrobactrum halosaudis,	phenanthrene	200	98	Jamal and Pugazhendi (2018)
Stenotrophomonas maltophilia,	fluorene	50	100	
Achromobacter xylosoxidans, Mesorhizobium halosaudis	pyrene	50	69	
Micrococcus sp.,	phenanthrene	100	100	Jamal (2018)
Pseudomonas stutzeri.,	fluorine			
Pseudomonas sp., Vibrio sp	pyrene	100	93	
Bacillus subtilis, Bacillus megaterium	benzo(k)fluoranthene	10	77	
Microbacterium steraromaticum	naphthalene	10	81.4	Kumari et al. (2018)
Pseudomonas aeruginosa	phenanthrene	3.5	67.1	
0	benzo(b)fluoranthene	6.5	61.0	
Stenotrophomonas maltophilia	fluorene	1.9	47.9	
Pseudomonas aeruginosa	fluorene	250	98	Fathi and Ebrahimipour (2018)
Serratia marcescens	phenanthrene	200	97	
	anthracene	250	94	
	pyrene	150	45	
Ochrobactrum halosaudia AJH1,	naphthalene	1000	97	Pugazhendi et al., (2017)
Ochrobactrum halosaudia AJH2	phenanthrene	50	100	
Pseudomonas aeruginosa	anthracene	100	90	
	fluorene	100	98	
	pyrene	50	90	
	benzo(e)pyrene	10	85	
	benzo(k)fluoranthene	10	81	
Microbacterium maritypicum CB7	benzo(a)pyrene	10	69.0	Mansouri et al. (2017)
Raoultella ornithinolytica	acenaphthene	50	98.60	Alegbeleye et al., (2016)
	fluorene		99.90	
Serratia marcescens	acenaphthene		95.70	
	fluorene		97.90	
Bacillus megaterium	acenaphthene		90.20	
	fluorene		98.40	

is quite high because phenanthrene is LMW PAH. However, this research has some flaws: the slow rate of PAH removal, the influence of abiotic factors such as pH and temperature, and the inefficient high molecular weight of PAHs exclusion (Patel et al., 2020).

# 5.1.2 Fungi

Unlike bacteria, some fungi can simply use PAHs as a sole carbon and energy source and co-metabolize them into a broad selection of detoxified-oxidized metabolites (Ghosal et al., 2016). Therefore, they

Fungi degrader	Isolated location	Country Isolation	References Obire et al. (2020)	
• Aspergillus niger, • Aspergillus sydowii, • Fusarium lichenicola	Oilfield wastewater	Nigeria		
<ul> <li>Aspergillus sp.</li> <li>Scopulariopsis brevicaulis</li> </ul>	Crude oil from Rumaila oilfield	Iraq China	Al-Hawash et al. (2018) Mao and Guan (2016)	
- Scopillar topsis brevieullis	- Geo	enna		

Table 4 Variety of fungi isolated from different environment for PAH-degrading

cannot fully remove the PAH contaminants. Several groups of fungi used in this remediation are *Phanerochaete*, *Chrysosporium*, *Aspergillus*, *Penicillium*, and *Trichoderma*. Table 4 shows the variety of fungi isolated from the different environments for the PAH-degrading process.

The research by Vieira et al. (2018) showed 97.2%, 60% and 30% removal of benzo[a]pyrene using *Marasmillus sp., Tinctoporellus sp.* and *Peniopora sp.*, respectively. These varied results showed that the different fungi used in the remediation could give a different removal rate. This finding is similarly supported by Obire et al. (2020), which used *Aspergillus niger* and *Aspergillus sydowii* in their study. There was the complete eradication of naphthalene and chrysene by *Aspergillus niger*. However, *Aspergillus sydowii* only showed the exclusion of chrysene. These findings proved that the fungi could eradicate one or more PAH components depending on the fungi's species. Table 5

showed the performance of PAH-degrading fungi to catabolize specific PAH from the previous studies.

The advantages of fungus are their resilience to diverse environmental settings, capability to develop on various media, and generating enzymes to degrade organic contaminants (Hyde et al., 2019). The fungal metabolism of PAHs is typically mediated by ligninolytic and non-ligninolytic enzymes (Gupta et al., 2017). Therefore, the enzymes would be different compared to the types of fungus used in the remediation process. From the previous study by Loss and Yu (2018), fungi have been recognized to have a distinctive set of ecological characteristics, making them potential contenders to be applied in the bioremediation method to eliminate PAHs.

