Envirotek : Jurnal Ilmiah Teknik Lingkungan
Vol.18, No.2, October, 2026, pp. 26-35
Journal Home Page: http://envirotek.upnjatim.ac.id/
e-ISSN 26231336 p-ISSN 2085501X

Potential of Microbial Consortia (Bacteria-Fungi) in Hydrocarbon-Contaminated Soil Bioremediation: Overcoming Wax Fraction Degradation Challenges

Fikri Daryat[1], Denny Helard[2], Shinta Indah[2], Delita Zul[3]

[1]Doctoral Program in Environmental Engineering, Universitas Andalas
[2]Department of Environmental Engineering, Universitas Andalas
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau

email correspondence: fikridaryat94@gmail.com

Received: *13-11-2026*
Accepted: *18-08-2026*
Published: *31-10-2026*

Keywords:
Bioremediation, microbial consortium, bacteria-fungi, petroleum hydrocarbons, wax fractions, biosurfactants

ABSTRACT
Soil contamination by petroleum hydrocarbons, including wax fractions, is a major global environmental issue causing ecosystem degradation, decreased soil fertility levels and effects on human health. Conventional physical-chemical remediation techniques are often costly and unsustainable. Alternatively, the application of bacterial-fungal microbial consortia has emerged as a promising bioremediation solution. This consortium offers superior metabolic synergy to break down complex hydrocarbons, facilitated by the production of biosurfactants and extracellular enzymes. This review article focuses on the potential of bacterial-fungal consortia in remediating hydrocarbon-contaminated soils, addressing aerobic and anaerobic degradation, biosurfactant performance and the challenges of degrading wax fractions. The review concludes that further research is essential to optimize the use of bacterial-fungal consortia for degrading wax fractions in heavy oil-polluted soils, leading to the development of viable bioremediation technologies

1. INTRODUCTION

One of the most significant environmental pollution issues to date is soil contamination by petroleum that has been made to date is the contamination of the soil by petroleum. A number of pollution control models have been developed continuously, such as physical and chemical procedures up to biological technology. The international petroleum sector has a big contribution to the environmental degradation due to hydrocarbon spills. This is through the increased demand of petroleum in the world as the main source of energy. As reported by Oltulular (2024), the daily consumption of crude oil in the world today stands at 97.3 million barrels/day in 2022. To meet this demand, the downstream, midstream, and upstream sectors of the petroleum industry operate more actively, resulting in both intentional and unintentional oil spill incidents that discharge crude oil into ecosystems worldwide. There has been a pressing need to eliminate the released hydrocarbons to avoid health and environmental risks of the compounds of petroleum. There are a number of remediation technologies that have been tested and used to treat petroleum contaminated soils. The sites with the relatively high amount of contamination can be optimally remedied by a combination of various processes in accordance with the existing conditions (Dindar et al., 2013).

Petroleum hydrocarbons are a mixture of complex and simple hydrocarbons, which are made up of carbon and hydrogen, and some traces of nitrogen, sulfur, and oxygen (Varjani and Upasani, 2017). There are four groups of hydrocarbons, including saturates, aromatics, asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and resins (pyridines, quinolines, carbazoles, sulfides, and amides) (Adeniji et al., 2017). Hydrocarbons are also composed of numerous volatile substances including benzene, toluene, ethylbenzene, and xylene (BTEX), and various toxic ones including polycyclic aromatic hydrocarbons (PAHs) and have numerous negative impacts on soil, like changes in the population of soil microorganisms, soil structure and composition, organic matter, and soil enzyme activity. Moreover, they are attached to the soil elements, which makes them difficult to decompose and cause pollution of groundwater and surface water (Pinedo-Gonzalez et al., 2018).

A variety of physicochemical techniques have been highly advanced to treat the polluted petroleum environments (Naeem and Qazi, 2020). These approaches can be viewed as fairly effective yet cannot actually offer the solution to the issue and are highly costly, may necessitate the increase of energy consumption and bear the rest of the technical implications that are difficult to meet. Bioremediation techniques have been preferred more because of their high efficiency, cost effective and due to higher degree of environmental friendliness. Several types of microorganisms may be used as degraders of hydrocarbon compounds and serve as a source of carbon and energy (Chunyan et al., 2023).

Not to mention that there are also fungi and bacteria among other types of microorganisms that play an important role in the process of removing hydrocarbons (Gaur et al., 2022).

Hydrocarbons can be degraded by over 100 fungi and bacteria and through a wide variety of mechanisms and with a wide range of conditions (Zhang et al., 2021). However, the petroleum hydrocarbon concentration is highly heterogeneous, and that is why it is projected that no single microorganism can completely degrade the entire petroleum compounds (Kaushik et al., 2021).

The most preferable variant is to employ a microbial consortium of various species or a blend of various fungi and bacteria so as to have total removal of pollutants. The mixed cultures of microbes are more effective in degrading pollutants than the pure ones and are less dependent on the genes as well as metabolism. It is because they are capable of being in various interactions with each other and attaching different desirable properties, such as stability, resilience, and division of labor, and can enable microorganisms to degrade pollutants more effectively (Espinosa-Ortiz et al., 2022).

The review article will cover the potential of bacterial fungal consortium in remediating hydrocarbon polluted soil with a specific concern on aerobic and anaerobic degradation processes, the efficacy of bio-surfactants and the obstacles of treating the wax fractions. The biodegradation capability, with a focus on the potential of bacterial and fungal consortium as a more environmentally friendly bioremediation technology solution, will be highlighted in comparison to conventional remediation mechanisms that generate secondary pollutants (Sanjana et al., 2024) in petroleum hydrocarbon-contaminated soil.

2. PETROLEUM HYDROCARBONS

Crude oil takes different physical features and appearance depending on the composition, and that, on average, is between black and dark brown. Nevertheless, there are yellow, red and green samples. It is also dependent on its composition as well as its viscosity. An illustration is that oil that has low viscosity and there are also those that have extremely high viscosity (Vicenzo et al., 2013). Overall, petroleum has a very intense and characteristic smell and its intensity may differ depending on the chemical composition of petroleum and the amount of sulfur in it. There is the possibility that some of the oils could fluoresce under the ultraviolet light, mainly because of the presence of a number of the polycyclic aromatic compounds in the oil blend (Vicenzo et al., 2011). The polycyclic aromatic compounds are dangerous chemical compounds that consist of benzene rings (Kanokpan et al., 2012). Figure 1 presents a diagram of various classes of hydrocarbons, which consist of organic material components, including aromatic and aliphatic hydrocarbons, as well as several other organic and mineral components. Gudde (2018) states that oil properties are complex combinations of many various hydrocarbons and the most common types of hydrocarbons are linear alkanes (paraffins), cycloalkanes (naphthenes), and aromatic hydrocarbons (arenes) and the asphaltene complex compounds. Oil mixtures possess different compositions of the constituent particles, which finally determines the overall physical characteristics of the oil mixtures like its color and viscosity.

