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Ability of *Pseudomonas* sp. and *Aspergillus* sp. on the Bioremediation of Petroleum Hydrocarbons Contaminated Water: A Review

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ABSTRACT. In the year 2022, an estimated 41,000 tonnes of petroleum hydrocarbon spills were officially reported, underscoring the urgent environmental concern linked with the contamination of water bodies. This comprehensive analysis highlights the notable species engaged in the process of bioremediation for petroleum hydrocarbons within marine environments, specifically focusing on the *Pseudomonas* and *Aspergillus* species. Petroleum hydrocarbons pose multifaceted toxicological hazards to both human and animal well-being. The objective of this study is to review the distinctive attributes of *Pseudomonas* and *Aspergillus* species that render them suitable candidates for the bioremediation of hydrocarbon-polluted water, encompassing their capacity to flourish in hydrocarbon-laden conditions, generate biosurfactants as emulsifying agents, and degrade a diverse spectrum of petroleum hydrocarbons. Furthermore, this review aims to assess how the formation of a consortium enhances the efficiency of the bioremediation process.

Keywords: Aspergillus species, Bioremediation, Hydrocarbons-contaminated water, Pseudomonas species

INTRODUCTION

The contamination of water bodies with petroleum hydrocarbons (PH) has become a serious environmental concern. Petroleum hydrocarbons are usually found as deposits in sedimentary rocks deep underground. They can be in the form of gases (natural gas), semisolids (bitumen), solids (wax or asphaltite), or liquids (petroleum crude oil, which is also called fossil fuel) (Varjani, 2017; Moubasher et al., 2015; Speight, 2015; Riazi, 2005). Natural gases are a mixture of gaseous hydrocarbons and contain mostly saturated aliphatic hydrocarbons such as methane, ethane, butane, and propane. Bitumens are thick, viscous fluids composed of heavy hydrocarbons, and wax is the wax obtained from petroleum, such as paraffin wax, microcrystalline wax, and petroleum jelly. Petroleum crude oil is a mixture of volatile liquid hydrocarbons that contains more than 30 parent polycyclic aromatic hydrocarbons (PAHs). Some examples of PAHs are pyrene, naphthalene, benzo[a]pyrene, phenanthrene and fluoranthene.

Although naturally occurring, the presence of PHs is primarily associated with contamination resulting from oil and gas exploration and production, transportation and storage activities, incidents such as tank leaks, accidental spills during loading and discharging procedures, ballasting and deballasting operations, bunkering, oil tanker accidents, petrochemical industry effluent discharge, fugitive emissions, and ruptures in aged underground pipelines (Sajna et

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al., 2015). Therefore, any discharge or release or permit the discharge or release of sewage onto or into any soil, or any inland waters or Malaysian waters must follow the Environmental Quality Act 1974 (Environmental Requirements, 2010). In 2022, the International Tanker Owners Pollution Federation Ltd. (ITOPF, 2023) reported that three significant oil spills (>700 tonnes) and four medium oil spills (7–700 tonnes) had occurred. This situation raises a matter of significant concern, as these pollutants possess the potential to induce a range of toxicological health complications in both human beings and animals. The expected routes of exposure to synthetic compounds from the oil spill are inhalation, dermal contact, ingestion of food and water, and contact with the beach sediment. This chronic exposure can adversely impact various physiological functions, affecting the hematologic, hepatic, respiratory, renal, and neurological systems (Alzahrani and Rajendran, 2019). The toxic effects of the compounds are primarily determined by their chemical composition and physical state.

The chemical composition of petroleum hydrocarbons can be classified based on their solubility in organic solvents, and these categories are aliphatics, aromatics, resins, and asphaltenes (Steliga, 2012). Compared to the others, the compounds that are mostly toxic and carcinogenic are polycyclic aromatic hydrocarbons (PAH) (Abdel-Shafy and Mansour, 2016). Petroleum hydrocarbon degradation is a complex process dependent on the nature, composition, and concentration of hydrocarbons in the impacted medium. For example, alkanes with a higher molecular weight are more toxic than those with a lower molecular weight. Due to their ability to swiftly volatilise, alkanes with a low molecular weight do not persist for extended periods in contaminated sites (Barnes *et al.,* 2018).