# 5.1.3 Fungi and Bacteria Co-cultures

The bacteria used to degrade HMW PAHs had several limitations which affected the degradation outcome (Kumari et al., 2018). Hence, the fungus role can be

Fungi	РАН	Initial concentration (mg/L)	Removal (%)	References
Aspergillus niger,	naphthalene	100	73.4	Obire et al. (2020)
Aspergillus sydowii,	acenaphthylene		73.1	
Fusarium lichenicola	acenaphthene		65.8	
• Aspergillus niger • Aspergillus sydowii • Fusarium lichenicola	chrysene		85.2	
Aspergillus sp.	naphthalene	100	97.4	Al-Hawash et al. (2018)
	phenanthrene		84.9	
	pyrene		90.7	
Marasmiellus sp.	benzo[a]pyrene	0.02	97.2	Vieira et al. (2018)
	pyrene	0.04	92.8	
Scopulariopsis brevicaulis	benzo[a]pyrene	20	82	Mao and Guan (2016)
	pyrene	100	64	

 Table 5
 Performance of PAH-degrading fungi to catabolize specific PAH

used in the remediation and the bacteria to resolve the drawbacks of bacteria. The benefit of fungi is that it is more tolerant in reducing toxic compounds similar to PAHs. Furthermore, this could aid in removing the HMW PAHs (Hamzah et al., 2018). Therefore, the bacteria-fungal cocultures can be applied as useful bioremediations for HMW PAHs reduction in wastewater. The positive removal outcome can be shown in the previous studies. It can be proven by Vieira et al. (2018), in which the fungus reduced the toxic compounds with total oxygen-carbon (TOC) at 17%. The fungus used in their study was *Marasmiellus sp.* 

Bhattacharya et al. (2017) reported that the individual species of Pleurotus ostreatus PO-3 had degraded 64.3% of benzo(a)pyrene. While, as compared to bacterial-fungal cocultures, P. ostreatus PO-3 with Penicillium chrysogenum MTCC 787 and P. ostreatus PO-3 with Pseudomonas aeruginosa MTCC 1688 can reduce approximately about 86.1% and 75.1% of benzo[a]pyrene, respectively. These positive results of bacteria-fungal coculture could be supported by Ma et al. (2016). Their study found that 30.6% of the fluoranthene was degraded by the Bacillus subtilis, a pure bacterial culture, during the Acremonium sp. P0997, pure fungal culture degraded 58.4% of fluoranthene. However, the fungal-bacterial consortium showed a maximum reduction of 64.1% fluoranthene. Apart from that, Ortega-González et al. (2014) found different outcomes for removing fluoranthene and pyrene when using the bacteriafungi coculture method. The removal of fluoranthene by the coculture was 87.95%, which is higher than the removal by the individual cultures, 68.95% for Fusarium sp. FPyF1 and 64.59% for O. anthropi BPyF3. However, the pyrene reduction by the coculture, which is 99.68%, showed a similar result obtained from pyrene reduction by Fusarium sp. FPyF1 of 99.75%. Those findings showed that the coculture of Fusarium sp. FPyF1 and O. anthropi BPyF3 have a larger capability to eliminate fluoranthene than pyrene, which can be degraded effectively by Fusarium sp. FPyF1 solely.

Jambon et al. (2018) stated that fungi and bacteria could be complementing one another in the degradation pathway of the contaminant. This co-metabolic degradation can indirectly eliminate contaminants, where one can substantially degrade the intermediates formed by the other during the PAH degradation process. These previous studies showed that the different combinations of the bacteria-fungi could affect PAH degradation.

## 5.1.4 Microbial—Plant Interaction

Phytoremediation implements various plants to degrade, extract, and immobilize pollutants in removing PAHs from the water or soil (Abdel-Shafy & Mansour, 2018). The plant parts such as leaves, stems, and roots were used as the habitats for a broad range of microbes. Here, the interaction between plants and microbes can readily degrade the toxic pollutants and elevate the treatment process (Kumar et al., 2018). Many plant species that already being used in this method, such as fescue grass (Festuca arundinacea), maize (Zea mays L.) and soybean (Glycine max L.) which could phyto-degrade the PAHs (Guo et al., 2017a, b; Lawal, 2017). This method is a green technology, environmentally friendly and acceptable to the public when properly implemented (Malik et al., 2017). However, the limitations of this method, such as longer remediation time needed, high toxicity, bioavailability, and slow growth rate of the plant (Fasani et al., 2018; Khodaverdiloo et al., 2020), affect the whole process of remediation. These limitations made this approach less chosen to eliminate the PAHs, and appropriate aid for better PAH removal is required.

