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REVIEW

Microbial and Plant-Based Bioremediation of Petroleum Hydrocarbons: Mechanisms, Strategies and Environmental Applications

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ABSTRACT

Petroleum hydrocarbon (PH) pollution poses a persistent threat to terrestrial and aquatic ecosystems, with wide-ranging implications for soil fertility, water quality, and human health. Conventional physicochemical remediation methods, although effective in some contexts, often entail high operational costs, generate secondary waste, and fail to achieve complete mineralization of complex hydrocarbon mixtures. In contrast, microbial bioremediation has emerged as a sustainable, cost-effective, and ecologically compatible strategy that harnesses the metabolic versatility of indigenous and exogenous bacteria to degrade a broad spectrum of aliphatic, aromatic, and heterocyclic hydrocarbons. Recent advances in biostimulation, bioaugmentation, phytoremediation, and engineered bioreactors have demonstrated field-scale removal efficiencies of total petroleum hydrocarbons (TPH) exceeding 70–90% within weeks to months, depending on site-specific conditions and pollutant load. Nevertheless, incomplete degradation, environmental variability, and the potential accumulation of intermediate metabolites constrain the robustness and predictability of bioremediation at scale. Emerging technologies such as nanobioremediation, bioelectrochemical systems, and "omics" driven microbial community engineering offer promising avenues to enhance degradation kinetics, extend substrate range, and mitigate antibiotic resistance gene (ARG) dissemination associated with exposure to contaminated matrices. This review synthesizes current knowledge on the classification, sources, and ecological impacts of petroleum hydrocarbons, critically evaluates the mechanisms and techniques underpinning microbial bioremediation, and identifies key research gaps and regulatory challenges. The article further outlines future perspectives for integrating

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multi-process, data-driven remediation strategies into national and international frameworks for environmental restoration.
Keywords: Bioremediation; Petroleum Hydrocarbons; Bioaugmentation; Phytoremediation; Bioaccumulative; Antibiotic Resistance Gene and Engineered Bioreactors

1. Introduction

Petroleum hydrocarbon (PH) pollution is a global environmental crisis, driven by extensive extraction, refining, transportation, and incomplete combustion of fossil fuels. Crude oil and its derivatives constitute complex mixtures of aliphatic, aromatic, and cyclic hydrocarbons, many of which are recalcitrant, toxic, and bioaccumulative, thereby posing long-term risks to ecosystems and public health. Despite the proliferation of spill-response protocols and engineered containment measures, persistent contamination of soils, sediments, and groundwater continues to undermine ecosystem services, agricultural productivity, and water security [1].

Conventional remediation approaches, including mechanical removal, chemical oxidation, thermal treatment, and solid-phase extraction, often provide rapid but localized relief at the expense of high energy inputs, secondary pollution, and incomplete mineralization of higher-molecular-weight fractions [2]. In contrast, bioremediation capitalizes on the intrinsic metabolic capacity of microorganisms, particularly bacteria, to transform PHs into less toxic or fully mineralized end products such as carbon dioxide, water, chloride, and biomass. Indigenous microbial communities in oil-impacted environments frequently harbor hydrocarbon-degrading taxa that can be stimulated or complemented with engineered consortia, thereby aligning remediation with principles of circularity and environmental sustainability [1,2].

Yet, the field lacks a unified, data-driven framework that systematically compares the efficiency, scalability, and ecological trade-offs of different bioremediation strategies across diverse PH fractions and environmental matrices. Knowledge gaps persist regarding the long-term behavior of microbial communities under fluctuating biogeochemical conditions, the fate of transformation intermediates, and the potential co-selection of antibiotic resistance in contaminated sites [3]. This review addresses these gaps by providing a comprehensive, mechanistically grounded synthesis of the functional role of bacteria in PH cleanup, with emphasis on classification and sources, environmental and health impacts, remediation strategies, and current limitations. The article further critically evaluates emerging technologies, including nanobioremediation, bioelectrochemical systems, and high-throughput "omics" tools, while offering a forward-looking perspective on the integration of microbial bioremediation into policy-oriented environmental management frameworks. In the subsequent section, we delineate the classification, sources, and compositional complexity of petroleum hydrocarbons that underpin their varying susceptibility to microbial degradation [3,4].

