

# **Microbial Bioremediation of Petroleum-Contaminated Soil: A Sustainable Approach**

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**ABSTRACT:** Petroleum-contaminated soil is a significant environmental concern caused by oil spills, leakage from storage tanks, industrial discharges, and improper disposal of petroleum products during extraction, refining, and transportation processes. Globally, approximately 6 million tonnes of petroleum are released into the environment each year, leading to soil contamination that poses toxic risks to groundwater, ecosystems, plant life, and human health. The primary aim of this paper is to evaluate the effectiveness and potential of microbial bioremediation for treating petroleum-contaminated soils, offering a sustainable alternative to conventional methods. Traditional remediation approaches such as soil excavation, washing, chemical oxidation, and incineration are often expensive and environmentally disruptive. In contrast, bioremediation using microbes is cost-effective, sustainable, and environmentally friendly. Several microbial strategies are discussed, including natural attenuation, bioaugmentation, and biostimulation. Natural attenuation relies on indigenous microbes, whereas bioaugmentation involves adding hydrocarbon-degrading microbes, and biostimulation enhances microbial activity by supplying nutrients. Among these, bioaugmentation and biostimulation are generally more effective than natural attenuation in degrading petroleum hydrocarbons. However, microbial bioremediation faces challenges such as long treatment durations, incomplete degradation with free microbes, and the need for site-specific optimal conditions. Future research should focus on enhancing microbial efficacy through genetic engineering or microbial consortia, developing faster, site-specific solutions, assessing long-term ecological impacts, and integrating bioremediation with other green technologies. Overall, microbial bioremediation presents a promising strategy for the sustainable management of petroleum-contaminated soils due to its low cost, minimal environmental impact, and adaptability. Key topics addressed include the environmental impact of petroleum pollution, conventional and biological remediation techniques,

comparative effectiveness, and future development needs. The relevant keywords are: bioremediation, petroleum hydrocarbons, bioaugmentation, soil contamination, and microbial degradation.

**KEYWORDS:** Bioremediation; petroleum hydrocarbons; bioaugmentation; soil contamination; microbial degradation

## 1. Introduction

The term "petroleum" is derived from the Latin words *petra* (meaning "rock") and *oleum* (meaning "oil"), translating to "rock oil" or "oil from the earth." It is also commonly referred to as crude oil, a naturally occurring fossil fuel extracted from underground reservoirs or found seeping at the Earth's surface. Petroleum is formed over millions of years through the decomposition of ancient marine organisms and organic matter such as bacteria, plankton, algae, and plants. These materials were buried under layers of sediment, subjected to immense heat, pressure, and geological processes that transformed them into complex hydrocarbon mixtures [1].

Petroleum plays a critical role in modern society, serving two primary purposes: as a major fuel source and as a raw material for synthesizing various organic compounds. It is the world's leading energy source, providing fuel for heating, electricity generation, and as a power source for internal combustion engines in vehicles, ships, and airplanes. Additionally, around 80% of global organic chemicals are derived from petroleum, forming the basis for essential products such as gasoline, diesel, and kerosene. Beyond fuels, petroleum is also used to manufacture asphalt, pharmaceuticals, plastics, synthetic fibers, fertilizers, pesticides, and personal care items such as clothing, perfumes, cosmetics, and soap [2].

In 2023 alone, global daily oil consumption reached approximately 100.2 million barrels, marking a 3% increase from the previous year [3]. The United States and China were the top consumers, using up to 19.1 and 14.3 million barrels per day in 2022, respectively [4]. Malaysia, although a smaller player in the global market, accounted for about 0.7% of global consumption, equivalent to 708,000 barrels per day [5]. In addition to its consumption, Malaysia was a key petroleum producer, extracting around 660,000 barrels per day [6]. This dual role in petroleum production and consumption underscored the importance of effective petroleum management and remediation strategies to ensure environmental sustainability and reduce potential ecological damage.

Petroleum is typically described as a foul-smelling, yellow-to-black liquid extracted from underground reservoirs. It consists of hydrocarbons in various forms: gaseous, liquid, and solid [2]. These hydrocarbons, over 17,000 distinct compounds varying in molecular weight and structure, are broadly classified into three main categories: alkanes (paraffins), cycloalkanes (naphthenes), and polycyclic aromatic hydrocarbons (PAHs) [7]. Alkanes, the largest group, account for approximately 90% of petroleum and include compounds such as methane, ethane, pentane, and octane, which are valuable for producing diesel and kerosene. Cycloalkanes, with their characteristic ring structures, include toluene, xylene, cyclopentane, and cyclohexane. PAHs, made up of multiple aromatic rings, include compounds like naphthalene, biphenyl, anthracene, and benzopyrene [2, 7]. Table 1 provides a summary of these hydrocarbon groups, which are critical for understanding petroleum's composition and its environmental impacts.

**Table 1.** Major hydrocarbon compounds in petroleum.

Compound Type	Description	Examples
Aromatic Hydrocarbons	Compounds with fused benzene rings	Naphthalene, Anthracene, Pyrene
Aliphatic Hydrocarbons	Non-aromatic, open-chain hydrocarbons	Alkanes, Alkenes
Heterocyclic Aromatics	Aromatic rings containing heteroatoms (N, S, O)	Quinoline (N), Dibenzothiophene (S), Furan (O)
Nitrogen-Containing PAHs (N-PAHs)	PAHs with nitrogen atoms incorporated	Quinoline, Acridine
Sulfur-Containing PAHs (S-PAHs)	PAHs with sulfur atoms	Dibenzothiophene
Oxygen-Containing PAHs (O-PAHs)	PAHs with oxygen atoms	Benzofuran

## 2. Occurrence and Fate of Petroleum-Contaminated Soil

Petroleum was among the most common soil pollutants globally due to various sources in petroleum exploitation, production, refining, transportation, and storage. The release of petroleum into the environment was caused either accidentally or by anthropogenic activities, such as petroleum spills, leakage from storage tanks, wastewater from oil extraction, industrial discharge, stacking of oily slag and sludge, vehicle exhaust emissions, petroleum exploration, improper disposal of petroleum products, and other pollution sources [8]. These activities allowed petroleum pollutants to permeate or infiltrate the soil, contaminating the environment. Studies showed that approximately 6 million tonnes of petroleum had been released into the environment worldwide, harming flora, fauna, and human health [9]. Another study reported that global petroleum leakage reached about 600,000 tons annually, contaminating 3.5 million locations in Europe [10, 11].

When petroleum entered the environment, it could be transported vertically and horizontally through soil due to rainfall, facilitating leaching and runoff. As rainwater infiltrated the soil, petroleum moved into deeper layers, potentially reaching groundwater. It could also spread horizontally across the soil surface, particularly in sloped or porous soils. Petroleum flowed through the soil matrix as a contact front, advancing when the soil was moist or wet [12, 13]. Due to its high hydrophobicity, petroleum was absorbed by soil particles, displacing water molecules and reducing oxygen and water infiltration in contaminated soil. Petroleum hydrocarbons had strong sorption and affinity for soil organic matter [14, 15]. Generally, hydrocarbons with lower molecular weights were more volatile and penetrated groundwater more easily than heavier hydrocarbons. However, their permeability and volatilization depended on soil properties such as hydraulic conductivity, compaction, vegetation, and climate conditions [10, 16].