Microbe-assisted phytoremediation such as rhizoremediation appears to be more effective for degrading organic pollutants, including PAHs (Chen et al., 2016). These microorganisms are competent in breaking down hazardous pollutants into harmless products through their metabolic activity (Kumar et al., 2018). Chaudhry et al. (2005) found the degradation by the phytoremediation method alone, without the microbial contribution, is not quite efficient for many PAH degradation. They also stated that the combined interaction of microbial-plants in the rhizosphere offers useful tools for PAH environmental remediation. They used the Populus sp. bacteria to remove PAHs in the environment. The plant's root exudates help remove the PAHs and act as substrates for microorganisms, resulting in the increment rate of PAHs biodegradation.

Regarding the interactions among plants and indigenous rhizobacteria, microbe-assisted phytoremediation can be an effective and inexpensive technique for removing organic pollutants from polluted mediums such as soils, water bodies, or air. According to Hussain et al., (2018a, b), firstly, the plants have to provide the substrates and then alter the pH for making the pollutants suitable for microorganisms in order for plant roots to stimulate the microbes. As the low removal process by microorganisms and plants in single systems, Supreeth (2021) also found that the combined systems of plant-microbes improve the remediation of contaminants efficiently.

The term "Photobiol remediation" with the definition of phytoremediation assisted by microorganisms had shown the symbiotic relationships between plants and microorganisms as the plant roots released the natural substances as a nutrient for microorganisms (Fernández-Luqueño et al., 2017). Later, the microorganisms improved the biological activities of the plant roots (Abdel-Shafy & Mansour, 2016). The plant roots promote microbial activity by increasing the bioavailability of organic carbon possess enzymes, carbohydrates, amino acids that enrich the microbes (Dowling et al., 2009). The microbes utilize the metabolites and exudates of plants as a source of energy and carbon throughout the removal process, and later, the plants release the biodegrading enzymes themselves.

In addition, Wei et al. (2020) stated that the plants used their metabolism through the interaction with microorganisms to improve their ecological environment. The success of this method mainly depends on the host plant, followed by the colonization of microbes. The selection of plants needs to be considered as it will affect the whole removal process and outcome. The plants chosen must be resistant to contaminants, adaptable to environmental conditions and have excellent growth (Singha & Pandey, 2021). Ali et al. (2013) found that the genus Pseudomonas species are the predominant organisms, and they are linked well with the roots. Besides that, many plants survived at very high concentrations of hydrocarbons, such as Buchanania acuminata (Anacardiaceae), Pasparlum vaginatum, Chloris barbata, Chromolaena odorata and Aspilia Africana (Anyasi & Atagana, 2018).

Moreover, Folwell et al. (2016) have discovered that *Pseudomonas* and *Bacillus* species can degrade HMW PAHs in soil. It can be denoted that, *Sphingomonas* sp. species play a crucial role in the early degradation of PAHs, and then the reduction of HMW PAHs were primarily performed by *Pseudomonas* and *Bacillus* (Guo et al., 2017a, b). Grass species mostly

used in the studies on removing petroleum hydrocarbons as the family of *Poaceae*, which is the fibrous root system of grasses, were used as they have a high surface area that can enhance the contact between contaminants and microbes (Gaskin & Bentham, 2010; Hussain et al., 2017).