Over the years, different strategies have been used to restore oil-contaminated habitats, including physical, chemical, and biological treatments. However, the first two aforementioned means of mitigating pollutants have high operational costs, are less effective, and are less sustainable (Sharma, 2020). Sharma et al. (2023) also stated that many physical and chemical processes (flotation, precipitation, oxidation, adsorption, etc.) used for wastewater treatment are expensive, demand high maintenance, and require a complicated functional setup. This is due to the fact that they are susceptible to secondary pollution, necessitating integrated pollution management to reduce and/or eliminate the toxic pollutants from the environment. Thus, bioremediation is one of the most promising technologies since it is a cost-effective, natural, and sustainable cleanup process (Kumar et al., 2018). Bioremediation is the utilisation of living organisms, such as bacteria or fungi, to naturally degrade, transform, or remove hazardous substances and pollutants from contaminated environments.

Every year, it is estimated that between 1 and 3 million tonnes of oil enter the global marine environment. The toxic effects of hydrocarbons have led to detrimental impacts on marine life and human communities that rely on these ecosystems. According to Amran et al. (2023), there are many types of microorganisms, such as bacteria, algae, and fungi, that decompose hydrocarbons in the environment, but bacteria and fungi are common and efficient members of them. Also, an investigation has been done using the red alga, *Agardhiella subulata* which secretes some phenolic chemicals that may be used for hydrocarbon degradation in marine sediments (Satpati et al., 2023). To date, only a few studies have investigated the fungal bioremediation potential of petroleum hydrocarbons in marine environments (Dell'Anno et al., 2021).

Compared to soil bioremediation of petroleum hydrocarbons using *Pseudomonas* sp. and *Aspergillus* sp, there is a lack of comprehensive knowledge regarding the effects of *Pseudomonas* sp. and *Aspergillus* sp. on the bioremediation of hydrocarbon-contaminated marine water. This hinders the development of effective and efficient bioremediation strategies. Thus, this review aims to bridge this knowledge gap and advance the application of *Pseudomonas* sp. and *Aspergillus* sp. in marine bioremediation (Figure 1).

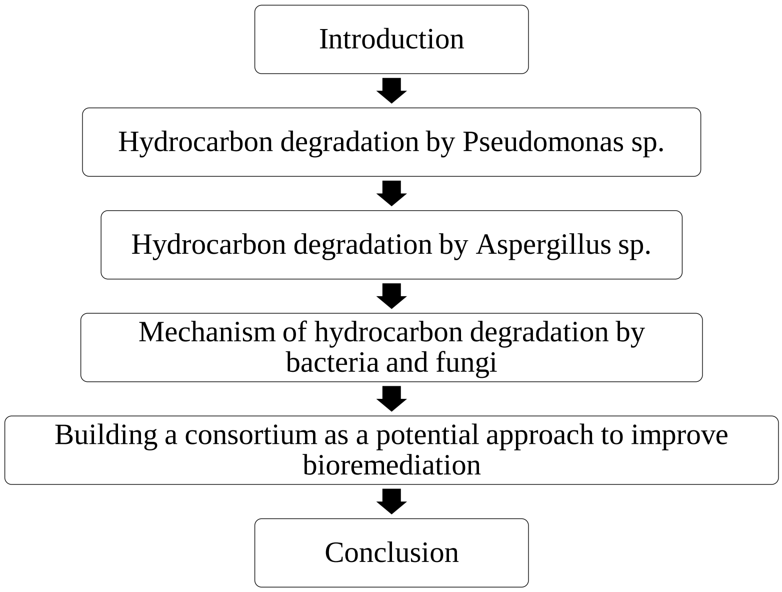

Figure 1. Schematic diagram of the study

Hydrocarbon Degradation by *Pseudomonas* sp.