## 3. Impact of Petroleum

Petroleum, which contained numerous toxic substances such as alkanes, cycloalkanes, and PAHs, posed risks to ecosystems and human health by polluting soil and groundwater. Soil contamination by petroleum led to long-term environmental issues, including soil degradation and groundwater pollution, which harmed soil quality, plant life, and human health. Petroleum degraded the physical and chemical properties of soil, as well as its ecological structure and function, altering hydrophobicity, moisture content, pH levels, total organic carbon, nitrogen and potassium content, and enzyme activity (catalase, dehydrogenase, and urease) [12, 17].

One negative effect of petroleum contamination was a change in soil color from dark brown to gray, which reduced light reflectivity and increased soil heating [18]. The hydrophobic coating from high-molecular-weight petroleum substances impaired the soil's ability to absorb and retain moisture. This resulted in significant losses in water conductivity and capacity, further increasing soil hydrophobicity. Consequently, the outer contaminated soil layer dried out, while the inner uncontaminated layers retained excessive moisture, disrupting air and water conditions and affecting anaerobic processes [19, 20].

Poor water conditions decreased nutrient solubility and availability for plants, inhibiting nitrification and ammonification. Additionally, petroleum could increase soil pH when sodium ions entered the soil sorption complex, displacing pH-balancing ions—a phenomenon often observed near petroleum-contaminated water sources [21, 22].

### *3.1. Effects on plants.*

Petroleum also had toxic effects on plants. As contamination increased, soil porosity and permeability decreased, while hydrophobicity rose, inhibiting root growth. A study showed that at a petroleum concentration of 7,791 mg/kg, the root length of *Sinapis alba*, *Sorghum saccharatum*, and *Lepidium sativum* decreased by 42.3%, 47.3%, and 65.1%, respectively [23]. Petroleum contaminants could penetrate plant surfaces and spread through intracellular spaces and vascular systems, reaching roots, leaves, and fruits. This stunted plant growth, reducing stem length, plant height, leaf area, and aboveground tissue length due to nutrient and oxygen deficiencies in contaminated soil [24, 25].

### *3.2. Human health risks.*

Exposure to petroleum whether through inhalation, skin contact, or ingestion of contaminated air, water, or food, posed serious health risks. Many petroleum components, such as benzene and PAHs, were toxic, carcinogenic, and mutagenic, harming kidney and liver function and increasing cancer risk [26]. Prolonged exposure to petroleum-contaminated environments could also cause fatigue, respiratory problems, headaches, eye irritation, and a higher risk of miscarriages in women [27].

## **4. Remediation**

Various remediation methods or soil clean-up strategies consisted of physical, chemical, thermal, and biological methods. The available and commonly used remediation methods are summarised in Table 1. In physical remediation, excavating petroleum-contaminated soil had been the fastest and simplest method to remove petroleum pollutants from a contaminated site. Typically, the contaminated soil was excavated, transported, and disposed of in appropriate landfills. While excavation provided an immediate solution for removing contaminants, it carried significant drawbacks. The primary disadvantage of excavation was its high cost, including expenses for transporting and disposing of the contaminated soil, as well as the cost of acquiring clean material to backfill the excavated area. Moreover, the long-term viability of the disposed material in landfills raised concerns about potential secondary pollution [7, 28].

Another physical method, soil washing, involved flushing petroleum-contaminated soil with organic solvents such as ethanol-water or ethyl acetate-acetone-water mixtures to extract hydrocarbons. This method had been simple in principle and demonstrated high effectiveness

in hydrocarbon removal. However, it was often costly and time-consuming, requiring careful handling of the wash fluids and the need for further treatment of the wash effluent to prevent secondary contamination [29, 30].

**Table 1.** Remediation methods for petroleum-contaminated soil.

Remediation Method	Advantages	Disadvantages	References
<b>Physical Methods:</b>			
Excavation	<ul style="list-style-type: none"> <li>• Fast and immediate removal of highly contaminated soil.</li> <li>• Suitable for sites with severe contamination where in-situ methods are impractical.</li> <li>• Can be combined with off-site treatment.</li> </ul>	<ul style="list-style-type: none"> <li>• Environmentally disruptive, removes large volumes of soil, affecting ecosystems.</li> <li>• Requires secure disposal or treatment at landfills.</li> <li>• High cost for excavation, transportation, and disposal.</li> <li>• Limited sustainability due to reliance on landfill capacity.</li> </ul>	[7, 28]
Soil Washing	<ul style="list-style-type: none"> <li>• Effective for reducing contaminant concentrations in soils with coarse particles.</li> <li>• Can recover and reuse valuable materials in some cases.</li> <li>• Relatively simple in concept and operation.</li> </ul>	<ul style="list-style-type: none"> <li>• High cost due to equipment and chemical use.</li> <li>• Less effective for clay-rich or fine-textured soils.</li> <li>• Time-consuming process.</li> <li>• Generates contaminated wash water requiring further treatment.</li> </ul>	[29, 30]
<b>Chemical Methods:</b>			
Chemical Oxidation	<ul style="list-style-type: none"> <li>• Rapid degradation of a wide range of organic contaminants.</li> <li>• Can achieve significant contaminant removal in a short time.</li> <li>• Applicable for both in-situ and ex-situ treatments.</li> </ul>	<ul style="list-style-type: none"> <li>• High cost of chemical reagents</li> <li>• Risk of secondary pollution from excess chemicals and by-products.</li> <li>• May lead to incomplete degradation if conditions are not optimal.</li> <li>• Potential for leaching into groundwater.</li> </ul>	[12, 31, 32]
<b>Thermal Methods:</b>			
Incineration	<ul style="list-style-type: none"> <li>• Complete destruction of organic contaminants.</li> <li>• Suitable for diverse and complex pollutant mixtures.</li> <li>• Reduces volume of waste.</li> </ul>	<ul style="list-style-type: none"> <li>• Extremely high energy demand and operational costs.</li> <li>• Can release toxic gases if combustion is incomplete.</li> <li>• Requires air pollution control systems to minimize emissions.</li> <li>• Not suitable for in-situ application.</li> </ul>	[33, 34, 35]
<b>Biological Methods:</b>			
Bioremediation	<ul style="list-style-type: none"> <li>• Environmentally friendly and sustainable.</li> <li>• Cost-effective, especially for large or low-concentration contamination sites.</li> <li>• Can access micropores and soil aggregates where contaminants are trapped.</li> <li>• Minimal site disruption compared to physical methods.</li> </ul>	<ul style="list-style-type: none"> <li>• Slow process, may take months or years.</li> <li>• Sensitive to environmental factors (temperature, pH, nutrients, oxygen).</li> <li>• Limited effectiveness for high contaminant concentrations.</li> <li>• May not fully degrade all petroleum fractions.</li> </ul>	[36, 37]

Despite the numerous advantages of bioremediation, significant challenges remained in its practical application for treating petroleum contaminants in soil. One major limitation of microbial bioremediation was the relatively long remediation periods required to achieve substantial pollutant degradation. Unlike physical methods such as excavation or thermal incineration, which could rapidly remove contaminants, bioremediation relied on the metabolic activity of microorganisms to break down hydrocarbons, a process that was inherently slower. The degradation rates of free, or planktonic, microorganisms in contaminated soils were often low, which limited the overall treatment efficacy [12]. For example, a study investigating

microbial degradation of petroleum hydrocarbons found that microorganisms could only destroy up to 62% of the contaminants over a 150-day period [38]. This slow pace made bioremediation unsuitable for situations demanding quick cleanup, such as emergency spill responses.