3. CHARACTERISTICS OF WAX FRACTIONS IN PETROLEUM HYDROCARBONS

Wax fractions are complex alkane mixtures of high molecular weight or high carbon numbers, which are straight-chain, branched, and cyclic structures (Demirbas, 2016). The maturity of the reservoir is one of the reasons why the crude oil contains wax fractions due to the ongoing production of crude oil in several regions across the world over the last several years (Sakthipriya et al., 2017).

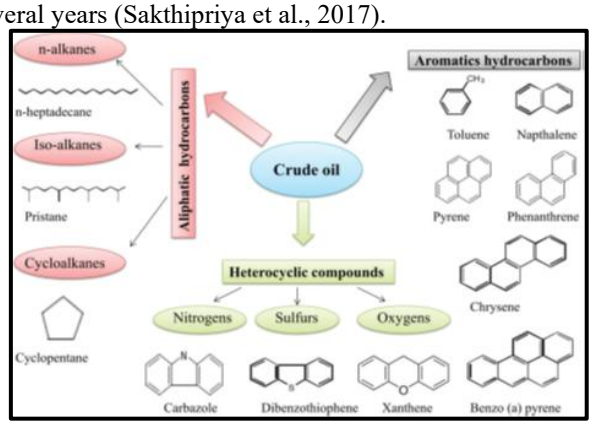

Figure 1. Classes of Hydrocarbons (Mahjoubi et al., 2018)

As illustrated in Figure 1, the structural complexity of hydrocarbons determines their degree of recalcitrance to microbial degradation. Short-chain aliphatic hydrocarbons are generally more easily oxidized compared to the complex ring structures of aromatic hydrocarbons, which require a series of specific enzymatic cleavages before they can enter the central metabolic cycle.

As Speight (1991) expounds, petroleum distillates and residues generally contain petroleum wax fractions that can be classified into two categories, such as the paraffin wax and microcrystalline wax. Paraffin wax is composed of straight-chain saturated hydrocarbons composed of carbon atoms between the C18 and C36 (Figure 2.a). Microcrystalline wax, on the other hand, is composed of branched and cyclic hydrocarbons, containing carbon atoms of C 30 down to C 60. This is in line with Hosseinipour et al. (2014), who stated that waxes are long-chain hydrocarbons with lengths varying from C15–C20 up to C75. Wax fractions undergo crystallization into two forms: orthorhombic and hexagonal. The crystallization of wax fractions into orthorhombic or hexagonal forms is significantly influenced by the temperature drop below the Wax Appearance Temperature (WAT) and the variations in the constituent carbon chain lengths. This thermodynamic temperature decrease triggers the agglomeration of long-chain hydrocarbon molecules, which physicochemically transforms the liquid phase of the pollutant into a crystalline solid. Ultimately, this condition significantly blocks soil pores and restricts microbial accessibility

The crude oil contains a high amount of wax, which is then crystallized into orthorhombic shapes and possesses needle-like structures (Figure 2.b) (Kane, 2023). Wen et al. (2025) add that wax deposition in the pipeline (Figure 2.c) is possible when the pour point and viscosity are high, the content of the wax and its complex rheological mechanisms of the oil are high, and the conditions at low temperatures of the fluids are poor. Ultimately, when these conditions co-occur, they become a threat to pipeline operations.

According to a study by Islam (2015), petroleum waste contains approximately 10.5% wax fractions in its total composition. Several other studies have also been conducted to determine the wax content in various types of crude oil worldwide. The wax content in crude oil from eastern Egypt is reported to be 3.3–4.5% (El-Ella & Nassef, 2014); in Venezuela and Russia, it has been identified as 4.1% and 9.4–12.2%, respectively (Lu & Redelius, 2007); while in Shengli, Quiling, Sudan, and Daqing, it is reported as 9.6%, 10.9%, 21.2%, and 26.3%, respectively (Chen et al., 2004). Meanwhile, Crude oil in the Arabian and South China Sea contains 12.7% and 32.5% meanwhile, respectively of the fraction content of the wax (Musser and Kilpatrick, 1998).

4. ENVIRONMENTAL IMPACTS OF PETROLEUM HYDROCARBON AND WAX FRACTION CONTAMINATION

Incidences of hydrocarbon contamination have impacts on the soil conditions and have serious impacts on the ecosystem and living organisms (Erdogan et al., 2012). According to Ikuesan (2017), when the soil is contaminated with hydrocarbons, it becomes infertile (the composition of the soil changes), microbiological, and physical properties. The development of phytotoxic plants symptoms because of the hydrocarbon contamination in the soil is the resultant effect on the plants and the soil ecosystem (Correa et al., 2022). A similar supporting study conducted by Das et al. (2022) also established that the persistence of hydrocarbons on the soil has undesirable effects on the soil whereby it becomes unable to support the plant growth and becomes aggregated and congested with soil pores that limit its aeration to the soil and water retention of water. It is also a fact that the presence and concentration of the hydrocarbons found in the soil highly depend on the nature of the distance between the contamination origin and the soil (Yuan et al., 2014).

The influence of the deposition of the wax on the soil influences the decrease in hydraulic pore diameter, porosity, and permeability of the soil (Sandyga et al., 2020). Wax characteristics create a film on the surfaces of soil particles, which blocks soil pores and leads to adverse outcomes regarding the soil fertility, and also suppresses the activities of soil microbesHydrocarbon contaminants can easily be absorbed into soil pores and persist for long periods such as the wax content easily and can last a long time (Wu et al., 2019).

Based on its effects on human health, acute exposure to hydrocarbons brings about effects like dermatitis, arrhythmia, acidosis, and encephalopathy. Several health issues arise as a result of exposure of the workers who are directly involved in handling petroleum and who include liver, bladder, and lung cancers. In some cases, neurological and reproductive disorders have also been reported (Tormoehlen et al., 2014).