2. Classification, Sources, and Composition of Petroleum Hydrocarbons

Petroleum hydrocarbons (PHs) constitute a heterogeneous class of organic compounds derived primarily from crude oil and its refined products, whose chemical architecture dictates their environmental persistence and biodegradability. PHs are broadly classified into four principal categories: aliphatic hydrocarbons (n-alkanes, branched alkanes, and cycloalkanes), aromatic hydrocarbons (monocyclic and polycyclic aromatic hydrocarbons, PAHs), heterocyclic compounds (e.g., sulfur- and nitrogen-containing molecules), and asphaltenes and resins, which collectively represent the most recalcitrant fractions [4]. Aliphatic compounds such as n-alkanes (C_8–C_{40}) are relatively labile and serve as preferred carbon sources for many hydrocarbonoclastic bacteria, whereas PAHs with three or more fused rings (e.g., phenanthrene, fluoranthene, pyrene) exhibit slower degradation kinetics and greater toxicity [4,5].

The composition of PHs varies significantly with crude origin, refining history, and weathering processes, thereby influencing both ecological risk and remediation strategy. For example, light crude oils and gasoline are dominated by low-molecular-weight aliphatics and mono-aromatics such as benzene, toluene, ethylbenzene, and xylene (BTEX), which are highly volatile and readily bioavailable but also acutely toxic. In contrast, heavy crude oils and bunker fuels contain higher proportions of high-molecular-weight n-alkanes, branched alkanes, and PAHs, which resist biodegradation, accumulate in sediments, and contribute to long-term contamination [5]. Asphaltenes and resins, which are primarily non-polar, high-molecular-weight aggregates, further complicate remediation by coating soil particles and limiting oxygen and water diffusion in contaminated matrices [6].

Sources of PH pollution are both anthropogenic and natural, with the former accounting for the largest share of environmental contamination. Major anthropogenic sources include oil spills from offshore platforms and tankers, pipeline ruptures, refinery effluents, urban and industrial runoff, fuel storage leaks, and incomplete combustion of fossil fuels in vehicles and power plants. Natural sources such as seeps and biogenic hydrocarbons contribute a smaller but non-negligible fraction, particularly in marine environments where microseepage can sustain background levels of hydrocarbon-degrading microbial activity [6,7]. Once released, PHs undergo complex weathering processes: evaporation, photo-oxidation, emulsification, and sedimentation that alter their physicochemical properties and partition between air, water, soil, and biota. These transformations often increase the proportion of higher-molecular-weight and aromatic fractions, shifting the substrate spectrum toward more recalcitrant compounds that challenge conventional remediation and demand tailored bioremediation approaches [7].

The comparative properties outlined above emphasize the relationship between molecular structure and toxicological impact across different hydrocarbon groups. Variations in solubility, volatility, and molecular weight govern their environmental mobility and biological interactions, ultimately determining their acute and chronic effects on living systems. Lower molecular weight compounds tend to exhibit rapid dispersion and immediate toxicity, whereas higher molecular weight hydrocarbons persist longer and contribute to cumulative ecological damage. These distinctions are critical for risk assessment and guide the prioritization of remediation approaches based on contaminant profile and exposure pathways, as the systematic organization in **Table 1** highlights how the structural architecture of petroleum hydrocarbons dictates their biodegradability, mobility, and persistence in the environment. Short-chain n-alkanes and BTEX compounds are typically removed with >70–90% efficiency under optimized biostimulation, as their relatively simple structures and high bioavailability facilitate rapid activation by oxygenase-mediated pathways. In contrast, four- to six-ring PAHs and asphaltene-rich fractions are often recalcitrant, requiring months to years for partial degradation and frequently yielding partially oxidized intermediates that may be more toxic than the parent compounds. This compositional hierarchy underscores the need for tailored remediation strategies that combine biostimulation, bioaugmentation, and, where necessary, physicochemical pretreatment to address the full spectrum of PH contamination across diverse environmental matrices [7,8].

Table 1. Classification, carbon ranges, environmental behavior, relative biodegradability, and references of key petroleum hydrocarbon (PH) classes.