Chen et al. (2016) used plants such as Sedum alfredii L., perennial ryegrass, and Lolium perenne L.) together with microorganisms (Microbacterium sp. strain KL5 and Candida tropicalis strain C10) in their study of wastewater-irrigated soil co-contaminated with PAHs and heavy metals. They found that the highest efficiency of PAH removal was obtained by interplanting ryegrass with S. alfredii associated with strain KL5 and C10 in the contaminated soil. Bisht et al. (2010) used the microbes-plants interaction method to remove naphthalene and anthracene. Here, Populus deltoides plant and varieties of microbes (Kurthia sp. SBA4, Micrococcus varians SBA8, Bacillus circulans SBA12) had been used for the removal process. Moreover, the same plant has a different microbe, for example, Bacillus sp. SBER3 in removing the PAHs (Bisht et al., 2014). Additionally, Fu et al. (2020) applied the same method in removing the phenanthrene (Orvza sativa-Phomopsis liguidambaris). Rodriguez-Campos et al. (2019) had found that the combination of the grass with P. corethrurus and the bacterial consortium increased the PAHs removal up to 90%. The findings by Xu et al. (2014) discovered that the combination of ryegrass and Kocuria sp. P10 achieved the maximum removal of total PAHs (69.6%). Here, the microbial-Phyto method showed the highest removal than phytoremediation or microbial remediation alone. He et al. (2016) have noticed that the V. spiralis can increase PAH degradation in sediment by changing the bacterial community during the process. Despite the same bacteria and plants used in their study, previous studies showed the inequality of the removal outcome. Hou et al. (2019) found that the combination of Fire Phoenix and Mycobacterium was more effective in removing the PAHs than Fire Phoenix alone in an aged petroleum-contaminated soil. Although using the same plant and bacteria, the results contrast with Zhao et al. (2021). It may be caused by the difference in soil properties, PAH concentration, and microbes' characteristics (Sivaram et al. 2018).

Verma and Rawat (2021) observed that the capability of the microbes, which reside in the rhizosphere

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of plants, are more potent to degrade the contaminants than the microbes of bulk soil. When involving both plant and microorganisms, the mutual interaction had enhanced the degradation of contaminants. Thus, the adaptability and versatility of microbes to any challenges posed by the environment make them suitable for the remediation of environmental pollutants. Besides, the microbes also improve soil health by adding nutrients when they convert the toxic pollutants into nontoxic minerals and make them available for plants (Ahammed et al., 2012; Verma & Rawat, 2021). The other advantages of this method are that it destroys the contaminants and converts hazardous compounds into harmless products, which also avoids the possibility of future liability (Abatenh, 2017). However, several factors can improve the efficiency of the PAH degradation as the removal process depends on plant species, the composition of root exudates changes as the plant stage develops (Abdel-Shafy & Mansour, 2016). The findings by Sun et al. (2014) stated that a steady supply of organic material through root exudation increased the availability of organic nutrients to the microbe as the different partners of combination (plant-microbe) ruled out the different degradation of PAHs, which is important to identify the relevant combination of them, to get the most effective outcome. The Orychophragmus violaceus interacts with the bacterial communities showed different PAH-degrading capabilities. Furthermore, various abiotic and biotic factors such as temperature, pH, moisture, organic matter, nutrient content, soil type, microbial competition also affected their activity and substrates (Chen et al., 2016).

# 6 Pathways of PAHs Degradation

The focus of bioremediation is to degrade organic pollutants solely to nontoxic compositions, such as carbon dioxide and water (Sharma, 2020). The early influence of certain PAH degradation differs from yielding several primary oxidation products, but only some regular intermediate metabolites are produced: catechol, protocatechuic and gentisic acids (Gupte et al., 2016). Among them, catechol is the generally common compound that has been developed. However, intermediate metabolites such as dihydrodiols, phenolic compounds, and arena oxides may be carcinogenic, mutagenic, and teratogenic (Sakshi & Haritash, 2020). Therefore, the degradation pathways ought to be explored to acknowledge any obstacles that could happen in the future. In addition, the capacity of enzymes made by microorganisms to combine oxygen into the ring structure will affect the degradation of PAHs (Ghosal et al., 2016; Gupte et al., 2016). The enzymes termed oxygenases are generated by the microorganisms that catalyze oxygen-fixing reactions in PAHs' initial activation and oxidation. The two groups of oxygenases are monooxygenases and dioxygenases (Dushyant & Bharti, 2018).