The genus *Pseudomonas* is one of the most diverse and ecologically significant groups of bacteria (Palleroni, 1984). *Pseudomonas* sp. is an aerobic Gram-negative bacterium in the form of a rod. Members of the genus are found in large numbers in all of the major natural environments, such as terrestrial, freshwater, and marine (Spiers et al., 2000) and also in urine, sputum, and environmental samples (Gomez-Martinez et al., 2023). This universal distribution is a reflection of how versatile their metabolic capacity and potential to adapt to fluctuating environmental conditions are. Additionally, *Pseudomonas* strains have a remarkable capacity for degrading a wide range of petroleum hydrocarbons with various chain lengths and structures, including aromatic compounds, halogenated derivatives, and recalcitrant organic residues, as studied by Stanier, Doudoroff, and other scientists at Berkeley (Bhattacharya et al., 2003). This is because *Pseudomonas* sp., hydrocarbon-degrading extremophiles, can tolerate a wide range of conditions and could be used for bioremediation of polluted extreme habitats. Also, Zang et al. (2021) stated that *Pseudomonas aeruginosa* is one of the most common biosurfactant-producing bacteria. Additionally, in a study done by Al-Jawhari et al. (2022), it was found that *P. aeruginosa* was more efficient in the bioremediation of crude oil compared to *B. subtilis* in mineral salt medium within the first 10 days of exposure. The high degradation yields may be partially due to their halophilic and halotolerant features, which allow them to operate under different levels of saline stress (Muriel-Millán et al., 2019). The diversity of *Pseudomonas* species capable of degrading petroleum hydrocarbons is presented in Table 1.

Table 1. An Overview of Potential *Pseudomonas* Species Isolated from Different Marine Ecosystems in Petroleum Hydrocarbons Degradation.

***Pseudomonas* species**	**Strain**	**Isolated from**	**Pollutants**	**% Degradation**	**Reference**
P. aeruginosa	GOM1	Southwestern Gulf of Mexico	Hexadecane	96	Muriel-Millan et al. (2019)
P. aeruginosa	PFL-P1	Paradip Port, Odisha, India.	Phenanthrene	74	Mahto and Das (2020)
P. aeruginosa	NAPH6	Fishing harbour of Sfax, Tunisia	Naphthalene	-	Hentati et al. (2021)
P. aeruginosa	WD23	Petrochemical refinery industry in Mangalore, India	Petroleum crude oil	27.25	Goveas (2020)
P. zhaodongensis	-	South Shetland Trench in Antarctica	Diesel	51.42	Xu et al., 2022

Table 1 demonstrates that *Pseudomonas* species are utilised effectively in petroleum hydrocarbon degradation research. Muriel-Millan et al. (2019) discovered that *P. aeruginosa* GOM1 isolated from the Southwestern Gulf of Mexico is capable of degrading hexadecane by 96%. The bacteria were incubated for 15 days. It is also worth noting that the addition of nitrogen and phosphate to the seawater culture medium enhanced hexadecane degradation by GOM1. Next, a study by Mahto and Das (2020) found that *P. aeruginosa* PFL-P1 isolated from Paradip Port, Odisha, India, is capable of degrading phenanthrene by approximately 74% when incubated for 5 days. Naphthalene can also be degraded by *Pseudomonas* sp. (Tirkey et al., 2021) and *P. aeruginosa* (Hentati et al., 2021) when incubated for 96 hours and 7 days, respectively. In a study done by Goveas (2020), it was found that *P. aeruginosa* WD23, isolated from the petrochemical refinery industry in Mangalore, India, degraded 27.25% of petroleum crude oil when incubated for 15 days. Last but not least, another species of *Pseudomonas* known as *P. zhaodongensis* (Xu et al., 2022) isolated from the South Shetland Trench in Antarctica degraded 51.42% diesel when incubated for 5 days.