Other studies have also been conducted on freshwater ecosystems, showing that hydrocarbon contamination has lethal effects on mammals, waterfowl, fish, insects, and aquatic microorganisms (EPA, 2016). Hydrocarbon contamination can become quite severe when it occurs in groundwater. The confined nature or relatively slow movement of groundwater, combined with the recalcitrant properties of hydrocarbons, leads to the persistence and accumulation of contaminants in groundwater (Fei-Baffoe, 2024).

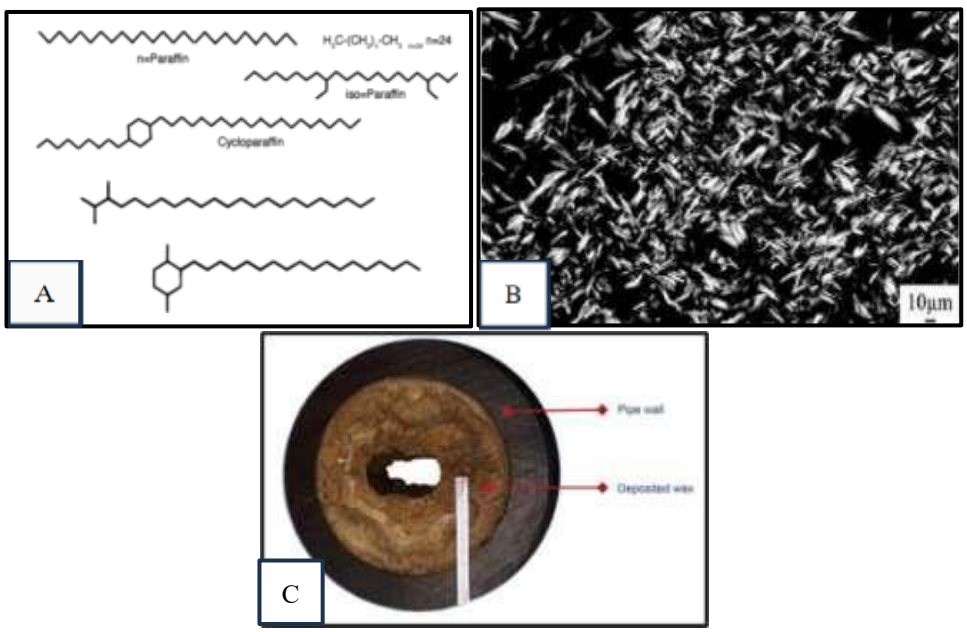

Figure 2a. Chemical structure of wax fractions, **Figure 2b**. Microscopic view of wax crystals after 6 hours of settling time (Yang et al., 2016), **Figure 2c.** Cross-sectional view of a pipeline with wax deposition (Kiyingi et al., 2022).

5. BIODEGRADATION OF PETROLEUM HYDROCARBONS AND WAX FRACTIONS BY MICROORGANISMS

5.1 Biodegradation of Petroleum Hydrocarbons by Microorganisms

Hydrocarbon contamination incidents can lead to a decline in environmental functions and may cause health impacts when accumulated in humans. The existence of oxidizing microbes facilitates the use of hydrocarbons as carbon and energy (electrons) sources. Microorganisms can do the degradation of hydrocarbons both in aerobic and anaerobic sources (Widdle et al., 2010). Aerobic microorganisms have

the capacity to degrade contaminants through mechanisms whereby oxygen is used to degrade pollutants like petroleum hydrocarbons, dioxins and other organic substances. Most organic pollutants are degraded best and most thoroughly in aerobic conditions by the degradation capability of microorganisms (Olajire and Essien, 2014). The first intracellular processes of degradation of organic pollutants as illustrated in Figure 3 entail oxidation processes, activation and use of oxygen which are major enzymatic reactions mediated by oxygenases and peroxidases. The peripheral pathway of degradation converts organic pollutants into the central metabolic pathways step-by-step developing to intermediates, which enter the central tricarboxylic acid cycle. Cellular biomass is biosynthesized using the major metabolites, which are acetyl-CoA, succinate, and pyruvate. In the meantime, sugars needed during different stages of biosynthesis and growth of the microorganisms are produced as a result of gluconeogenesis.

Microbial degradation of petroleum hydrocarbons fundamentally relies on either aerobic or anaerobic pathways, each with distinct metabolic characteristics and efficiencies. A comprehensive comparison of these two mechanisms, highlighting their oxygen requirements, enzymatic catalysts, and degradation rates, is presented in the table 1 below.

Table 1. Comparison of Aerobic and Anaerobic Hydrocarbon Degradation Mechanisms

Parameter	Aerobic Degradation	Anaerobic Degradation
Oxygen Requirement	Absolute (as the primary electron acceptor)	None (utilizes SO_4^{2-}, NO_3^-, Fe^{3+})
Primary Enzyme Catalyst	Oxygenases (Mono/Dioxygenase)	Reductases, Specific Hydrolases
Degradation Rate	Very Fast	Relatively Slow
Subsequent Cycle	Tricarboxylic Acid (TCA) Cycle	Fermentation, Methanogenesis
Reliability on Heavy	Optimal on porous surfaces with sufficient oxygenation	Susceptible to nutrient and electron limitations

The hydrocarbon degradation pollution process can take place in two steps, which are anaerobic. Such reactions have the potential to produce energy either by oxidizing substances (e.g. hydrocarbons) and reducing substances (e.g. sulfate and nitrate) or by reducing non-oxygen terminal electron acceptors (e.g. sulfate and nitrate) or by converting other energy sources (e.g. fermentation). For some types of bacteria and archaea, this process is referred to as fermentative degradation (Zwolinski et al., 2000).