The degradation pathways by microorganisms will be different when reducing different PAHs. Figure 3 shows the major pathways of PAHs degradation in bacteria and fungi. First, aerobic bacteria begin to oxidize benzene ring PAHs through the influences of dioxygenase enzymes to generate cis-dihydrodiols (Wackett & Robinson, 2020). Next, these dihydrodiols are dehydrogenases producing dihydroxylated



Fig. 3 The main pathways of PAHs degradation in bacteria and fungi (Haritash & Kaushik, 2009; Shahsavari et al., 2015) intermediates, later metabolized by catechols to carbon dioxide and water (Pérez-Pantoja et al. 2016). Fungi treated PAHs applying two pathways: nonligninolytic fungi employ the P450 monooxygenase pathway and the white-rot fungi, a ligninolytic fungus, degrade the PAHs employing ligninolytic enzymes (Kadri et al., 2016). In general, fungi generate monooxygenases, which incorporate one oxygen atom into the substrate to form arene oxides (Prenafeta-Boldú et al., 2019; Siddiqi et al., 2020). It is pursued by the enzymatic inclusion of water to produce trans-dihydrodiols and phenols. The degradation pathways need to be identified to avoid harm when using the method.

#### 7 Factors Affecting the PAHs Bioremediation

Bioremediation efficiency has been primarily examined in ideal laboratory environments, with a certain pH and temperature (Abatenh et al., 2017). Nevertheless, during the actual condition, bioremediation will be efficient merely at the locations where their requirements allow microbial development and enzymatic action (Azubuike et al., 2016). It permits the microorganisms to enzymatically eradicate the pollutants, converting them to harmless products or outcomes (Sharma et al., 2016). Furthermore, many elements such as pH, temperature, and PAH concentration differ from one location to another, impacting the bioremediation process in these environments and accelerating the growth of pollutant-degrading microorganisms (Patel et al., 2020).

#### 7.1 Temperature

The temperature strongly influences the biodegradation of PAHs in polluted places by which these sites are constantly at an ambient temperature for the action or development of the occupant microorganisms (Mehetre et al., 2019). Some remediation techniques are very sensitive to the environment's temperature (Azubuike et al., 2016). The increment of the bioavailability of PAH molecules is affected due to the temperature and high solubility of PAHs. Apart from that, the increasing temperature will reduce the dissolved oxygen (DO) level. The previous study (Othman et al., 2016) showed that the optimum temperature was  $30^{\circ}$ C, with 87.2% degradation. Less reduction is observed when using low temperature,  $25^{0}$ C, as the biodegradation process improves. After the optimum temperature, the degradation rate decreases along with the increasing temperature.

According to Ismail et al. (2020), the optimum pH condition for the bacteria and fungi growth were  $32^{\circ}C$ and 33<sup>o</sup>C, respectively. Sphingobacterium spiritovorum and Aspergillus brasiliensis were the bacteria and fungi employed in their analysis. Their analysis also showed minimal bacteria and fungus development at the lowest and highest temperature, indicating that these biomasses could not grow fairly in too low and too high temperatures, affecting the degradation rate. The influence of temperature on the degradation of PAHs also can be seen by the study of Ibrahim et al. (2018). The research showed that temperatures less than 30°C and more than 40°C did not support the growth of bacteria and the degradation of PAHs. The bacteria used in this study was Mycobacteria confluentis. Apart from that, the analysis by Ghosal et al. (2016), some pollutants would transform into a new compound at high temperatures, and it would appear to be more toxic than the original compound, which affects the biodegradation rate. However, Al-Hawash et al. (2018) found that temperature increment affected the degradation when using the Aspergillus sp. fungi. The degradation of PAH increased along with the rise of temperature until they reached the optimum temperature, 30 °C and then showed the decrement at 40 °C. Al-Hawash et al. (2018) stated that this condition happened due to the increment of the energy efficiency in the system. This energy facilitated the attachment of substrates on the cell surface, and with the temperature increment, at one phase, it affects the availability of the cell sites for adsorption. Pugazhendi et al. (2017) also revealed the same outcome with their study using the bacterial consortium. The phenanthrene degradation increased until they reached the optimum temperature, 60 °C and started to drop after 60 °C.