Furthermore, the effectiveness of bioremediation using free microorganisms could be significantly enhanced when microbes were immobilized or supported by carriers like biochar. In one experiment, free microorganisms alone degraded a relatively low 2.3 to 6.8% of petroleum hydrocarbons. However, when biochar was introduced as a carrier to support microbial colonization and activity, the degradation rate improved dramatically, increasing from 7.2% to 30.3% [39]. This enhancement occurred because biochar provided a favorable habitat for microbes, increasing their survival and metabolic efficiency. Such carriers also helped in retaining moisture and nutrients, making the microenvironment more conducive for biodegradation.

Another critical challenge affecting microbial bioremediation was its strong dependency on site-specific environmental conditions. Factors such as temperature, pH, soil moisture, nutrient availability, and oxygen content played pivotal roles in determining the success of bioremediation efforts. Microbial metabolism and enzymatic activity are highly sensitive to these factors, and any deviation from optimal conditions could slow down or even halt the degradation process. For example, low soil temperatures below 10°C were shown to inhibit microbial activity, leading to markedly slower hydrocarbon degradation [40]. Similarly, soil pH influenced microbial community structure and enzyme function. While many hydrocarbon-degrading microbes thrived in a pH range of approximately 6 to 8, more acidic or alkaline conditions (pH below 4 or above 9) adversely affected microbial populations and their degradation capabilities [41]. Soil moisture also needed to be maintained within an optimal range to support microbial activity; excessive dryness or waterlogging could both negatively impact biodegradation rates.

In addition, oxygen availability was essential for aerobic microbial degradation of petroleum hydrocarbons. Oxygen acted as a terminal electron acceptor in many biodegradation pathways, and limited oxygen levels in soil could restrict microbial respiration and slow hydrocarbon breakdown. Conversely, anaerobic conditions often required specialized microorganisms and longer degradation times. The balance of nutrients such as nitrogen and phosphorus was also crucial since microbial growth and enzyme production depended on adequate nutrient supply. Nutrient deficiencies in contaminated soils often limited biodegradation efficiency, necessitating nutrient amendment strategies as part of biostimulation to enhance microbial activity.

Despite the advantages of bioremediation, there are still challenges in its application for treating petroleum contaminants in soil. Bioremediation using microbes has drawbacks, including long remediation periods and low treatment efficacy of free microorganisms [12]. Bioremediation using microbes is time-consuming due to its slow degradation rates, so it could not offer immediate removal like excavation or incineration. Study shows microorganisms destroyed up to 62% of petroleum hydrocarbons in the soil in a 150-day experiment [38]. In another study, free microorganisms successfully degraded 2.3 to 6.8% of petroleum hydrocarbons, but the degradation rate increased from 7.2% to 30.3% when biochar was used as a carrier [39]. Furthermore, the performance of the bioremediation using microbes is highly dependent on site-specific conditions such as temperature, pH level, soil moisture, and nutrient

and oxygen content. Extreme environmental conditions, including soil temperature under 10°C and a pH range of 4 to 9, may inhibit microbial activities, slowing down the degradation of hydrocarbons [40, 41].

#### *4.1. Natural attenuation.*

Natural attenuation was the simplest form of bioremediation using microbes. It relied on indigenous microbes and natural conditions to perform natural degradation processes. This method was simple, cost-effective, and required low maintenance, as minimal to no active intervention was needed for the natural microbial degradation of petroleum contaminants. However, natural attenuation was not always effective, and continuous monitoring was required to ensure microbial degradation was progressing as expected and pollutant levels were reducing [15]. Typically, this method was employed to treat sites with low contamination levels [42]. According to Stroud et al. about 25% of all petroleum-polluted sites were treated using the natural attenuation method [43]. Natural attenuation was reported to remove up to 57% of total petroleum hydrocarbons (TPH) in PAH-contaminated soil [44]. In another study, petroleum hydrocarbon-polluted soil that had undergone prior treatment showed a 70% improvement in trinitrotoluene removal compared to the control set, indicating that prior hydrocarbon exposure may have increased microbial activity in the soil and the capacity to decompose other pollutants [45].

#### *4.2. Bioaugmentation.*

Bioaugmentation was an enhanced bioremediation method that introduced either indigenous or exogenous metabolically active microbes into petroleum-contaminated soil. This method was normally used when natural degrading microbes were insufficient or their metabolic activity was low, and it was suitable for remediating sites with high petroleum contamination levels [46, 47]. Typically, in the bioaugmentation process, native or indigenous microbes were employed to ensure the organisms were more resilient to local environmental changes and had greater tolerance to the toxicity of petroleum pollutants such as PAHs [48]. In contrast, exogenous microbes were helpful when dealing with more complex petroleum hydrocarbon structures, which caused biodegradation rates to be lower than those of simple hydrocarbons. Therefore, introducing microbes with specific metabolic activity improved the effectiveness of microbial remediation of contaminated soil [49]. Normally, microbes were chosen for bioaugmentation due to their high tolerance against contaminant toxicity, low cost, ease of culture, and fast growth [50, 51].

Bioaugmentation remediation proved to be more effective than other bioremediation approaches. In one study of diesel-polluted soil, representing light petroleum hydrocarbons with carbon chains from C12 to C23 (2800 mg/kg), the degradation rate in the bioaugmentation process was as high as 75.20%, whereas natural attenuation treatment exhibited only 48.70% degradation. Bioaugmentation also showed high remediation performance in treating heavy petroleum hydrocarbon fractions from C23 to C40 (9450 mg/kg); bioaugmented remediation exhibited 72.70% hydrocarbon removal using Long Beach soil, while biostimulation remediation demonstrated only 45.70% hydrocarbon removal [52]. Numerous studies on bioaugmentation of petroleum carbon have showcased successful reductions in total petroleum hydrocarbons (TPH), as summarized in Table 2. These case studies demonstrated that introducing indigenous or exogenous metabolically active microbes into petroleum-

contaminated soil effectively enhanced petroleum contaminant biodegradation and reduced degradation time.

**Table 2.** Case studies of bioaugmentation for petroleum hydrocarbon-contaminated soil.

Contaminated Soil Type	Microbes Used	TPH Removal Rate (% in Days)	Key Findings	References
Diesel	<i>P. aeruginosa</i>	66% in 30 days	Bioaugmentation improved diesel degradation to 66% after 30 days.	[53]
Crude oil	<i>Acinetobacter baumannii</i> T30C	43% in 35 days	Limited improvement; not significantly better than control.	[54]
Oil refinery waste	Bacteria consortium	96.51% in 10 months	TPH reduced from 83.5–531.3 g/kg to below 10 g/kg in 2–12 months.	[55]
Oil sludge	Bacteria consortium	76% in 90 days	TPH removal improved to 76% after bioaugmentation.	[56]
Oil sludge	<i>Rhodococcus erythropolis</i> (Rhedor)	52.75% in 160 days	TPH removal increased from 15.46% to 52.75%.	[57]
Petroleum-contaminated soil	<i>Acinetobacter</i> SZ-1 strain KF453955	34% in 70 days	TPH removal improved after bioaugmentation for 6 weeks.	[58]
TOH and PAHs contaminated soil	Bacteria consortium	69% in 195 days	TPH removal increased from 10–32% (control) to 69% with bioaugmentation.	[59]