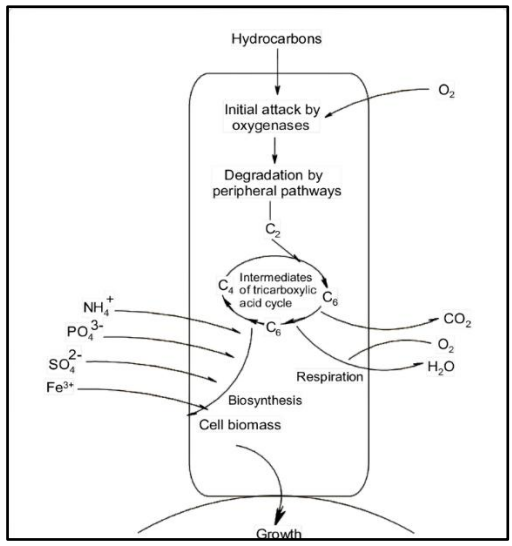

Figure 3. Stages of aerobic degradation hydrocarbons by microorganisms (adapted from Fritsche & Hofrichter, 2000)

As illustrated in Figure 3, the aerobic degradation pathway is fundamentally dependent on the initial attack by oxygenase enzymes. In the context of hydrocarbon breakdown, this step is critical because oxygen acts directly as a co-substrate to cleave stable carbon bonds, converting them into intermediates that subsequently enter the tricarboxylic acid (TCA) cycle. This rapid initial enzymatic cleavage provides the fundamental reasoning as to why aerobic conditions yield significantly higher hydrocarbon degradation efficiencies compared to anaerobic pathways. A study conducted by Foght (2008) indicates that some anaerobic species of bacteria also use other substitutes in obtaining energy; some of these substitutes include sulfate, nitrate, iron, manganese, or carbon dioxide. It can also be seen that microbial strains growing under particular conditions utilize unusual electron acceptors like vanadium, cobalt, and even uranium (Greene et al., 2016). Humic materials, sulfonates, e.g., cysteate, isethionate, taurine and a variety of other halogenated compounds, azo compounds (including dye), and some nitro compounds (including TNT) have been reported to be used by other anaerobic microorganisms (Hong and Gu, 2009).

5.2 Biodegradation of Wax Fractions by Microorganisms

The degradation ability of microorganisms is quite promising due to their natural presence in the environment, non-carcinogenic nature, non-pathogenic characteristics, environmental friendliness, and economic feasibility for controlling wax deposition (Mokhatab & Towler, 2009). According to Alaidaroos (2023), the biodegradation of hydrocarbons by microorganisms can occur through three mechanisms: (1) Direct degradation using the catalytic activity of microbial enzymes in the oxygenation process with oxygenase enzymes (monooxygenase and dioxygenase) as catalysts during the aerobic degradation stage (Figure 4); (2) Production of biosurfactants to enhance the biodegradation process by emulsifying and releasing hydrocarbons from the substrate; and (3) Under certain conditions, microorganisms utilize hydrocarbons as sources of energy and carbon. This is supported by Varjani's (2017) previous statement, which explains that microorganisms are capable of producing

catalytic enzymes to increase the degradation rate of pollutants.

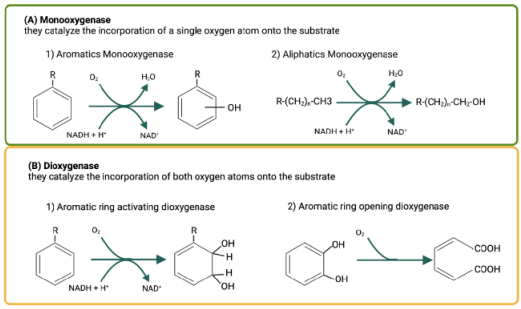

Figure 4. Oxygenation Reaction Catalyzed by Oxygenase Enzymes (Monooxygenase and Dioxygenase) (Pandolfo et al., 2023)

The microbial degradation of long-chain waxes into shorter paraffins is driven by complex metabolic mechanisms. This process relies heavily on the microbial secretion of extracellular enzymes and secondary metabolites, such as biosurfactants, organic acids, and alcohols. Furthermore, the efficiency of this metabolic capability strongly depends on the adequate availability of essential nutrients and water (Wenger et al., 2002).

As analytically demonstrated in Figure 5, biosurfactants play a crucial role in overcoming the hydrophobicity of wax deposits on metal surfaces. Biosurfactant molecules possess both hydrophobic and hydrophilic functional groups. During the interaction, the hydrophobic tail aligns with the petroleum hydrocarbons, while the hydrophilic head aligns with the aqueous phase, leading to the formation of micelles. This micellar agglomeration effectively emulsifies the crude oil, thereby increasing the substrate's bioavailability for microbial colonization (Ron & Rosenberg, 2002; Biktasheva et al., 2024). Consequently, this enhanced bioavailability facilitates optimal contact between microbes and hydrocarbon substrates, which is a fundamental prerequisite for oxygen permeation and the initiation of oxygenase enzymatic activity. Various microbial taxa, including yeasts, bacteria, and specific *Candida* species, have been documented to efficiently produce these extracellular biosurfactants for bioremediation applications (Panjiar et al., 2017; Khanna et al., 2023)

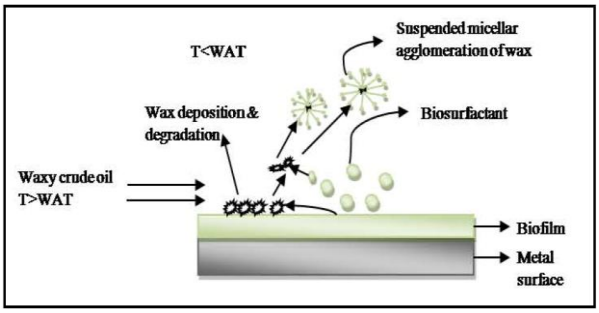

Figure 5. Schematic Representation of Biosurfactants Assisting in Wax Degradation on Metal Surfaces (Sakthipriya et al., 2017)

5.3 Biodegradation of Petroleum Hydrocarbons and Wax Fractions by Microbial Consortium (Bacteria–Fungi)

Remediation technology is a microbial consortium comprising of a combination of bacteria and fungi that are synergistic thus allowing them to break down hydrocarbons and wax fractions. These two microorganisms are members of different kingdom and this is of great promise because they have complementary characteristics of the ability to degrade pollutants. In general, the investigation related to bioremediation technology has been reduced to the use of bacteria since they reproduce extremely rapidly, can proliferate easily, and their oxidation is enzymatic. However, it has several physiological drawbacks because bacteria are quite small and simple which causes a decrease in optimal efficacy. On the other hand, fungi possess higher bioremediation capacity in the degradation of stronger and recalcitrant pollutant compounds because they utilize extracellular enzymes and metabolites to degrade even stronger recalcitrant pollutants (Atagana et al., 2006).