The different optimum conditions may be due to the different microbial used in their research. It is important to get the optimum condition as they found that 100 mg/L phenanthrene was completely degraded in 3 days at 30 °C. Nontoxic forms of 90% CO<sub>2</sub> and 10% biomass with water resulted from this mineralization of phenanthrene. Fathi and Ebrahimipour (2018) also found the same outcome when conducting the PAH degradation by mixing two bacteria. They conducted

the study from temperature 25 to 45 °C, and 35 °C was found to be the optimum condition for the degradation. According to McGenity, (2010), the increment of temperature was effective as long as the proteins and membranes of microorganisms were undamaged. Another outcome for the temperature is the temperature increase in parallel with the degradation process increment. Later, after reaching the optimum condition, the degradation process will start to retard. Othman et al. (2010) study showed that after a temperature of 25 °C, the degradation began to rise until 30 °C (optimum temperature). At 20 °C, the condition is uneffective to support the enzyme to speed up the reaction rate. Next, the catalyzed reaction needs to be done rapidly to reach the optimum condition. At 40 °C, the degradation decreased as the enzyme was rapidly denatured and reduced its activity. Generally, many PAHs biodegradation outcomes would be assessed in moderate temperatures, identifying the best temperature suitable for removing contaminants.

#### 7.2 pH

pH, in addition, portrays a vital part in the biodegradation process, in which the microorganisms favored the near-neutral pH (6.5–7.5) for their normal activity (Ghosal et al., 2016). However, in real situations, the pH needs to be checked thoroughly as the value at some places is far from the neutral pH. For example, different wastewater sources such as factories and houses would have different pH. These sources can result in neither increase nor a decrease in the pH value of the wastewater. These acidic or alkaline conditions can generate undesirable situations for microbial actions, affecting the biodegradation of PAHs in these sites. Othman et al. (2016) showed that the optimum value for pH is 7, where the phenanthrene has degraded at 88% using Corynebacterium uroalyticum bacteria. At pH 5 and pH 6, a gradual biodegradation rate was detected with the array of phenanthrene degradation of 61 to 75%, respectively. For the pH value greater than 7, the biodegradation process is lower than the optimum level. The outcome may be varied when using different bacteria. It can be supported by Ismail et al. (2020), which conducted the study on Sphingobacterium spiritovorum bacteria. The optimum condition for bacteria growth in their research was at pH 6.2. At lower pH, bacteria growth is minimal and would affect the degradation of benzo(a) pyrene. It is also suggested to modify the pH at these locations by inserting sodium hydroxide and hydrochloric acid to generate a buffer condition for effective biodegradation (Deshmukh et al., 2020; Huang et al., 2020).

According to Al-Hawash et al. (2018), the increment of pH affects the degradation by Aspergillus sp. fungi. The degradation improves with the increasing pH until it reaches the optimum pH of 7. After pH 7, the degradation started to drop. Jalali et al. (2002) suggested that the result showed that the increment of hydrogen ions enhance the PAHs adsorption to cation-binding sites. They also stated that the acidic pH effect of the low adsorption might be due to the cation-binding site's competition between hydrogen  $(H^+)$ and hydronium  $(H_30^+)$  ions in the solution. This finding could be supported by Othman et al. (2010). They degraded PAH by Corynebacterium urealyticum bacteria. Although different microbial used, the optimum pH was found the same as their study, at pH 7. After reaching pH 7, the biodegradation rate slowed and affected the removal process. However, Arulazhagan et al. (2016) found that the rise of the pH corresponds to the reduction of the PAHs removal. As the optimum pH was found at pH 2, the removal started to drop after pH 2.

#### 7.3 PAH Concentration

The initial concentration of PAHs influences the efficiency of bioremediation treatment. Rabani et al. (2020) has a study on naphthalene removal by Bacillus licheniformis, has found that at 100 mg/L and 200 mg/L with, 73% and 48% of the naphthalene were eradicated, respectively. From their analysis, it proved that initial concentration affects the degradation. Therefore, excessive concentrations may negatively affect the bacteria in the remediation process. They also conducted the degradation study on naphthalene by the same genus of bacteria, Bacillus sonorensis, which showed the same outcome. The 52% naphthalene was degraded at a low concentration of 100 mg/L, while only 29% naphthalene was reduced at a high concentration of 200 mg/L. Thus, the same genus of bacteria did not replicate the same results for several initial PAH concentrations. Some studies can support it, whereas Barman et al. (2017) analyzed the naphthalene removal by Pseudomonas mendocina. For the low initial concentration at 6 mg/L, 97% of naphthalene was removed. However, Tirkey et al. (2021) showed a greater removal at a high concentration of 98.74% with an initial concentration of 500 mg/L. The bacteria used was *Pseudomonas* sp. strain SA3. These outcomes had become the reason for deeper investigation on the optimum concentration of PAH for this study.