#### 4.3. Biostimulation.

Biostimulation was one of the most commonly used and environmentally friendly techniques for microbial degradation of petroleum-contaminated soil. In the biostimulation process, nutrients such as carbon, nitrogen, phosphorus, oxygen, and organic biostimulants were added to stimulate microbial growth and metabolic activities, enhancing the native microorganism populations and their capability to mineralize organic contaminants [15, 51]. Since petroleum mainly consisted of carbon and hydrogen, contamination of soil often caused an imbalance in the carbon-nitrogen-phosphorus (C:N:P) ratio, which inhibited most bioremediation processes of indigenous microbes [37]. Therefore, the addition of nutrients such as inorganic fertilizers rich in nitrogen and phosphorus or organic materials, including animal manure, biochar, and agricultural wastes, improved soil properties and enhanced the biodegradation ability of the microbes [60]. However, it was critical to add appropriate amounts of nutrients during biostimulation, as over-fertilization could result in soil eutrophication and ammonia toxicity [61]. The optimal C:N:P ratio for biostimulation of petroleum-contaminated soil was suggested to be 100:5:1 but needed re-examination based on different petroleum-contaminated sites and environmental conditions [62].

One main advantage of biostimulation was that it utilized existing indigenous microbes already adapted to the subsurface environment and well-dispersed across the subsurface. This approach was more cost-effective and time-saving than bioaugmentation, since culturing and introducing foreign microbes was unnecessary [63]. However, biostimulation also faced challenges in delivering nutrients to subsurface microbes. The success of nutrient accessibility depended significantly on local geology, where tight and impermeable subsurface formations such as clays and fine-grained soils could prohibit effective nutrient distribution across contaminated areas [63]. Another challenge was that nutrient addition might promote the growth of microbes not native to petroleum degradation, resulting in competition with indigenous petroleum-degrading microbes [64–66]. Many other case studies summarized in



Table 3 further supported the high effectiveness of biostimulation in biodegrading petroleum-contaminated soil.

**Table 3.** Case studies of biostimulation of petroleum hydrocarbon-contaminated soil.

Contaminated Soil Type	Nutrients Added for Biostimulation	TPH Removal Rate (% in Duration)	Key Findings	References
5% and 15% lubricating oil	10% brewery spent grain (BSG); banana skin (BS); spent mushroom compost (SMC)	5% oil: BSG 92%, BS 84%, SMC 79% in 84 days; 15% oil: BSG 55%, BS 49%, SMC 36% in 84 days	BSG showed the highest TPH removal, followed by BS and SMC.	[67]
Crude oil	Sugar cane bagasse; oil palm empty fruit bunch	100% (sugarcane); 97% (palm) in 20 days	Both substrates stimulated microbial growth (bacteria counts increased to 10.3 CFU/g and 9.5 CFU/g).	[68]
Total petroleum hydrocarbon (TPH)	Cow dung	69.85% in 70 days	TPH reduced from 12,419.89 mg/kg to 3,743.98 mg/kg over 70 days.	[69]
Diesel fuel	Tea leaf (TL); potato skin (PS); soy cake (SC)	SC 82%, PS and TL lower, in 126 days	Soy cake showed the highest TPH removal rate; order of effectiveness: SC > PS > TL.	[70]
Total petroleum hydrocarbon (TPH)	Compost from wood chips and sewage sludge	100% in 19 months	TPH removal increased from 17% to 100% using compost.	[71]

Biostimulation has consistently shown strong potential in enhancing the degradation of total petroleum hydrocarbons (TPHs) in contaminated soils by stimulating native microbial communities through nutrient enrichment. In a study focusing on the bioremediation of hydrocarbon-contaminated burned woodland soil, three approaches were compared: biostimulation using a commercial microbial growth-promoting formulation, bioaugmentation with *Trichoderma* sp. mycelium, and natural attenuation. Among these, biostimulation achieved the highest TPH removal rate of 70% within 60 days, outperforming bioaugmentation (55%) and natural attenuation (45%) [65]. This highlights the critical role of nutrient supplementation in accelerating microbial degradation. Another investigation on diesel-contaminated arable soil further supported the effectiveness of biostimulation. Organic manure application led to a remarkable TPH reduction of 93.31%, followed by inorganic NPK fertilizer at 71.40%, while natural attenuation lagged behind at 57.9% [66]. These findings underline that biostimulation, especially when utilizing organic amendments, significantly improves remediation outcomes. Organic waste materials like cow dung, banana peels, sugarcane bagasse, and soy cake have been effectively used as cost-efficient nutrient sources in various studies. These amendments not only provide essential nutrients but also support microbial proliferation and enzymatic activity, promoting eco-friendly and sustainable remediation. Compared to bioaugmentation, biostimulation is often more accessible and economical, particularly in regions where indigenous microbial populations can be effectively activated. Table 4 summarizes and compares the efficiency of biostimulation, bioaugmentation, and natural attenuation, demonstrating the superior performance and practicality of biostimulation in diverse soil conditions affected by petroleum hydrocarbons.

**Table 4.** Summary of remediation technologies using microbes for petroleum-contaminated soil.

Remediation Technology	Mechanism	Pros	Cons	References
<b>Natural Attenuation</b>	Relies on indigenous (native) microbes and natural environmental conditions (oxygen, moisture, nutrients) to gradually degrade petroleum hydrocarbons without human intervention.	<ul style="list-style-type: none"> <li>• Minimal intervention required</li> <li>• Low cost, environmentally friendly</li> <li>• No need for external microbial inoculation</li> <li>• Suitable for low-contaminant sites</li> </ul>	<ul style="list-style-type: none"> <li>• Slow process; may take years to decades</li> <li>• Ineffective for sites with high contamination or poor nutrient conditions</li> <li>• Requires regular monitoring to track progress</li> <li>• Potential accumulation of intermediate toxic byproducts</li> </ul>	[15, 42]
<b>Bioaugmentation</b>	Involves introducing specific hydrocarbon-degrading microbes (often isolated from petroleum-polluted environments) into contaminated soil to enhance degradation rates. These microbes possess specialized enzymes for breaking down hydrocarbons.	<ul style="list-style-type: none"> <li>• Accelerates degradation of specific hydrocarbons</li> <li>• Effective for sites with high contamination or poor native microbial populations</li> <li>• Can tailor microbes for particular pollutants</li> </ul>	<ul style="list-style-type: none"> <li>• Higher cost due to microbial culture production and transport</li> <li>• May disrupt native microbial communities</li> <li>• Risk of poor survival or activity of introduced microbes</li> <li>• Repeated application may be necessary; potential ecological risks</li> </ul>	[46–49]
<b>Biostimulation</b>	Involves adding limiting nutrients (typically nitrogen, phosphorus, or organic matter like compost, manure, or agricultural waste) to stimulate the growth and activity of native hydrocarbon-degrading microbes.	<ul style="list-style-type: none"> <li>• Cost-effective compared to bioaugmentation</li> <li>• Enhances native microbial populations, minimizing ecological disturbance</li> <li>• Effective for large-scale applications</li> <li>• Promotes in-situ degradation under natural conditions</li> </ul>	<ul style="list-style-type: none"> <li>• Risk of promoting non-target microbes</li> <li>• Nutrients may not be evenly distributed; accessibility for microbes can be limited</li> <li>• Excess nutrients can cause eutrophication, secondary pollution, or toxicity</li> <li>• Requires monitoring and balancing of nutrient levels</li> </ul>	[61–64]