Bacterial and fungal consortium has been used in previous works on a variety of hydrocarbons and different target remediation compounds (Table 1). There is limited research on bioremediation technology on the use of bacterial and fungal consortium to degrade hydrocarbons in the heavy oil polluted soils with a particular emphasis on TPH and the wax components. Most studies have employed either a bacterial or a fungal consortium. Synergistic treatments between bacteria and fungi in bioremediation mechanisms have not yet been observed. Studies using bacterial and fungal consortium have only targeted other hydrocarbon compounds, such as n-alkanes, aromatic compounds, and branched alkanes (Zhang et al., 2021), pyrene and tetracosane (Ghorbannezhad et al., 2021), naphthalene, fluorene, phenanthrene, anthracene, and pyrene (Khandelwal et al., 2021). Other studies have also targeted compounds such as TPH, n-alkanes, and PAHs (Atakpa et al., 2022). The study by Monteiro et al. (2024) demonstrated the effectiveness of bacterial and fungal consortium in degrading PAH and TPH compounds.

Table 2. Studies on Microbial Consortium (Bacteria–Fungi) in Hydrocarbon Degradation (2021–2025)

Source of Isolates	Microbes	Remediation Target	Results	References
Soil and sludge contaminated with crude oil	**Bacteria:** *Acinetobacter baumannii* **Fungi:** *Talaromyces* sp.	n-alkanes, aromatic compounds, branched alkanes	• The bacterial–fungal consortium degraded n-alkanes in crude oil at a 1:1 ratio and at a pH of 9 (representing an alkaline soil condition) over 14 days • Achieving a total crude oil degradation rate of up to 80% • Both strains produced small amounts of biosurfactants, accompanied by high cell viability.	Zhang et al. 2021
Soil samples from Pazanan, Kohgiluyeh and Boyer-Ahmad Province, Iran	**Bacteria:** *Bacillus*, *Geobacillus*, *Glutamicibacter*; yeasts *Naganishia*, *Silicoccozyma aerius* Y123 **Fungi:** *Aspergillus*, *Alternaria soliaridae* F22 and other diverse strains	Pyrene and tetracosane	• Sequential mixed culture (SMC) of bacteria and fungi, in which bacteria were inoculated one week after the fungus was applied to pyrene and tetracosane showed that 60.76% and 73.48% pyrene and tetracosane were degraded, respectively • This was approximately ten percent more than Traditional Mixed Culture (TMC) • Later inoculation of bacteria caused increased fungal growth and degradation of pyrene • Introduction of rhamnolipid enhanced the growth of pyrene and tetracosane by 16 and 23 percent respectively, which was better than introduction of Tween-80.	Ghorbannezhad et al. 2021
Agricultural soil, Nanjing City, Jiangsu Province, China	**Increase in** Bacteria: *Alphaproteobacteria*, *Gammaproteobacteria* **Increase in** Fungi: *Tremellomycetes* (Basidiomycota)	Polycyclic Aromatic Hydrocarbons (PAHs), Benzoanthracene (BaA)	• The addition or modification of lignin can significantly enhance the mineralization of Benzo[a]anthracene (BaA) into CO_2, accompanied by an increase in the abundance of *Basidiomycota* • Fungi are also significant in biodegradation of the BaA which may take place via ligninolytic enzymes mediated pathways • This diversification of bacterial populations like the *Methylophilaceae* and *Sphingomonadaceae* helps in the use of the methyl and aryl groups of lignin by the bacteria	Gu et al. 2021
Hydrocarbon-contaminated soil	**Bacteria:** *Lysinibacillus*, *Arthrobacter*, *Bacillus*, *Kocuria, Arthrobacter* sp. *Pseudomonas, Nocardioides*, *Sporosarcina, amyloliquefaciens*, *Staphylococcus* **Fungi:** *Trichoderma, Aspergillus, Aspergillus*	Naphthalene, fluorene, phenanthrene, anthracene, pyrene	• The heat map and Tukey HSD analysis indicated the maximum degradation of PAH was in the treatment of Kocuria rosea and the Tween 80 • Percentages of degradation (without/with Tween 80) naphthalene 96.6/98.9; fluorene 93.8/95.1; phenanthrene 58.1/70.9; anthracene 19.9/32.5; pyrene 13.8/54.7 • The products of decomposition of PAH were phthalic acid, antron, phenanthrene-9,10 oxide and 9-phenanthrol.	Khandelwal et al. 2021
Sediments contaminated with petroleum hydrocarbons	**Bacteria:** *Acinetobacter* sp. Y2 **Fungi:** *Scedosporium* sp. ZYY	Total petroleum hydrocarbons (TPH), n-alkanes, polycyclic aromatic hydrocarbons (PAHs)	• The capability of microbial consortium demonstrated the decrease of the hydrocarbon surface tension, which indicates the production of biosurfactants in the culture of the consortium • The overall rate at which petroleum hydrocarbons were degraded rose to 58.61 as compared to 23.36 at the expiry of the 7 days incubation period • Considerable degradation occurred, n-alkanes between 3789.27 and 940.33 mg/L and polycyclic aromatic hydrocarbons between 1667.33 and 661.5 µg/L • Co-culture was found to have high levels of the genes alkB and CYP52 that were highly expressed by Acinetobacter sp. Y2 and	Atakpa et al. 2022