Pugazhendi et al. (2017) used bacterial consortium to degrade low molecular weight (LMW) and high molecular weight of HMW PAHs. Different concentrations were used to identify the best removal. At 50 mg/L, the phenanthrene (LMW PAH) was completely degraded only in one day. When the concentration increased to 100 mg/L, the degradation decreased to 96%. For the pyrene, one of the HMW PAHs, the 50 mg/L was degraded to 98% in 5 days, which took longer than phenanthrene. It showed that along with the increment of concentration, the increasing number of benzene rings made the degradation process extended. The condition where the concentration increment decreased the degradation also can be seen in the study by Fulekar (2017). The Pseudomonas aeruginosa bacteria degraded the naphthalene and anthracene, LMW PAH with different concentrations and both PAH showed the decrement of removal as the concentration increased. Both were completely removed at 250 mg/L concentration, with different durations of 10 days and 14 days for naphthalene and anthracene, respectively. When the concentration increases to 500 mg/L, the degradation drops to 80% and 60.8% for naphthalene and anthracene. The same pattern of degradation was shown using the consortium bacteria. Arulazhagan et al. (2016) also supported the outcome while degrading the HMW PAH. The increment of pyrene concentration in their study reduced the degradation and increased the time required for degradation. At 100 mg/L concentration, pyrene was degraded 86% in 11 days, and the degradation declined as the concentration rose to 200 mg/L, with 77% in 14 days.

The study by Ghosh and Mukherji (2017) found that the concentration increments also affected the decrement of the PAH degradation when PAH existed in the group. According to their study, the high concentration of phenanthrene and fluoranthene reduced pyrene removal. However, the high concentration of acenaphthalene gave minimal effect. At 25 mg/L, pyrene was degraded more than 95%, when the other PAH also existed in the same concentration.

## 7.4 Surfactants

The bioavailability of pollutants as carbon sources for microorganisms is necessary for efficient bioremediation. The surfactants are synthetic (chemical) and natural (bio), divided by sources. The surfactant is also classified into hydrophilic and hydrophobic groups (Liang et al., 2017) and based on the ionic charge of the hydrophilic group, and they are also categorized as non-ionic cationic, anionic or zwitterionic (Paria, 2008). The use of surfactants can reduce surface tension and interfacial tension (surfactantenhanced remediation (SER)). SER could be used for increasing the solubility and bioavailability of PAHs. According to Lamichhane et al., 2017, many factors such as the temperature, pH, type and concentration of surfactants and PAH hydrophobicity would affect the PAHs removal.

There are many synthetic surfactants such as Tween80, Brij35 and Triton X100. The previous research showed that different types of surfactants would affect PAH removal. Lu et al. (2020) discovered that acenaphthene was 24% higher for the TX100-facilitated immobilized bacterial beads than the beads in the absence of TX100. Balaji et al. (2014) also found the outcome, where Triton X-100 improved and maximized the degradation. Apart from that, the study by Iglesias et al. (2014) conducted the degradation of phenanthrene with the help of surfactants. The phenanthrene removal with the addition of Tween80 was better than Brij35 and TX100, other non-ionic surfactants. Tween80 as the surfactant is widely used in the other research, as it is more biodegradable and less toxic than anionic and cationic synthetic surfactants (Lamichhane et al., 2017). Chen et al. (2013) degraded the pyrene by Burkholderia cepacian, with Tween80.

Similarly, Gharibzadeh et al. (2016) degraded the 99% phenanthrene in the presence of Tween80. Besides the bacteria remediation showing the positive outcome for the addition of surfactants, the fungi remediation also appeared to have a positive result. According to Wang et al. (2008), the surfactants improved the growth of fungal spores and increased the bioavailability of less soluble substrates for the fungi.