One of the most highly utilised *Pseudomonas* species in the degradation of hydrocarbons is *P. aeruginosa*. Even though this species has been recognised as an opportunistic pathogen in humans, certain strains of *P. aeruginosa* found in crude oil-polluted environments exhibit significant abilities in degrading alkanes, as observed in studies by Chaerun et al. (2004), Liu et al. (2014), and Thomas et al. (2014). What makes this species one of the most effective hydrocarbon-degrading bacteria is the ability for it to thrive in a variety of habitats. *Pseudomonas aeruginosa* can withstand temperatures from 4°C to 42°C (Smith and Iglewski, 2003), a pH range between 4.5 to 9.5, and significant salt concentrations. Furthermore, *P. aeruginosa* is extensively studied for its ability to produce glycolipid-type biosurfactants that enhance the efficiency of hydrocarbon biodegradation.

Rhamnolipids are biosurfactants generated by bacteria. Rhamnolipids have relatively high surface activities and high production yields after brief incubation times. Jarvis et al. (1949) and Edwards et al. (1965) determined the precise chemical composition of these biomolecules. Figure 2 shows the common structures of rhamnolipids. There are two common structures of rhamnolipids, namely mono-rhamnolipids and di-rhamnolipids. The mono-rhamnolipids consist of mono-rhamno-di-lipid and mono-rhamno-mono-lipid, whereas the di-rhamnolipids consist of di-rhamno-di-lipid and di-rhamno-mono-lipid. The diversity in structures results from the varying quantities of rhamnose moieties and the length and number of carbon chains. A connection exists between the surfactant type and the specific hydrocarbons (HCs) degraded, as demonstrated by Rahmati et al. (2022). Specifically, *P. aeruginosa*-produced rhamnolipids are effective in degrading hexadecane. Moreover, Abdel-Mawgoud *et al.* (2011) demonstrated the successful enhancement of PAH degradation through the application of rhamnolipids.

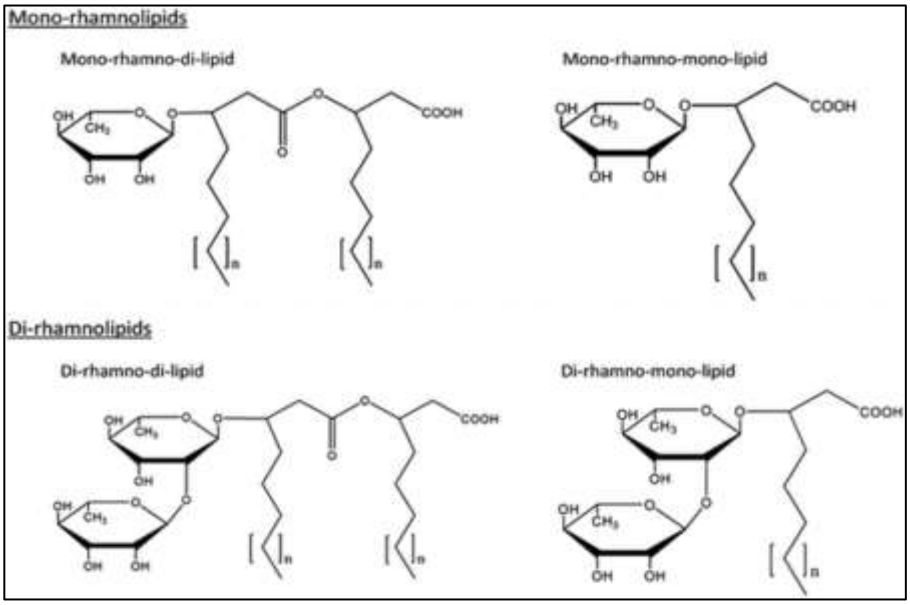

Figure 2. Common Structures of Rhamnolipids (Grosso-Becerra et al., 2014)

Hydrocarbon Degradation by *Aspergillus* sp.