Source of Isolates	Microbes	Remediation Target	Results	References
			Scedosporium sp. ZYY respectively and correlated positively with the n-alkane degradation.	
Hydrocarbon-contaminated soil (bioaugmentation mechanism)	**Bacteria:** *Pseudomonas* XH-1 **Fungi:** *Aspergillus* sp. LJD-29	Phenanthrene (PHE)	• The study was done to investigate the bioaugmentation processes of Phenanthrene (PHE) degradation with the use of an indigenous fungal strain Aspergillus sp. LJD-29 and Pseudomonas XH-1 bacterial strain through the application of Stable Isotope Probing (SIP) to track the active PHE degrading microorganisms. • Single microorganisme might considerably degrade PHE, the concomitant action of both strains showed more complicated degradation interaction. • SIP analysis showed that bacterial strain contributed a lot to the break of PHE, whereas the fungus was very important in the breakdown of PHE by extracellular enzymes that promoted assimilation of metabolites by bacteria.	Luo et al. 2024
Ressacada Experimental Farm, Florianopolis, Southern Brazil	**Bacteria:** *Bacillus*, *Burkholderia*, *Stenotrophomonas*, *Dyella*, *Paraburkholderia*, *Serratia.* **Fungi:** *Penicillium*, *Trichoderma* and other diverse strains	PAHs and TPH	• The sources of microbes were associated with native species in soil contaminated with a blend of diesel and biodiesel (20% biodiesel and 80% diesel) • The total number of identified fungi was 7 and bacteria number was 12 • The drop collapse test revealed that, all fungal strains and four bacterial strains could produce biosurfactant, and the surface tension decreased by at least 20 • The PAH and TPH (C5 -C40) degradation of the diesel oil were examined and revealed that the microbial consortium had a higher degradation capacity • Optimal outcomes were witnessed in the bacterial-fungal consortium of Trichoderma koningiopsis P05R2, Serratia marcescens P10R19, and Burkholderia cepacia P05R9 with degradation spectrum of 91 per cent in the PAHs under analysis and a 93.61 per cent in total PAHs and 93.52 per cent in TPH reduction.	Silva Monteiro et al. 2024
1. Activated sludge (Xuzhou, China) and copper mine sludge (Tongling, China) 2. Acid-tolerant PAH-degrading microbial consortium (Shanghai, China)	**Bacteria:** *Pseudomonas*, *Achromobacter*, *Stenotrophomonas*, *Burkholderia*, *Citrobacter*, *Brucella*, *Gammaproteobacteria*, *Luteibacter*, *Pseudomonadota*, *Ochrobactrum*, *Brucellaceae*, *Enterobacteriaceae*, *Leifsonia*, *Acinetobacter*, *Methylobacterium*, *Xanthomonas*, *Methylorubrum* **Fungi:** *Talaromyces*, *Rhizopus*, *Penicillium janthinellum*, *Trichoderma koningiopsis*,	Pyrene and n-docosane	• Lignin-enriched bacterial–fungal consortium achieved the highest pyrene and n-docosane removal efficiencies of 74.8% and 99.3%, respectively, in 12 days at a 5:5 fungi-to-bacteria inoculation ratio • Lignin addition enhanced the activities of ligninolytic enzymes, dioxygenase and dehydrogenase.	Wu et al. 2025

Source of Isolates	Microbes	Remediation Target	Results	References
	Penicillium pulvillorum, *Penicillium simplicissimum*			

Research by Zhang et al. (2021) demonstrated that in alkaline soil treatments, the consortium managed to decrease crude oil percentage up to 80 with no loss in microbial cell viability. This shows how microbial consortium has high resistance to adverse living conditions.

The two microbial cultures/strains will be utilized and this will reflect a more complex hydrocarbon degradation potential. The bacteria and fungi strain will cooperate and perform their respective activities and improve hydrocarbon reduction that can be determined through Stable Isotope Probing (SIP) (Luo et al., 2024).

The utility of utilizing bacterial and fungal cultures at different points also turned out to be more successful in the reduction of the target compounds at the bioremediation steps (Ghorbannezhad et al., 2021). This was observed when two forms of microbial culture mechanism were used and they are sequential mixed culture application and conventional bacterial-fungal mixed culture application.

The natural or artificial materials also may be introduced in a manner that will enhance the ability of the degradable microorganisms. Gu et al. (2021) further suggest that the more natural materials like lignin can have a positive impact on the mineralization not only of Benzo[a]anthracene (BaA) or the reactions of the microbes in the bioremediation stages. Another added supplement to the pyrene/n-docosane degradation is the lignin which enhances the activity of ligninolytic enzymes that are dioxygenase and dehydrogenase (Wu et al., 2025).

Other studies that included the use of surfactants such as Tween-80 were also seeing the effects of the degradation of PAHs being significant (Khandelwal et al., 2021). The solubility of PAHs in tween-80 surfactant will improve, and therefore, its degradation ability will be increased. Tween-80 is a non-ionic hydrophilic surfactant that is not toxic in nature (Kachieng'a & Momba, 2017). The ability of natural surfactants formed by bacterial and fungal consortium to increase the degradation rate of n-alkanes, TPH, and PAHs is also supported by the study by Atakpa et al. (2022). Other research demonstrated that biosurfactants produced by bacterial and fungal consortium can significantly reduce the surface tension of hydrocarbons, resulting in an increased percentage of PAH and TPH degradation (Silva Monteiro et al., 2024).

6. CONCLUSION

The presence of hydrocarbon contamination, particularly heavy wax fractions, causes severe degradation of soil productivity and poses significant environmental health risks. Based on the synthesized literature, applying a cross-kingdom microbial consortium (bacteria-fungi) emerges as the most promising and environmentally sustainable bioremediation technology. The metabolic synergy between bacteria and fungi facilitates robust extracellular enzyme activity and optimal biosurfactant production, which are crucial for emulsifying recalcitrant hydrocarbons. However, a critical research gap remains: while the consortium demonstrates high efficacy in controlled laboratory settings, its performance in real-world scenarios fluctuates due to dynamic environmental temperatures that trigger the rapid crystallization of wax fractions, severely limiting substrate bioavailability.

To overcome these unresolved challenges, a rigorous future research direction framework is required. Subsequent empirical studies must transition from in vitro assays to pilot-scale field treatability studies. Future research should prioritize evaluating the efficacy of this microbial consortium within precisely controlled ex-situ landfarming designs. Furthermore, the degradation performance must be quantitatively validated through mathematical modeling, specifically utilizing kinetics analysis, to accurately predict the reduction rate of the wax fractions over time. Integrating this kinetic modeling with recent bioaugmentation optimization strategies, will provide a highly robust operational framework for scaling up this bioremediation technology in heavy oil-polluted environments.

REFERENCES

Alaidaroos, B. A. (2023). Advancing Eco-Sustainable Bioremediation for Hydrocarbon Contaminants: Challenges and Solutions. *Processes*, 11, 3036.

Atagana, H. I., Haynes, R. J., & Wallis, F. M. (2006). Fungal bioremediation of creosote-contaminated soil: A laboratory-scale bioremediation study using indigenous soil fungi. *Water, Air, & Soil Pollution*, 172(1), 201–219.

Atakpa, E. O., Hanghai, Z., Lijia, J., Yinghui, M., Yanpeng, L., Yanhong, L., Dongdong, Z., & Chunfang, Z. (2021). Improved degradation of petroleum hydrocarbons by co-culture of fungi and biosurfactant-producing bacteria. *Chemosphere*, 290.

Biktasheva, L., Gordeev, A., Usova, A., Kirichenko, A., Kuryntseva, P., & Selivanovskaya, S. (2024). Bioremediation of oil-contaminated soils using biosurfactants produced by bacteria of the genus *Nocardiopsis* sp. *Microbiology Research*, 15, 2575–2592.