## 5. Challenges and Prospects

As there was increasing demand for sustainable and low-cost remediation technologies, bioremediation methods using microbes such as natural attenuation, bioaugmentation, and biostimulation showed significant potential as effective treatments for petroleum-contaminated soil, potentially replacing conventional remediation technologies. However, many challenges remained to be addressed through further research. Firstly, more studies were needed to deepen knowledge and understanding of the biodegradation mechanisms of microbes, including their physio-chemical properties and behavior, interactions with petroleum hydrocarbon contaminants, and the properties of contaminated soils. Research on microbial pathways, as well as synergistic and antagonistic interactions with contaminants, remained inadequate [72]. This understanding was crucial for selecting and implementing suitable bioremediation methods, which depended on various factors such as microbial characteristics, contaminant type and concentration, site conditions, remediation time, pollutant removal standards, space constraints, monitoring challenges, geohydrologic location, risk assessment, cost-benefit ratio, life cycle analysis, and more. All these factors influenced the biodegradation process, effectiveness, efficiency, and suitability of the remediation strategy [73–75]. Therefore, the optimal bioremediation method—whether natural attenuation, bioaugmentation, or

biostimulation, needed to be carefully selected based on site-specific conditions, advantages and disadvantages, cost, and feasibility [76].

Moreover, bioremediation of petroleum-contaminated soil using microbes had drawbacks such as slow processing times, regular monitoring and maintenance requirements, and incomplete degradation [77]. Additionally, the mineralization process of petroleum contaminants sometimes altered soil pH, potentially destabilizing contaminant residues. Consequently, further research was necessary to address microbial limitations through approaches such as genetic modification and detailed investigation of microbial metabolic pathways and dynamics, aiming to enhance microbes' petroleum hydrocarbon-degrading capabilities [72]. Combining two or more remediation technologies could also improve treatment efficiency for petroleum-contaminated soil. Furthermore, nanotechnology such as the use of nanoparticles to improve microbial access to hydrocarbons or deliver nutrients to contaminated subsurface zones, showed promise for future implementation.

Many studies demonstrated high bioremediation performance by microbes in restoring soil at laboratory and field scales. Despite these promising findings, no single method had yet proven adaptable to all environmental conditions for real-world treatment of petroleum hydrocarbons. Therefore, more attention was required on evaluating the effectiveness of bioremediation for in-situ remediation to enable broader application across diverse environments [75].

The future of microbial bioremediation held promising prospects for both the environment and communities. As interest in sustainable remediation continued to rise, bioremediation using microbes played a significant role in restoring petroleum-contaminated soils, improving soil and groundwater quality, and minimizing the long-term environmental impacts of petroleum pollution. Academia, industry stakeholders, and government agencies all bore responsibility for advancing sustainable microbial remediation and overcoming existing challenges. By addressing these issues, bioremediation could not only improve treatment of petroleum-contaminated soil but also contribute to a safer, healthier environment and community by recovering contaminated sites sustainably and cost-effectively [75].

## 6. Conclusion

Petroleum was the world's most common soil pollutant, resulting from human activities such as spills or leaks from storage tanks, industrial discharge, accumulation of oily slag and sludge, petroleum exploration, and improper disposal of petroleum products. Approximately 6 million tonnes of petroleum were released into the global environment annually, contaminating soil and infiltrating groundwater, thus posing toxic risks to the environment, plants, and human health. Consequently, remediation of petroleum-contaminated soil was critical to protecting plants and humans from hydrocarbon toxicity, preventing further pollution and environmental harm. Remediation methods included physical approaches such as excavation and washing of contaminated soil, chemical oxidation, incineration of petroleum pollutants, and bioremediation using microbes. Conventional methods often had drawbacks, including high operational costs and potential environmental disruption. In contrast, bioremediation using microbes was promising due to its cost-effectiveness, sustainability, and environmental friendliness. However, it also faced limitations such as lengthy remediation times, incomplete treatment efficacy when using free microbes, and the need for optimal site-specific conditions to perform effectively. Common microbial bioremediation techniques included natural

attenuation, bioaugmentation, and biostimulation. Bioaugmentation (adding microbes) and biostimulation (adding nutrients) generally achieved higher petroleum hydrocarbon degradation rates than natural attenuation. Challenges in microbial bioremediation mainly arose from limited understanding of the biodegradation mechanisms, which depended on multiple environmental and biological factors. Therefore, further research was essential to identify and optimize the appropriate bioremediation approach for different conditions.

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## Competing Interests

The authors declare no competing interests.

## Data Availability

The datasets generated and/or analyzed during the current study are available from the author upon reasonable request.

## Author Contributions

Ahmad Rizal Roslan Nordin contributed to conceptualization, methodology, investigation, original draft writing, and supervision. Ariela Rose Navarro was responsible for data curation, visualization, and review and editing of the manuscript. Juan Carlos Reyes handled formal analysis, validation, and review and editing. S. Maragathavalli contributed resources, conducted the literature review, and participated in review and editing. Risky Ayu Kristanti worked on methodology, data curation, and provided resources. Retno Wulandari oversaw supervision and project administration, and contributed to review and editing. Seng Bunrith supported funding acquisition, supervision, and review and editing. All authors have read and approved the final manuscript.

## References

- [1] Edo, G.I.; Okoro, O.E.; Nwosu, A.C.; Udo, N. (2024). Petroleum discovery, utilization and processing in the world and Nigeria: a comprehensive literature review. *Sustainable Chemistry and Engineering*, 2024. <http://doi.org/10.37256/sce.5120243970>.
- [2] Eneh, O.C. (2011). A review on petroleum: source, uses, processing, products and the environment. *Journal of Applied Sciences*, 11, 2084–2091. <http://doi.org/10.3923/jas.2011.2084.2091>.
- [3] Global oil consumption 2023. (accessed Oct. 22, 2024) Available online: <https://www.statista.com/statistics/265239/global-oil-consumption-in-barrels-per-day/>.
- [4] OECD oil demand breakdown by sector 2022. (accessed Oct. 22, 2024) Available online: <https://www.statista.com/statistics/307194/top-oil-consuming-sectors-worldwide/>.