PH Class	Representative Compounds/ CarBon Range	Environmental Behavior and Fate	Relative Biodegradability	References
Short-chain n-alkanes	C_8–C_{15} (e.g., octane, decane)	Highly volatile, water-soluble, rapidly evaporate; highly bioavailable in soil and groundwater	Very high; typically >80–90% removal within days–weeks under aerobic conditions	Chen et al., 2020 [9]
Mid-chain n-alkanes	C_{16}–C_{25}	Moderate volatility; partition between soil organic matter and pore water	High; ~70–90% removal within weeks–months under optimized biostimulation	Yang et al., 2016 [10]
Long-chain n-alkanes	C_{26}–C_{40}+	Low volatility; strongly adsorbed to soil; limited diffusion and bioavailability	Moderate; slower degradation with increasing chain length	Haritash and Kaushik, 2009 [11]
Branched alkanes	Isoalkanes (e.g., pristane, phytane)	Resistant to weathering; accumulate due to low solubility	Low–moderate; slower degradation due to structural complexity	Liu et al., 2021 [12]

Table 1. *Cont.*

PH Class	Representative Compounds/ CarBon Range	Environmental Behavior and Fate	Relative Biodegradability	References
Cycloalkanes	Naphthenes (e.g., cyclohexane, decalin)	Intermediate solubility; persist in soil and groundwater	Moderate; requires specialized enzymatic pathways	Abu Bakar et al., 2020 [13]
BTEX (mono-aromatics)	Benzene, toluene, ethylbenzene, xylenes (C_6–C_8)	Highly volatile and mobile; toxic and groundwater-contaminating	High; often >80% removal within days–weeks under aerobic bioremediation	Chen et al., 2020 [9]
Low-ring PAHs	2–3 rings (e.g., naphthalene, phenanthrene, fluorene; C_{10}–C_{18})	Semi-volatile; adsorb to organic matter; moderately mobile	Moderate high; ~60–80% removal within weeks months	Xu et al., 2018 [14]
High-ring PAHs	4–6 rings (e.g., pyrene, benzo[a]pyrene; C_{18}–C_{30})	Highly hydrophobic; strongly adsorbed; persistent and bioaccumulative	Low; degradation may take months–years; often incomplete	Chen et al., 2020 [9]
Heterocyclic compounds	S- and N-containing (e.g., dibenzothiophene, carbazole)	Resistant due to heteroatoms; influence soil chemistry upon degradation	Low–moderate; requires specialized microbial consortia	Chen et al., 2020 [9]
Asphaltenes and resins	High molecular weight (C_{30}–C_{100}+) polyaromatic–aliphatic complexes	Extremely hydrophobic; form aggregates; strongly adsorbed in soils and sediments	Very low; highly recalcitrant; often require physicochemical pretreatment	Eliaz et al., 2018 [15]

Note: This table illustrates how the structural complexity of petroleum hydrocarbon (PH) classes systematically modulates their environmental behavior and susceptibility to microbial degradation.

Visual roadmap of the structural diversity underpinning petroleum hydrocarbon (PH) persistence and degradability, illustrating how carbon chain length, degree of branching, ring fusion, and heteroatom content modulate bioavailability, toxicity, and susceptibility to microbial enzyme attack. Short, linear, and unsubstituted aliphatic chains and mono-aromatics such as BTEX appear in the upper-left quadrant, symbolizing their high solubility, bioavailability, and rapid biodegradation under aerobic conditions, whereas fused-ring PAHs and bulky asphaltene-resin aggregates in the lower-right quadrant reflect low aqueous solubility, strong sorption to soil and sediment, and recalcitrance to microbial attack [8] (**Figure 1**). This hierarchical organization directly informs the subsequent discussion of environmental and health impacts, where the more soluble and bioavailable fractions (e.g., BTEX) tend to dominate acute toxicity and early-phase exposure, while high-molecular-weight PAHs and asphaltenes drive long-term ecological damage and pose challenges for complete bioremediation.