Chen, J., Zhang, J., & Li, H. (2004). Determining the wax content of crude oils by using differential scanning calorimetry. *Thermochimica Acta*, 410, 23–26.

Chunyan, X., Qaria, M. A., Qi, X., & Daochen, Z. (2023). The role of microorganisms in petroleum degradation: Current development and prospects. *Science of the Total Environment*.

Correa, H. D. S., Christoper, T. B., Franklin, G., & Leila, T. M. (2022). Effects of oil contamination on plant growth and development: a review. *Environmental Science and Pollution Research*, 29(29), 43501–43515.

Das, N., Bhrigu, B., & Piyush, P. (2022). Correlation of soil microbiome with crude oil contamination drives detection of hydrocarbon degrading genes which are independent to quantity and type of contaminants. *Environmental Research*, 215(1).

Demirbas, A. (2016). Deposition and flocculation of asphaltenes from crude oils. *Petroleum Science and Technology*, 34, 6–11.

Dindar, E., Olcay, F., Sagban, T., & Baskaya, H. S. (2013). Bioremediation of petroleum-contaminated soil petrol ile kirlenmiş toprakların biyoremediasyonu. *Journal of Biological and Environmental Sciences*, 7, 39–47.

El-Ella, A., & Nassef, E. (2014). Determination of wax content in Egyptian crude oils. *International Journal of Engineering Trends and Technology*, 8, 93–97.

EPA. (2016). *Sensitivity of freshwater habitats*. Emergency Management.

Erdogan, E. E., Fikrettin, S., & Ayten, K. (2012). Determination of petroleum-degrading bacteria isolated from crude oil-contaminated soil in Turkey. *African Journal of Biotechnology*, 11(21), 4853–4859.

Espinosa-Ortiz, E. J., Rene, E. R., & Gerlach, R. (2022). Potential use of fungal-bacterial co-cultures for the removal of organic pollutants. *Critical Reviews in Biotechnology*, 42(3), 361–383.

Fei-Baffoe, B., Esther, B., Kwodwo, M., Lyndon, N. A. S., Alhassan, S., & Ebenezer, E. Y. A. (2024). Contamination of groundwater by petroleum hydrocarbons: Impact of fuel stations in residential areas. *Heliyon*, 10.

Foght, J. (2008). Anaerobic Biodegradation of Aromatic Hydrocarbons: Pathways and Prospects. *Journal of Microbiology and Biotechnology*, 15, 93–120.

Fritsche, W., & Hofrichter, M. (2000). Aerobic degradation by microorganisms. In *Environmental Processes – Soil Decontamination* (pp. 123–145). Wiley-VCH. Weinheim, Germany.

Gaur, V. K., Tripathi, V., & Manickam, N. (2022). Bacterial- and fungal-mediated biodegradation of petroleum hydrocarbons in soil. In *Microbial Degradation of Xenobiotics through Bacterial and Fungal Approach*. Elsevier, 407–427.

Ghorbannezhad, H., Hamid, M., & Seyed, M. M. D. (2021). Evaluation of pyrene and tetracosane degradation by mixed cultures of fungi and bacteria. *Journal of Hazardous Materials*, 416.

Greene, A., Wright, M., & Aldosary, H. (2016). *Bacterial diversity and metal reducing bacteria in Australian thermal environments*. Brown Walker Press.

Gu, D., Xingjia, X., Yucheng, W., Jun, Z., & Xiangui, L. (2021). Synergy between fungi and bacteria promotes polycyclic aromatic hydrocarbon cometabolism in lignin-amended soil. *Journal of Hazardous Materials*, 425, 127958.

Gudde, N. J. (2018). Adaptation of oil refineries to make modern fuels. *Proceedings of the Institution of Mechanical Engineers, Part D: Journal of Automobile Engineering*, 232(1), 5–21.

Hong, Y., & Gu, J. D. (2009). Bacterial anaerobic respiration and electron transfer relevant to the biotransformation of pollutants. *International Biodeterioration & Biodegradation*, 63, 973–980.

Hosseinipour, A., Khalik, M. S., Andhy, A. E., Azuraein, B. J., & Lukman, I. (2014). The impact of the composition of the crude oils on the wax crystallization. *Applied Mechanics and Materials*, 625, 196–200.

Ikuesan, F. A. (2017). Evaluation of crude oil biodegradation potentials of some indigenous soil microorganisms. *Journal of Scientific Research and Reports*, 1–9.

Islam, B. (2015). Petroleum sludge, its treatment and disposal: a review. *International Journal of Chemical Science*, 13(4), 1584–1602.

Kachieng'a, L., & Momba, M. N. B. (2018). Kinetics of petroleum oil biodegradation by a consortium of three protozoan isolates (*Aspidisca* sp., *Trachelophyllum* sp., and *Peranema* sp.). *Biotechnology Reports*, 15, 125–131.

Kané, M., Djabourov, M., Volle, J.-L., Lechaire, J.-P., & Frebourg, G. (2003). Morphology of paraffin crystals in waxy crude oils cooled in quiescent conditions and under flow. *Fuel*, 82, 127–135.

Kanokpan, J., Onruthai, P., & Panan, R. (2012). Degradation of polycyclic aromatic hydrocarbons by newly isolated *Curvularia* sp. F18, *Lentinus* sp. S5, and *Phanerochaete* sp. T20. *Science Asia*, 38, 147–156.

Kaushik, S., Alatawi, A., Djiwanti, S. R., Pande, A., Skotti, E., & Soni, V. (2021). Potential of extremophiles for bioremediation (Vol. 1, pp. 293–328).

Khandelwal, A., Lata, N., Shashi, B. S., Eldho, V., Anamika, S., Suman, G., & Neera, S. (2021). Bacteria and fungi mediated degradation of polyaromatic hydrocarbons and effect of surfactant Tween-80. *International Journal of Environmental Analytical Chemistry*, 104(1), 27–42.

Khanna, A., Shristi, H., Samriti, R., Ashish, S., Sanjeev, P., & Mary, C. (2023). Biosurfactant from *Candida*: sources, classification, and emerging applications. *Archives of Microbiology*, 205, 149.

Kiyingi, W., Guo, J. X., Rui, Y. X., Li, S., Xiao, H. Y., & Shi, L. Z. (2022). Crude oil wax: A review on formation, experimentation, prediction, and remediation techniques. *Petroleum Science*, 19, 2343–2357.