Although research has been mostly focused on bacteria for bioremediation purposes, the utilisation of fungi, also known as myco-remediation, has garnered increased interest over the past several decades. Due to their extensive mycelial networks, enzyme-secreting activities, and resistance to environmental conditions that affect their proliferation, such as pH, temperature, nutrients, and aeration, filamentous fungi have been reported as bioremediation agents (Xenia and Refugio, 2016). In a study conducted by Barnes et al. (2018), *Aspergillus* was one of 10 fungal isolates that demonstrated the ability to use crude oil as the primary carbon source and was chosen to quantify the degradation. They can be found in terrestrial and aquatic habitats, including freshwater, brackish, and marine environments. Despite the fact that various terrestrial fungal taxa have been reported as playing an important role in hydrocarbon biodegradation, only a few studies have investigated the potential for fungal bioremediation of petroleum hydrocarbons in marine environments (Dell'Anno et al., 2021). The diversity of *Aspergillus* species isolated from marine environments capable of degrading hydrocarbons is presented in Table 2. Based on Table 2, it was found by Elshafie et al. (2007) that *A. niger* isolated from Oman beaches had the greatest capability to degrade C15 n-alkanes after being incubated for 70 days. Additionally, *A. niger* (Al-Nasrawi, 2012) isolated from the Gulf of Mexico, *A. terrus* (Kottb et al., 2019) from the Gulf of Suez, *A. niger* from swamp in the Niger Delta proved to be able to degrade crude oil when incubated for 3 weeks, 21 days, and 28 days, respectively.

Table 2. An Overview of Potential *Aspergillus* Species Isolated from Marine Environments in Hydrocarbon Degradation.

***Aspergillus* species**	**Isolated from**	**Pollutants**	**% Degradation**	**Incubation time**	**Reference**
A. niger	Oman beaches	C15 n-alkanes	6.19	70 days	Elshafie et al. (2007)
A. niger	Gulf of Mexico	Crude oil	8.6	3 weeks	Al-Nasrawi, (2012)
A. flavus	Arabian Sea	Crude oil	62	23 days	Barnes et al. (2018)
A. flavus	Gulf of Suez	Crude oil (accommodated fraction)	98.53	5 weeks	Kottb et al. (2019)
A. terrus	Gulf of Suez	Crude oil (water-soluble fraction)	97.45	21 days	Kottb et al. (2019)
A. niger	Mangrove swamp in the Niger Delta	Crude oil	~97	28 days	Kottb et al. (2019)

These species can degrade crude oil by up to 8.6%, 97.45%, and 97%, respectively. A study conducted by Barnes et al. (2018) found that *A. flavus* NIOSN-SK56S22 isolated from the Arabian Sea effectively degraded crude oil by 62% after 23 days of incubation. This is consistent with the findings of Kottb et al. (2019), where the isolate of *A. flavus* proved to be the most effective fungus, degrading crude oil (accommodate fraction) by 98.79% after 5 weeks of incubation.

Aspergillus niger is one of the filamentous fungi that contribute significantly to the degradation of hydrocarbons. One of the most crucial factors in the biodegradation of hydrocarbons by *A. niger* is the hyphae, which help discharge the enzymes into the environment. The hyphae of the species contribute to a significant contact surface with the contaminated substrate or medium. Such hyphal systems are capable of swiftly colonising and penetrating substances, as well as transporting and distributing the substances that provide vital nutrients for their vegetative parts' growth and survival. This increases the efficiency of nutrient assimilation and the fungus' interaction with the target contaminants. Numerous studies have elucidated the mycelial degradation mechanisms of various complex hydrocarbon pollutants (Varjani and Upasani, 2021).

Some extracellular enzymes produced by *A. niger* are lipase, amylase, cellulase, pectinase, and glucose oxidase. Lipases (triacylglycerol-acylhydrolase) catalyse a variety of reactions. According to Lee et al. (2015), lipase enzymes possess the ability to break down hydrocarbon-containing pollutants, such as oils and lipids, through processes like partial or complete hydrolysis of triacylglycerols and reactions involving esterification, transesterification, and interesterification. This degradation facilitates the assimilation of broken-down products like fatty acids. Utilising fungal lipases in the biodegradation of hydrocarbons is advantageous due to their thermal stability, high turnover rate, and simple recovery of extracellular enzymes (Fleuri et al., 2014). Additionally, the biosurfactants produced by *A. niger* are known as monoglucosylox octadecenoic (glycolipid). Glycolipids consist of amphiphilic glycosyl and lipid fractions, endowing them with surfactant properties. These are intracellular metabolites or secondary metabolites (Abdel-Mawgoud et al., 2011) that aid fungi in their dominance over other microorganisms in a particular environmental niche.