- [5] Malaysia oil reserves, production and consumption statistics. (accessed Oct. 22, 2024) Available online: <https://www.worldometers.info/oil/malaysia-oil/>.
- [6] Production. (accessed Oct. 22, 2024) Available online: <https://www.petronas.com/mpm/malaysia-e-p/production>.
- [7] Xuezhai, D. (2020). Remediation methods of crude oil contaminated soil. *World Journal of Agricultural Soil Science*, 4, 000595. <http://doi.org/10.33552/wjass.2020.04.000595>.
- [8] Fanaei, F.; Moussavi, G.; Shekoohiyan, S. (2020). Enhanced treatment of the oil-contaminated soil using biosurfactant-assisted washing operation combined with H<sub>2</sub>O<sub>2</sub>-stimulated biotreatment of the effluent. *Journal of Environmental Management*, 271, 110941. <http://doi.org/10.1016/j.jenvman.2020.110941>.
- [9] Refugio, X.M. (2016). Microorganisms metabolism during bioremediation of oil contaminated soils. *Journal of Bioremediation & Biodegradation*, 7, 340. <http://doi.org/10.4172/2155-6199.1000340>.
- [10] Adipah, S. (2018). Introduction of petroleum hydrocarbons contaminants and its human effects. *Journal of Environmental Science and Public Health*, 3, 43. <http://doi.org/10.26502/jesph.96120043>.
- [11] Elgazali, A.; Althalb, H.; Elmusrati, I.; Ahmed, H.M.; Banat, I.M. (2023). Remediation Approaches to Reduce Hydrocarbon Contamination in Petroleum-Polluted Soil. *Microorganisms*, 11, 2577. <https://doi.org/10.3390/microorganisms11102577>.
- [12] Sui, X.; Wang, X.; Li, Y.; Ji, H. (2021). Remediation of petroleum-contaminated soils with microbial and microbial combined methods: advances, mechanisms and challenges. *Sustainability*, 13, 9267. <http://doi.org/10.3390/su13169267>.
- [13] Ra, T.; Zhao, Y.; Zheng, M. (2019). Comparative study on the petroleum crude oil degradation potential of microbes from petroleum-contaminated soil and non-contaminated soil. *International Journal of Environmental Science and Technology*, 16, 7083–7096. <http://doi.org/10.1007/s13762-018-2114-z>.
- [14] Sakshi; Singh, S.K.; Haritash, A.K. (2019). Polycyclic aromatic hydrocarbons: soil pollution and remediation. *International Journal of Environmental Science and Technology*, 16, 6393–6413. <http://doi.org/10.1007/s13762-019-02414-3>.
- [15] Daâssi, D.; Qabil Almaghribi, F. (2022). Petroleum-contaminated soil: environmental occurrence and remediation strategies. *3 Biotech*, 12, 198. <http://doi.org/10.1007/s13205-022-03198-z>.
- [16] Zahermand, S.; Vafaeian, M.; Bazyar, M.H. (2020). Analysis of the physical and chemical properties of soil contaminated with oil (petroleum) hydrocarbons. *Earth Science Research Journal*, 24, 138–147. <http://doi.org/10.15446/esrj.v24n2.76217>.
- [17] Stepanova, A.Y.; Gladkov, E.A.; Osipova, E.S.; Gladkova, O.V.; Tereshonok, D.V. (2022). Bioremediation of soil from petroleum contamination. *Processes*, 10, 1224. <http://doi.org/10.3390/pr10061224>.
- [18] Salimnezhad, A.; Soltani-Jigheh, H.; Soorki, A.A. (2021). Effects of oil contamination and bioremediation on geotechnical properties of highly plastic clayey soil. *Journal of Rock Mechanics and Geotechnical Engineering*, 13, 618–627. <http://doi.org/10.1016/j.jrmge.2020.11.011>.
- [19] Hewelke, E.; Szatyłowicz, J.; Hewelke, P.; Gnatowski, T.; Aghalarov, R. (2018). The impact of diesel oil pollution on the hydrophobicity and CO<sub>2</sub> efflux of forest soils. *Water, Air, & Soil Pollution*, 229, 72. <http://doi.org/10.1007/s11270-018-3720-6>.
- [20] Wang, L.; Cheng, Y.; Naidu, R.; Bowman, M. (2021). The key factors for the fate and transport of petroleum hydrocarbons in soil with related in/ex situ measurement methods: an overview. *Frontiers in Environmental Science*, 9, 756404. <http://doi.org/10.3389/fenvs.2021.756404>.
- [21] Klamerus-Iwan, A.; Błońska, E.; Lasota, J.; Kalandyk, A.; Waligórski, P. (2015). Influence of oil contamination on physical and biological properties of forest soil after chainsaw use. *Water, Air, & Soil Pollution*, 226, 299. <http://doi.org/10.1007/s11270-015-2649-2>.

- [22] Camenzuli, D.; Freidman, B.L. (2015). On-site and in situ remediation technologies applicable to petroleum hydrocarbon contaminated sites in the Antarctic and Arctic. *Polar Research*, 34, 24492. <http://doi.org/10.3402/polar.v34.24492>.
- [23] Steliga, T.; Kluk, D. (2020). Application of *Festuca arundinacea* in phytoremediation of soils contaminated with Pb, Ni, Cd and petroleum hydrocarbons. *Ecotoxicology and Environmental Safety*, 194, 110409. <http://doi.org/10.1016/j.ecoenv.2020.110409>.
- [24] Lorestani, B.; Ghasemi, M.; Cheraghi, M.; Yousefi, N. (2012). Survey the effect of oil pollution on morphological characteristics in *Faba Vulgaris* and *Vicia Ervilia*. *Journal of Chemical Health Risks*, 2, 5–8.
- [25] Uzoho, B.; Oti, N.; Onweremadu, E. (2006). Effect of crude oil pollution on maize growth and soil properties in Ihiagwa, Imo State, Nigeria. *International Journal of Agriculture and Rural Development*, 5, 1–7. <http://doi.org/10.4314/ijard.v5i1.2568>.
- [26] Kuppusamy, S.; Maddela, N.R.; Megharaj, M.; Venkateswarlu, K. (2020). Impact of total petroleum hydrocarbons on human health. In *Total Petroleum Hydrocarbons*. [http://doi.org/10.1007/978-3-030-24035-6\\_6](http://doi.org/10.1007/978-3-030-24035-6_6).
- [27] O’Callaghan-Gordo, C.; Orta-Martínez, M.; Kogevinas, M. (2016). Health effects of non-occupational exposure to oil extraction. *Environmental Health: A Global Access Science Source*, 15, 39. <http://doi.org/10.1186/s12940-016-0140-1>.
- [28] Treatment of petroleum contaminated soils in cold, wet, remote regions. (accessed Oct. 22, 2024) Available online: [https://www.fs.usda.gov/t-d/pubs/pdfpubs/pdf02712801/pdf02712801\\_72dpi.pdf](https://www.fs.usda.gov/t-d/pubs/pdfpubs/pdf02712801/pdf02712801_72dpi.pdf).
- [29] Khodadoust, A.P.; Suidan, M.T.; Acheson, C.M.; Brenner, R.C. (1999). Solvent extraction of pentachlorophenol from contaminated soils using water-ethanol mixtures. *Chemosphere*, 38, 2433–2445. [http://doi.org/10.1016/S0045-6535\(98\)00458-5](http://doi.org/10.1016/S0045-6535(98)00458-5).
- [30] Silva, A.; Delerue-Matos, C.; Fiúza, A. (2005). Use of solvent extraction to remediate soils contaminated with hydrocarbons. *Journal of Hazardous Materials*, 124, 223–228. <http://doi.org/10.1016/j.jhazmat.2005.05.022>.
- [31] Goi, A.; Kulik, N.; Trapido, M. (2006). Combined chemical and biological treatment of oil contaminated soil. *Chemosphere*, 63(10). <http://doi.org/10.1016/j.chemosphere.2005.09.023>.
- [32] Kwan, W.P.; Voelker, B.M. (2003). Rates of hydroxyl radical generation and organic compound oxidation in mineral-catalyzed Fenton-like systems. *Environmental Science & Technology*, 37(6). <http://doi.org/10.1021/es020874g>.
- [33] Brejea, R.; Boroş, M.; Roşca, S.; Traian, J.E.; Budău, R.; Borza, I.M.; Păcurar, I. (2023). Bioremediation of Oil Contaminated Soil and Restoration of Land Historically Polluted with Oil Products in the Agricultural Circuit in the Plain and Western Hills, Romania. *Applied Sciences*, 13, 10245. <https://doi.org/10.3390/app131810245>.
- [34] Giannopoulos, D.; Kolaitis, D.I.; Togkalidou, A.; Skevis, G.; Founti, M.A. (2007). Quantification of emissions from the co-incineration of cutting oil emulsions in cement plants - Part I: NO<sub>x</sub>, CO and VOC. *Fuel*, 86(7–8). <http://doi.org/10.1016/j.fuel.2006.10.025>.
- [35] Vidonish, J.E.; Zygourakis, K.; Masiello, C.A.; Sabadell, G.; Alvarez, P.J.J. (2016). Thermal treatment of hydrocarbon-impacted soils: A review of technology innovation for sustainable remediation. *Engineering*, 2(4). <http://doi.org/10.1016/J.ENG.2016.04.005>.
- [36] Das, N.; Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotechnology Research International*, 2011, 941810. <https://doi.org/10.4061/2011/941810>.
- [37] Safdari, M.S.; [other authors]; (2018). Development of bioreactors for comparative study of natural attenuation, biostimulation, and bioaugmentation of petroleum-hydrocarbon contaminated soil. *Journal of Hazardous Materials*, 342, 270–278. <http://doi.org/10.1016/j.jhazmat.2017.08.044>.