Like synthetic surfactants, natural (bio) surfactants are also found to increase the degradation of PAHs (Tecon & van der Meer, 2010). The biosurfactants are classified as plant-based surfactants such as saponin (Iglesias et al., 2014) and microbial-based surfactants such as glycolipids (Gudina et al., 2016). According to previous reports, the addition of biosurfactants improved PAH removal efficiency from 34.2 to 63.0% (Bezza & Chirwa, 2017a). Apart from that, 83.5% of pyrene was removed with the addition of a lipopeptide biosurfactant (Bezza & Chirwa, 2017b). Sponza and Gok (2010) found that the rhamnolipid biosurfactants increased the degradation of 5-6 rings PAHs in petrochemical wastewater. In addition, the biosurfactants became more widely used as they demonstrated higher biodegradability, lower toxicity, and better environmental compatibility (Banat et al., 2014; Patel & Patel, 2020; Pi et al., 2017). However, Decesaro et al. (2017) found that the biosurfactants at higher concentrations could reduce biodegradation efficiency because of microbial growth inhibition. For example, Wang et al. (2016) stated that their study observed the biosurfactant rhamnolipids addition in degrading 95% of PAHs, but only 92% with Tween80 and 90% with sodium dodecylbenzene sulfonate (SDBS). Zhou et al. (2011) found that the saponin effectively helped the phenanthrene degradation much better than Tween80, Brij58 and TX100.

# 7.5 Scalability

This microbial remediation has been widely studied for the PAHs removal in wastewater. However, most studies only covered the pilot scale, which conducts the experiments in the laboratory, and yet to implement in the wastewater treatment plant.

Abo-State et al. (2017) conducted the research of naphthalene degradation by *Bordetella avium*, isolated from petroleum refinery wastewater in Egypt. The 95% naphthalene removal from their research showed that this bacteria strain could be used in the bioremediation method to clean up the petroleum refinery wastewater at full-scale. Then, Arulazhagan et al. (2016) used acidophilic *S. maltophilia* strain AJH1 for degrading the low molecular weight (LMW) PAHs and high molecular weight of (HMW) PAHs. The lab-scale reactor study was conducted with refinery wastewater from Petro Rabigh, Saudi Arabia, in a continuous stirred tank reactor (CSTR). The removal rates of LMW PAH were up to 95% and 80% for HMW PAH, hence showing the strain can be a good candidate as a degrader for biological treatment of PAHs contaminated wastewater at acidic pH. The research by Obire et al. (2020) conducted the polycyclic aromatic hydrocarbon (PAHs) degradation by Aspergillus niger, Fusarium lichenicola and Aspergillus sydowii fungi. The study's outcome supported that these fungi can be effectively removed from PAHs in contaminated environments. In addition, the study by Goswami et al. (2018) used the bacteria strain, which is Rhodococcus opacus, for eliminating PAHs. High removal of 91.6%, 82.3%, and 80.7% for naphthalene, phenanthrene and fluoranthene, respectively, showed the potential as a degrader for bioremediation and industrial wastewater treatment. These previous studies demonstrate the potential utility of these microorganisms for microbial bioremediation in the full-scale treatment of PAHs.

Fatone et al. (2011) investigated the fate of aromatic hydrocarbons in Italian municipal wastewater treatment plant systems (WWTPs). The study of WWTPs showed the PAH degradation by remediation method, including microbial remediation. They observed both pilot-scale and full-scale treatment of WWTPs, for removing the PAHs. The study by Sun et al. (2018) also explored the occurrence and fate of PAHs in a wastewater treatment plant of Harbin, Northeast China. They observed the PAH degradation using several methods, including bioremediation. Similarly, Zhang et al. (2019a and b) studied the PAHs removal in wastewater treatment plants by biodegradation and other several methods.

#### 8 Conclusion

This review describes current works on PAHs as one of the harmful pollutants in the environment. Their comprehensive sources became a significant part of instant evolution in understanding PAHs. Bioremediation such as phytoremediation and microbial bioremediation has been conducted to cover the PAH removal limitations. These methods attempt to eradicate or lessen the pollutants in a sustainable green approach for long term best management practices. However, due to PAHs' differing physical and chemical properties, their degradation of PAHs is also affected by various factors. Therefore, the optimum condition of the parameters such as temperature, pH and PAHs concentration of the wastewater needs to be addressed in preparing the suitable condition for removing PAHs and high growth of bacteria and fungi. The kinetic degradation of PAHs by microbial bioremediation should be investigated for an insightful overview of the mechanism pathways. In addition, the previous study stated that heavy metals could affect PAHs degradation by shifting the surface properties of microbes and obstructing enzymes of microbes also can be explored. It showed that the PAH degradation could affect the physicochemical parameters of the wastewater containing heavy metals pollutants that prolong the need for more remediation techniques to be discovered.

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Data Availability All data during this study are included from previous studies.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

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Conflict of Interest The authors declare no competing interests.

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