Mechanism of Hydrocarbon Degradation by Bacteria and Fungi

The utilisation of microorganism-produced surfactant molecules is a viable strategy to improve the bioremediation efficacy of contaminated settings (Radmann et al., 2015). Figure 3 shows the effect of a rhamnolipid produced by *Pseudomonas* sp. on hydrocarbons, the formation of micelles and the uptake of hydrocarbons.

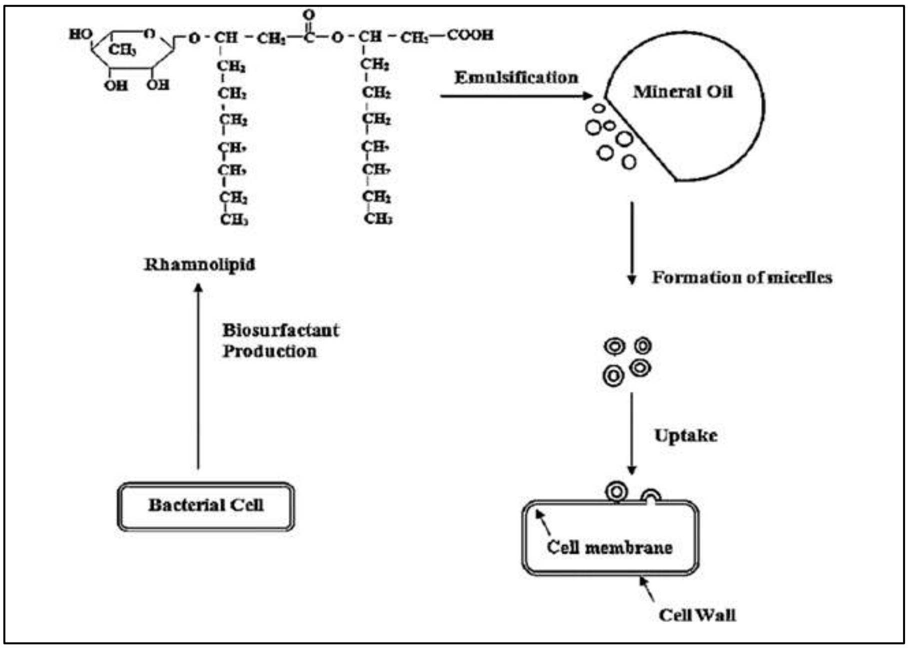

Figure 3. Effect of Rhamnolipids on Hydrocarbons (Fritsche and Hofrichter, 2005).

Based on Figure 3, it is shown that biosurfactants, or rhamnolipids, function as emulsifiers by lowering surface tension and forming micelles. This is done by the emulsification of hydrocarbons, which leads to the formation of micelles. Due to its aggregation within the surfactants' micelles, the apparent solubility of oil increases dramatically. Inside the micelles, the hydrophobic ends of the surfactant molecules clump together to form a hydrophobic environment capable of solubilising hydrophobic substances, whereas the hydrophilic ends exposed to the aqueous phase allow the entire structure to remain in solution (Urum and Pekdemir, 2004). The micelles may be encased in the hydrophobic microbial cell surface, which will then enter the cells of the microorganism to be degraded.

Additionally, *A. niger* and *P. aeruginosa* are examples of microorganisms that degrade PAH via a cytochrome P450 monooxygenase-mediated oxidative pathway. Figure 4 shows the mechanism of degradation of polycyclic aromatic hydrocarbon PAHs by bacteria and fungi.