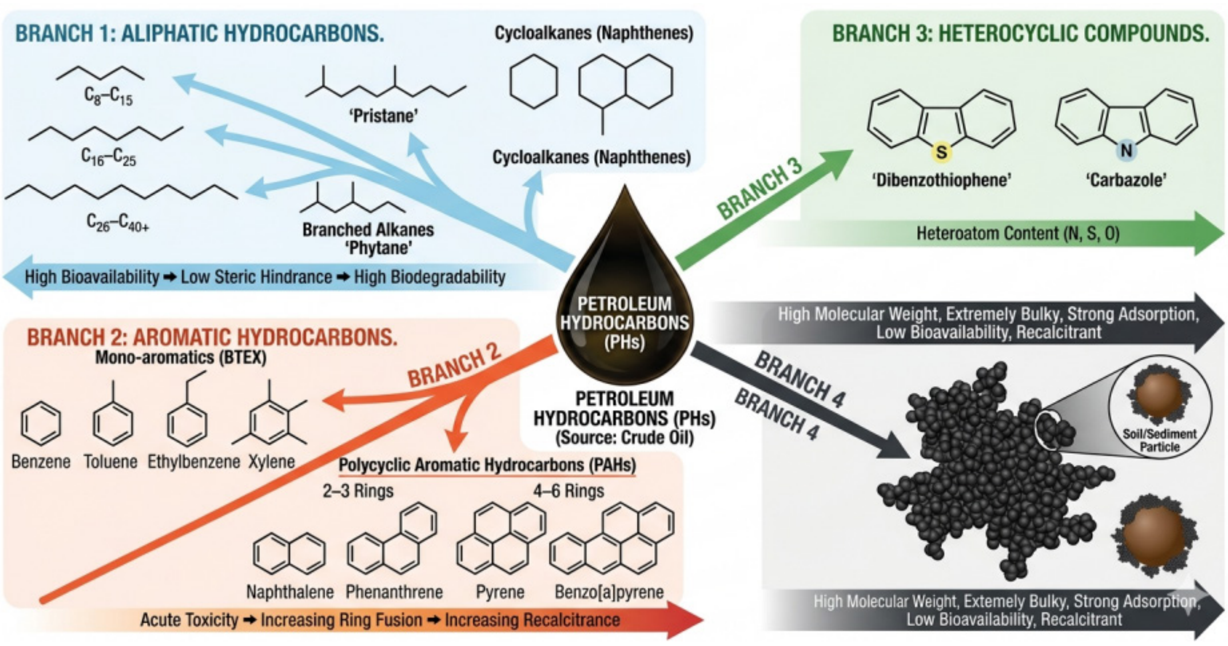

Figure 1. Schematic classification of petroleum hydrocarbon groups (e.g., aliphatics, aromatics, heterocyclics, asphaltenes).

Figure 1 represents a visual roadmap of the structural diversity underpinning the persistence and degradability of Petroleum Hydrocarbons (PHs), illustrating how carbon chain length, degree of branching, ring fusion, and heteroatom content modulate bioavailability, toxicity, and susceptibility to enzymatic attack. **Branch 1** (Aliphatic Hydrocarbons, top-left): Exhibits a structural gradient from high-molecular-weight n-alkanes (low bioavailability) toward more bioavailable forms, driven by lower steric hindrance, leading to high biodegradability. Note the sub-categories: simple alkane chains (C_8–C_{15}, C_{16}–C_{25}, C_{26}–C_{40}+); branched alkanes (e.g., 'Pristane', 'Phytane'); and saturated ring systems (cycloalkanes/naphthenes). **Branch 2** (Aromatic Hydrocarbons, bottom-left): Follows a trajectory from highly bioavailable mono-aromatics (BTEX: benzene, toluene, ethylbenzene, xylene) that often dominate acute toxicity, toward polycyclic aromatic hydrocarbons (PAHs) with increasing ring fusion (2–3 rings → 4–6 rings, e.g., naphthalene, phenanthrene, pyrene, benzo[a]pyrene), which drives increasing recalcitrance. **Branch 3** (Heterocyclic Compounds, top-right): Specifically highlights non-hydrocarbon additions to ring systems, such as Nitrogen (N) (e.g., 'Carbazole') and Sulfur (S) (e.g., 'Dibenzothiophene'), increasing the chemical complexity. **Branch 4** (Asphaltenes & Resins, bottom-right): Shows high-molecular-weight, extremely bulky aggregated clusters. These components drive long-term ecological damage due to low bioavailability, extreme recalcitrance, and strong adsorption to soil/sediment particles (as detailed in the inset visual).

The structural diversity of petroleum hydrocarbons illustrated above reflects the complexity of their environmental behavior and degradation potential. Aliphatic and aromatic fractions differ significantly in terms of volatility, solubility, and resistance to microbial breakdown, which directly influences their persistence in contaminated ecosystems. While simpler aliphatic compounds are generally more susceptible to rapid biodegradation, complex aromatic hydrocarbons, particularly polycyclic structures, exhibit greater stability and toxicity. This classification, therefore, provides a foundational basis for understanding not only their environmental distribution but also the selection of appropriate remediation strategies, which are discussed in the subsequent sections.