- [38] Chaîneau, C.H.; Rougeux, G.; Yéprémian, C.; Oudot, J. (2005). Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil. *Soil Biology and Biochemistry*, 37(8), 1490–1497. <http://doi.org/10.1016/j.soilbio.2005.01.012>.
- [39] Pino, N.J.; Muñera, L.M.; Peñuela, G.A. (2016). Bioaugmentation with immobilized microorganisms to enhance phytoremediation of PCB-contaminated soil. *Soil, Sediment and Contamination*, 25(4), 419–430. <http://doi.org/10.1080/15320383.2016.1148010>.
- [40] Das, M.; Adholeya, A. (2012). Role of microorganisms in remediation of contaminated soil. In *Microorganisms in Environmental Management: Microbes and Environment*; Satyanarayana, T., Johri, B., Eds.; Springer: Dordrecht. [http://doi.org/10.1007/978-94-007-2229-3\\_4](http://doi.org/10.1007/978-94-007-2229-3_4).
- [41] Zou, C.; Wang, M.; Xing, Y.; Lan, G.; Ge, T.; Yan, X.; Gu, T. (2014). Characterization and optimization of biosurfactants produced by *Acinetobacter baylyi* ZJ2 isolated from crude oil-contaminated soil sample toward microbial enhanced oil recovery applications. *Biochemical Engineering Journal*, 90, 49–58. <http://doi.org/10.1016/j.bej.2014.05.007>.
- [42] Wijekoon, W.; Priyashantha, H.; Gajanayake, P.; Manage, P.; Liyanage, C.; Jayarathna, S.; Kumarasinghe, U. (2025). Review and Prospects of Phytoremediation: Harnessing Biofuel-Producing Plants for Environmental Remediation. *Sustainability*, 17, 822. <https://doi.org/10.3390/su17030822>.
- [43] Adedeji, J.A.; Tetteh, E.K.; Opoku Amankwa, M.; Asante-Sackey, D.; Ofori-Frimpong, S.; Armah, E.K.; Rathilal, S.; Mohammadi, A.H.; Chetty, M. (2022). Microbial Bioremediation and Biodegradation of Petroleum Products—A Mini Review. *Applied Sciences*, 12, 12212. <https://doi.org/10.3390/app122312212>.
- [44] Guarino, C.; Spada, V.; Sciarrillo, R. (2017). Assessment of three approaches of bioremediation (Natural Attenuation, Landfarming and Bioaugmentation – Assisted Landfarming) for a petroleum hydrocarbons contaminated soil. *Chemosphere*, 170. <http://doi.org/10.1016/j.chemosphere.2016.11.165>.
- [45] Corredor, D.; Duchicela, J.; Flores, F.J.; Maya, M.; Guerron, E. (2024). Review of Explosive Contamination and Bioremediation: Insights from Microbial and Bio-Omic Approaches. *Toxics*, 12, 249. <https://doi.org/10.3390/toxics12040249>.
- [46] Ite, A.E.; Semple, K.T. (2012). Biodegradation of petroleum hydrocarbons in contaminated soil. In *Microbial Biotechnology for Energy and Environment*, pp. 250–278. <http://doi.org/10.1079/9781845939564.0250>.
- [47] Dell’ Anno, F.; Rastelli, E.; Sansone, C.; Brunet, C.; Ianora, A.; Dell’ Anno, A. Bacteria, (2021). Fungi and Microalgae for the Bioremediation of Marine Sediments Contaminated by Petroleum Hydrocarbons in the Omics Era. *Microorganisms*, 9, 1695. <https://doi.org/10.3390/microorganisms9081695>.
- [48] Pszczółkowski, P.; Sawicka, B.; Barbaś, P.; Skiba, D.; Krochmal-Marczak, B. (2023). The Use of Effective Microorganisms as a Sustainable Alternative to Improve the Quality of Potatoes in Food Processing. *Applied Sciences*, 13, 7062. <https://doi.org/10.3390/app13127062>.
- [49] Kiamarsi, Z.; Kafi, M.; Soleimani, M.; Nezami, A.; Lutts, S. (2020). Conjunction of *Vetiveria zizanioides* L. and oil-degrading bacteria as a promising technique for remediation of crude oil-contaminated soils. *Journal of Cleaner Production*, 253. <http://doi.org/10.1016/j.jclepro.2019.119719>.
- [50] Wojtowicz, K.; Steliga, T.; Kapusta, P. (2023). Evaluation of the Effectiveness of Bioaugmentation-Assisted Phytoremediation of Soils Contaminated with Petroleum Hydrocarbons Using *Echinacea purpurea*. *Applied Sciences*, 13, 13077. <https://doi.org/10.3390/app132413077>.
- [51] Ali, M.H.; Khan, M.I.; Naveed, M.; Tanvir, M.A. (2023). Microbe-Assisted Rhizoremediation of Hydrocarbons and Growth Promotion of Chickpea Plants in Petroleum Hydrocarbons-Contaminated Soil. *Sustainability*, 15, 6081. <https://doi.org/10.3390/su15076081>.