Lu, X., & Redelius, P. (2007). Effect of bitumen wax on asphalt mixture performance. *Construction and Building Materials*, 21, 1961–1970.

Luo, C., Guoqing, G., Yeliang, D., Xixi, C., Qihui, H., Jibing, L., & Gan, Z. (2024). Determination of soil phenanthrene degradation through a fungal–bacterial consortium. *Applied and Environmental Microbiology*, 90(6).

Mahjoubi, M., Simone, C., Yasmine, S., Atef, J., & Ameur, C. (2018). Microbial bioremediation of petroleum hydrocarbon–contaminated marine environments. In *Recent Insights in Petroleum Science and Engineering*.

Mokhatab, S., & Towler, B. (2009). Wax prevention and remediation in subsea pipelines and flowlines: deepwater technology. *World Oil*.

Musser, B. J., & Kilpatrick, P. K. (1998). Molecular characterization of wax isolated from a variety of crude oils. *Energy & Fuels*, 12, 715–725.

Naeem, U., & Qazi, M. A. (2020). Leading edges in bioremediation technologies for removal of petroleum hydrocarbons. *Environmental Science and Pollution Research*, 27(22), 27370–27382.

Olajire, A. A., & Essien, J. P. (2014). Aerobic degradation of petroleum components by microbial consortia. *Journal of Petroleum & Environmental Biotechnology*, 5, 195.

Oltulular, S. 2024. Circular behavior of oil consumption and the impact of oil prices with the Fourier approach. *Energy Strategy Reviews*, 53, 1-13.

Pandolfo, E., Barra Caracciolo, A., & Rolando, L. (2023). Recent advances in bacterial degradation of hydrocarbons. *Water*, 15, 375.

Panjiar, N., Shashwati, G. S., & Ashish, S. (2017). Biosurfactants: A multifunctional microbial metabolite. In *Microbial Applications* (Vol. 2, pp. 213–229). India.

Pinedo-González, P., West, A. J., Tovar-Sanchez, A., Duarte, C. M., & Sañudo-Wilhelmy, S. A. (2018). Concentration and isotopic composition of dissolved Pb in surface waters of the modern global ocean. *Geochimica et Cosmochimica Acta*, 235, 41–54.

Ron, E. Z., & Rosenberg, E. (2002). Biosurfactants and oil bioremediation. *Current Opinion in Biotechnology*, 13(3), 249–252.

Sakthipriya, N., Mukesh, D., & Jitendra, S. S. (2017). Enhanced microbial degradation of waxy crude oil: A review on current status and future perspective. *International Journal of Oil, Gas and Coal Technology*, 16(2).

Sandyga, M. S., Struchkov, I. A., & Rogachev, M. K. (2018). Formation damage induced by wax deposition: laboratory investigations and modeling. *Journal of Petroleum Exploration and Production Technology*.

Sanjana, M., Prajna, R., Urvi, S. K., & Kavitha, R. V. (2024). Bioremediation – the recent drift towards a sustainable environment. *Environmental Science Advances*, 3, 1097–1110.

Sen, R. (2008). Biotechnology in petroleum recovery, the microbial EOR. *Progress in Energy and Combustion Science*, 34(6), 714–724.

Silva Monteiro, J. P., da Silva, A. F., Delgado Duarte, R. T., & José Giachini, A. (2024). Exploring novel fungal–bacterial consortia for enhanced petroleum hydrocarbon degradation. *Toxics*, 12, 913.

Tormoehlen, L. M., Tekulve, K. J., & Nanagas, K. A. (2014). Hydrocarbon toxicity: A review. *Clinical Toxicology*, 52(5), 479–489.

Varjani, S. J. (2017). Microbial degradation of petroleum hydrocarbons. *Bioresource Technology*, 223.

Varjani, S. J., & Upasani, V. N. (2017). A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants. *International Biodeterioration and Biodegradation*, 120, 71–83.

Vincenzo, G., Stefano, M., & Alessandro, I. (2013). A permeability model for naturally fractured carbonate reservoirs. *Marine and Petroleum Geology*, 40, 115–134.

Vincenzo, G., Stefano, V., & Sabatino, C. (2011). Improved statistical multi-scale analysis of fractures in carbonate reservoir analogues. *Tectonophysics*, 504(1–4), 14–24.

Wen, J., Lu, Y., Jia, Y., Luo, H., You, C., Huang, Z., Wang, C., & Luo, Y. (2025). Progress on wax deposition characteristics and prediction methods for crude oil pipelines. *Processes*, 13, 1651.

Wenger, L. M., Cara, L., Davis, C. L., & Isaksen, G. H. (2002). Multiple control on petroleum biodegradation and impact on oil quality. *SPE Reservoir Evaluation & Engineering*, 5(5), 1–9.

Widdel, F., Knittel, K., & Galushko, A. (2010). Anaerobic hydrocarbon-degrading microorganisms: An overview. Springer, Berlin Heidelberg, 1997–2021.

Wu, H., Sun, B., & Li, J. (2018). Polycyclic aromatic hydrocarbons in sediments/soils of the rapidly urbanized lower reaches of the River Chaohu. *International Journal of Environmental Research and Public Health*, 16, 2302.

Wu, L., Jiajie, W., Tao, J., Xiangyuan, Z., & Liping, W. (2025). Lignin-enhanced fungal–bacterial consortium for degradation of petroleum hydrocarbon contaminants. *Journal of Environmental Management*, 388, 125973.

Yang, F., Jinyang, C., Liang, C., Chuanxian, L., Zhongyuan, J., Bo, Y., Yansong, Z., & Guangzheng, Z. (2016). Development of asphaltenes-triggered two-layer waxy oil gel deposit under laminar flow: An experimental study. *Energy & Fuels*, 30, 9922–9932.

Zhang, X., Bao, D., Li, M., Tang, Q., Wu, M., & Zhou, H. (2021). Bioremediation of petroleum hydrocarbons by alkali–salt-tolerant microbial consortia and their community profiles. *Journal of Chemical Technology and Biotechnology*, 96(3), 809–817.

Zhang, X., Dewen, K., Xiaoyan, L., Huanhuan, X., Xinyi, L., & Cheng, Z. (2021). Combined microbial degradation of crude oil under alkaline conditions by *Acinetobacter baumannii* and *Talaromyces* sp. *Chemosphere*, 273.

Zwolinski, M., Harris, R., & Hickey, W. (2000). Microbial consortia involved in the anaerobic degradation of hydrocarbons. *Biodegradation*.