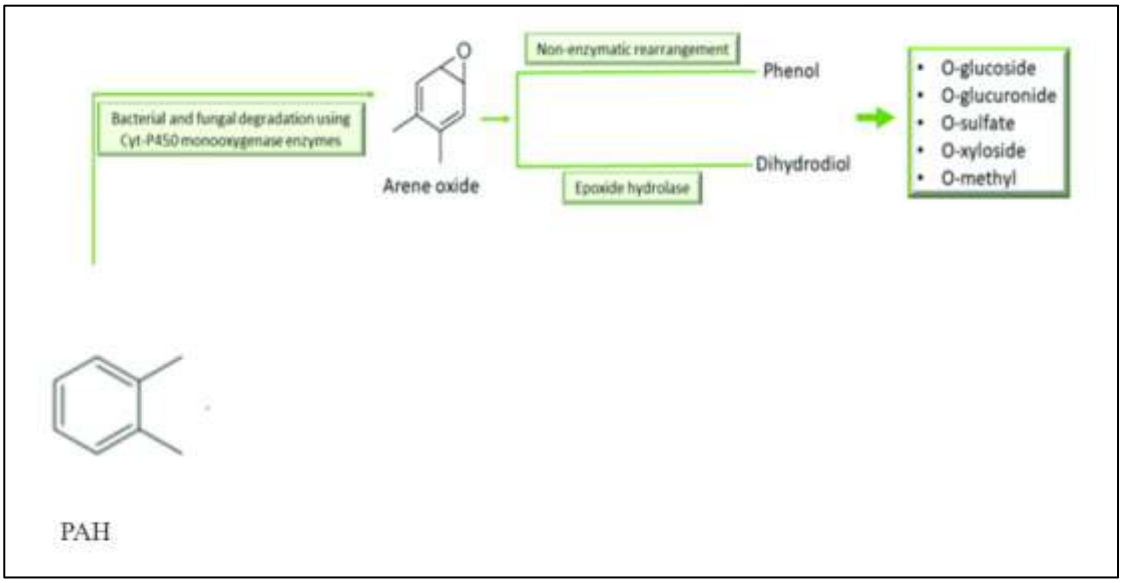

Figure 4. Mechanism of Degradation of Polycyclic Aromatic Hydrocarbon PAHs by Bacteria and Fungi (Rehm and Reiff, 1981).

According to Rehm and Reiff (1981), cytochrome P450 (CYP) monooxygenases play a role in the proposed metabolic pathways for aliphatic assimilation by bacteria and fungi. In general, CYP monooxygenases are the last oxidases in electron transfer chains that are linked to NADPH reductases. These enzymes provide the electrons needed to add one oxygen atom to an aliphatic chain while reducing the other oxygen atom to water (Chen et al., 2014). For cytochrome P450 monooxygenases to work, the aromatic ring must be monooxygenated (epoxidised and hydroxylated) with the help of oxygen and water must be released. Microsomes are responsible for detoxifying xenobiotics (Chen et al., 2014). The CYP system catalyses the ring-epoxidation of aromatic structures to produce arene oxides, which either undergo enzymatic hydration by epoxide hydrolase to form trans-dihydrodiols or rearrange nonenzymatically to form phenols (Sariaslani, 1991). According to Juhasz and Naidu (2000), arene epoxides formed at the "bay-region" that is, between angled benzene rings of PAHs such as benzo[a]pyrene are highly reactive and operate as carcinogens. Hydroxylation products can undergo further detoxification via conjugation to numerous intermediate forms, including methoxyls, O-glucosides, O-glucuronides, sulphates, and O-xylosides. These molecules are more soluble and are ultimately expelled from the body.

Building a Consortium as a Potential Approach to Improve Bioremediation

Due to the intricacy of petroleum as a substrate, it cannot be completely degraded by a single microbial species, as no species possesses all the necessary enzymes. This is due to differences in hydrocarbons' volatility, solubility, and sensitivity. For its complete biodegradation, a diverse population of active microbial species is required. Due to their metabolic diversity, a consortium of microorganisms outperforms pure cultures when it comes to utilising hydrocarbon contaminants as a sole carbon source (Cerqueira et al., 2011). Janbandhu and Fulekar (2011) demonstrated that a PAH-contaminated environment cannot be remedied by a single axenic microorganism culture. In contrast, mixed populations can degrade both pure and complex PAH compounds under a wide range of environmental conditions.