- [52] Bento, F.M.; Camargo, F.A.O.; Okeke, B.C.; Frankenberger, W.T. (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technology*, 96(9), 1049–1055. <http://doi.org/10.1016/j.biortech.2004.09.008>.
- [53] Sharma, A.; Kumar, P.; Rehman, M.B. (2014). Biodegradation of diesel hydrocarbon in soil by bioaugmentation of *Pseudomonas aeruginosa*: A laboratory scale study. *International Journal of Environmental Bioremediation & Biodegradation*, 2(4), 202–212.
- [54] Lee, K.C.; Darah, I.; Ibrahim, C.O. (2011). A laboratory scale bioremediation of Tapis crude oil contaminated soil by bioaugmentation of *Acinetobacter baumannii* T30C. *African Journal of Microbiology Research*, 5(18), 2609–2615. <http://doi.org/10.5897/ajmr11.185>.
- [55] Sayed, K.; Baloo, L.; Sharma, N.K. (2021). Bioremediation of Total Petroleum Hydrocarbons (TPH) by Bioaugmentation and Biostimulation in Water with Floating Oil Spill Containment Booms as Bioreactor Basin. *International Journal of Environmental Research and Public Health*, 18, 2226. <https://doi.org/10.3390/ijerph18052226>.
- [56] Vasudevan, N.; Rajaram, P. (2001). Bioremediation of oil sludge-contaminated soil. *Environment International*, 26(5–6), 409–411. [http://doi.org/10.1016/S0160-4120\(01\)00020-4](http://doi.org/10.1016/S0160-4120(01)00020-4).
- [57] 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. <https://doi.org/10.3390/microbiolres15040171>.
- [58] Myazin, V.A.; Korneykova, M.V.; Chaporgina, A.A.; Fokina, N.V.; Vasilyeva, G.K. (2021). The Effectiveness of Biostimulation, Bioaugmentation and Sorption-Biological Treatment of Soil Contaminated with Petroleum Products in the Russian Subarctic. *Microorganisms*, 9, 1722. <https://doi.org/10.3390/microorganisms9081722>.
- [59] Udume, O.A.; Abu, G.O.; Stanley, H.O.; Vincent-Akpu, I.F.; Momoh, Y.; Eze, M.O. (2023). Biostimulation of Petroleum-Contaminated Soil Using Organic and Inorganic Amendments. *Plants*, 12, 431. <https://doi.org/10.3390/plants12030431>.
- [60] Gkorezis, P.; Daghighi, M.; Franzetti, A.; Van Hamme, J.D.; Sillen, W.; Vangronsveld, J. (2016). The interaction between plants and bacteria in the remediation of petroleum hydrocarbons: An environmental perspective. *Frontiers in Microbiology*, 7, 1836. <http://doi.org/10.3389/fmicb.2016.01836>.
- [61] Yavari, S.; Malakahmad, A.; Sapari, N.B. (2015). A review on phytoremediation of crude oil spills. *Water, Air, and Soil Pollution*, 226(8), 279. <http://doi.org/10.1007/s11270-015-2550-z>.
- [62] Shahi, A.; Aydin, S.; Ince, B.; Ince, O. (2016). Evaluation of microbial population and functional genes during the bioremediation of petroleum-contaminated soil as an effective monitoring approach. *Ecotoxicology and Environmental Safety*, 125, 153–160. <http://doi.org/10.1016/j.ecoenv.2015.11.029>.
- [63] Omokhagbor Adams, G.; Tawari Fufeyin, P.; Eruke Okoro, S.; Ehinomen, I. (2020). Bioremediation, biostimulation and bioaugmentation: A review. *International Journal of Environmental Bioremediation & Biodegradation*, 3(1), 28–39. <http://doi.org/10.12691/ijeabb-3-1-5>.
- [64] Panchenko, L.; Muratova, A.; Dubrovskaya, E.; Golubev, S.; Turkovskaya, O. (2023). Natural and Technical Phytoremediation of Oil-Contaminated Soil. *Life*, 13, 177. <https://doi.org/10.3390/life13010177>.
- [65] Andreolli, M.; Lampis, S.; Brignoli, P.; Vallini, G. (2015). Bioaugmentation and biostimulation as strategies for the bioremediation of a burned woodland soil contaminated by toxic hydrocarbons: A comparative study. *Journal of Environmental Management*, 153, 121–131. <http://doi.org/10.1016/j.jenvman.2015.02.007>.



- [66] Nwankwegu, A.S.; Orji, M.U.; Onwosi, C.O. (2016). Studies on organic and in-organic biostimulants in bioremediation of diesel-contaminated arable soil. *Chemosphere*, 162, 148–156. <http://doi.org/10.1016/j.chemosphere.2016.07.074>.
- [67] Abioye, O.P.; Agamuthu, P.; Abdul Aziz, A.R. (2012). Biodegradation of Used Motor Oil in Soil Using Organic Waste Amendments. *Biotechnology Research International*, 2012, 587041. <http://doi.org/10.1155/2012/587041>.
- [68] Hamzah, A.; Phan, C.W.; Yong, P.H.; Mohd Ridzuan, N.H. (2014). Oil Palm Empty Fruit Bunch and Sugarcane Bagasse Enhance the Bioremediation of Soil Artificially Polluted by Crude Oil. *Soil Sediment Contamination*, 23, 751–762. <http://doi.org/10.1080/15320383.2014.870528>.
- [69] Rahmati, F.; Asgari Lajayer, B.; Shadfar, N.; van Bodegom, P.M.; van Hullebusch, E.D. (2022). A Review on Biotechnological Approaches Applied for Marine Hydrocarbon Spills Remediation. *Microorganisms*, 10, 1289. <https://doi.org/10.3390/microorganisms10071289>.
- [70] Agamuthu, P.; Dadrasnia, A. (2013). Potential of biowastes to remediate diesel fuel contaminated soil. *Global Nest Journal*, 15, 474–484. <http://doi.org/10.30955/gnj.001031>.
- [71] Castro Rodríguez, D.J.; Gutiérrez Benítez, O.; Casals Pérez, E.; Demichela, M.; Godio, A.; Chiampo, F. (2022). Bioremediation of Hydrocarbon-Polluted Soil: Evaluation of Different Operative Parameters. *Applied Sciences*, 12, 2012. <https://doi.org/10.3390/app12042012>.
- [72] Shahsavari, E.; Rouch, D.; Khudur, L.S.; Thomas, D.; Aburto-Medina, A.; Ball, A.S. (2021) Challenges and Current Status of the Biological Treatment of PFAS-Contaminated Soils. *Frontiers in Bioengineering and Biotechnology*, 8, 602040. <https://doi.org/10.3389/fbioe.2020.602040>.
- [73] Ossai, I.C.; Ahmed, A.; Hassan, A.; Hamid, F.S. (2020). Remediation of soil and water contaminated with petroleum hydrocarbon: A review. *Environmental Technology and Innovation*, 17, 100526. <http://doi.org/10.1016/j.eti.2019.100526>.
- [74] Kuppusamy, S.; Thavamani, P.; Venkateswarlu, K.; Lee, Y.B.; Naidu, R.; Megharaj, M. (2017). Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future directions. *Chemosphere*, 168, 944–968. <http://doi.org/10.1016/j.chemosphere.2016.10.115>.
- [75] Sui, X.; Wang, X.; Li, Y.; Ji, H. (2021). Remediation of Petroleum-Contaminated Soils with Microbial and Microbial Combined Methods: Advances, Mechanisms, and Challenges. *Sustainability*, 13, 9267. <https://doi.org/10.3390/su13169267>.
- [76] Sher, S.; Waseem, M.; Leta, M.K. (2023). Review of Techniques for the Removal of Polycyclic Aromatic Hydrocarbons from Produced Water. *Environments*, 10, 40. <https://doi.org/10.3390/environments10030040>.
- [77] Sumathi, K.; Manian, R. (2023). Bioremediation of polycyclic aromatic hydrocarbons contaminated soils: recent progress, perspectives and challenges. *Environmental Monitoring and Assessment*, 195(12), 1141. <http://doi.org/10.1007/s10661-023-12042-7>.