Mixed populations may consist of organisms from a single microbial group, such as bacteria, or they may consist of organisms from disparate groups, such as fungi and bacteria. To ensure the strain's compatibility, an antibiosis test can be performed before combining the strains in a consortium. Antibiosis is the antagonism that results from the toxicity of secondary metabolites produced by one microorganism for other microorganisms. A study done by Daccò (2020), who performed the antibiosis test, found that *Scedosporium apiospermum* F10 can form a consortium with *Penicillium oxalicum* F15 by studying the partition of the inoculated strains on the Petri plate. Whereas *T. harzianum* F26 and *Coniochaeta sp.* F37 gave no positive results. *T. harzianum* F26, on the other hand, is the strain that most inhibited the growth of the others, as Trichoderma is a genus known to be a hyperparasitic species of other fungi (Juliatti et al., 2019 and Inayati et al., 2020).

It has been established that bacterial and fungal co-cultures enhance the degradation of numerous polyaromatic hydrocarbons under laboratory conditions (Varjani and Upasani, 2021). In a study done by Anih et al. (2019), a microbial consortium of *A. niger* and *P. aeruginosa* was utilised to remediate crude oil-polluted water. The result of this study showed that 95.64% of hydrocarbon was degraded after 49 days. On the other hand, Odili et al. (2020) conducted the degradation of hydrocarbons using a fungal-fungal consortium of *Monocillum* sp. and *A. niger* showed that the degradation of crude oil was higher by the individual isolates than when they were used in combination. This might be due to the fact that competition for the same active site might slow down the degradative process or that the metabolites resulting from the degradation are affecting the enzyme's ability to perform effectively.

Understanding the ecological impact of bioremediation processes on aquatic ecosystems and other environmental factors is crucial for researchers seeking to enhance their studies. The sustainability and long-term success of bioremediation efforts can be ensured by evaluating all potential side effects and unintended consequences. In addition, conducting on-site field trials and large-scale applications of hydrocarbon bioremediation will aid in determining the practicability and efficacy of these techniques in real-world situations. This will provide vital insight into their implementation in practice and potential obstacles. Lastly, the integration of bioremediation with other remediation techniques, such as phytoremediation or chemical remedies, may offer comprehensive and effective solutions for complex contamination scenarios, according to a recommendation for further research.

CONCLUSION

In summary, *Pseudomonas* sp. and *Aspergillus* sp. have emerged as highly effective and efficient bioremediation agents for hydrocarbon-contaminated water. The suitability of *Pseudomonas* sp. and *Aspergillus* sp. as viable candidates stems from their capacity to thrive within hydrocarbon-contaminated water, owing to their inherent adaptive mechanisms, as well as their ability to produce biosurfactants, and their remarkable capacity for degrading a wide range of petroleum hydrocarbons. Notably, *A. niger*'s filamentous nature further elevates its candidacy for petroleum hydrocarbon bioremediation due to the advantageous presence of hyphae. Furthermore, petroleum hydrocarbons, which commonly are in the form of PAHs, are degraded by *Pseudomonas* sp. and *Aspergillus* sp. through the cytochrome P450 monooxygenase-mediated oxidative pathway. Biosurfactants produced by the microorganisms function as emulsifiers, which naturally improves the bioremediation efficacy. Of equal significance, the formation of a consortium amplifies the effectiveness of petroleum hydrocarbon bioremediation within water bodies due to the diverse enzymatic profiles of various microorganisms, facilitating a complete degradation of hydrocarbons. This is due to the fact that distinct enzymes are requisite for the degradation of different hydrocarbon constituents. Extensive research has showcased their remarkable capabilities in degrading diverse hydrocarbon pollutants, positioning them as promising candidates for practical bioremediation applications. Thus, this review sheds light on the potential of *Pseudomonas* sp. and *Aspergillus* sp. in addressing hydrocarbon pollution and offers valuable insights for advancing bioremediation strategies.

AUTHOR CONTRIBUTIONS

Nurul Izzati Liyana Yusne was responsible for drafting this manuscript. Wan Razarinah Wan Abdul Razak was responsible for reviewing and editing the manuscript.

COMPETING INTEREST

The authors declare that there are no competing interests.

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