3. Environmental and Health Impacts

Petroleum hydrocarbon (PH) pollution exerts multifaceted pressures on ecosystems and human populations, spanning acute toxicity, chronic ecological degradation, and long-term public-health risks. The environmental and health impacts of PHs are closely tied to their physicochemical properties: water-soluble compounds such as BTEX and certain low-molecular-weight aliphatics readily partition into aqueous phases, leading to rapid contamination of groundwater and surface water, whereas higher-molecular-weight PAHs and asphaltenes preferentially adsorb to soils and sediments, creating persistent contamination hotspots that can remain for decades [13].

In terrestrial ecosystems, PH contamination alters soil structure, reduces porosity, and displaces water from the pore space, thereby impairing hydraulic conductivity and discouraging root penetration and microbial colonization. Total petroleum hydrocarbon (TPH) concentrations exceeding 1–5% (w/w) in soils have been associated with severe inhibition of seed germination, root elongation, and microbial respiration, leading to reduced biomass yield and loss of agricultural productivity [13,15]. In aquatic environments, oil slicks and emulsified hydrocarbons decrease light penetration, disrupt gas exchange at the air water interface, and suffocate benthic organisms by coating gills and sediments. Marine oil spills have been documented to cause mass mortalities of fish, invertebrates, and seabirds, with sublethal effects including compromised immunity, reproductive dysfunction, and developmental abnormalities mediated by oxidative stress and endocrine disruption [15].

Health impacts on humans arise primarily through exposure to PH-laden air, water, and food, as well as dermal contact with contaminated soil or sediments. BTEX compounds are volatile and can be inhaled near spill sites, refineries, and fuel-handling facilities; benzene, in particular, is a known human carcinogen (International Agency for Research on Cancer (IARC) Group 1) linked to leukemia and other hematological malignancies, with chronic exposure at elevated atmospheric concentrations significantly increasing risk [14]. PAHs (Polycyclic Aromatic Hydrocarbons), such as benzo[α]pyrene and chrysene, are genotoxic and mutagenic, capable of forming DNA adducts and contributing to lung, skin, and bladder cancers, especially in occupational settings without adequate respiratory protection. Ingestion of contaminated drinking water or consumption of hydrocarbon-bioaccumulating seafood further elevates exposure, with vulnerable populations such as children, pregnant women, and immunocompromised individuals facing disproportional risk [14,16].

The synthesis of quantitative toxicological dimensions of petroleum hydrocarbon (PH) pollution illustrates how dose-response relationships and bioaccumulation patterns differ markedly across PH classes and trophic levels. The steeper dose response curves for BTEX and low-ring PAHs reflect their high bioavailability and acute toxicity at relatively low environmental concentrations, whereas the shallower but persistent curves for 4 to 6-ring PAHs underscore chronic, long-term risks even at sub-acute exposure levels. The trophic bioaccumulation panel further highlights how these compounds magnify through food webs, with bioaccumulation factors increasing several-folds from primary producers to higher predators, thereby amplifying ecological and human health risks (**Figure 2**). This toxicity landscape rationalizes the urgency of targeted remediation strategies discussed in the following section, where bioremediation, phytoremediation, and complementary technologies are evaluated not only for their removal efficiency but also for their ability to mitigate the ecological and health burdens imposed by these structurally and toxicologically diverse PH fractions [16]. The observed toxicity patterns and bioaccumulation behavior across petroleum hydrocarbon classes provide a coherent framework for understanding their ecological and human health risks. Compounds with higher solubility, such as BTEX and low-molecular-weight PAHs, tend to exert rapid and acute toxic effects due to their mobility and bioavailability, whereas high-molecular-weight PAHs persist in environmental matrices and contribute to long-term, chronic toxicity through bioaccumulation and biomagnification across trophic levels. These differential behaviors highlight the need for remediation strategies that are not only efficient in removal but also tailored to the structural and toxicological complexity of each hydrocarbon fraction. Accordingly, the following section examines integrated remediation approaches, including microbial and plant-based systems, designed to address both immediate toxicity and long-term environmental persistence in contaminated ecosystems.

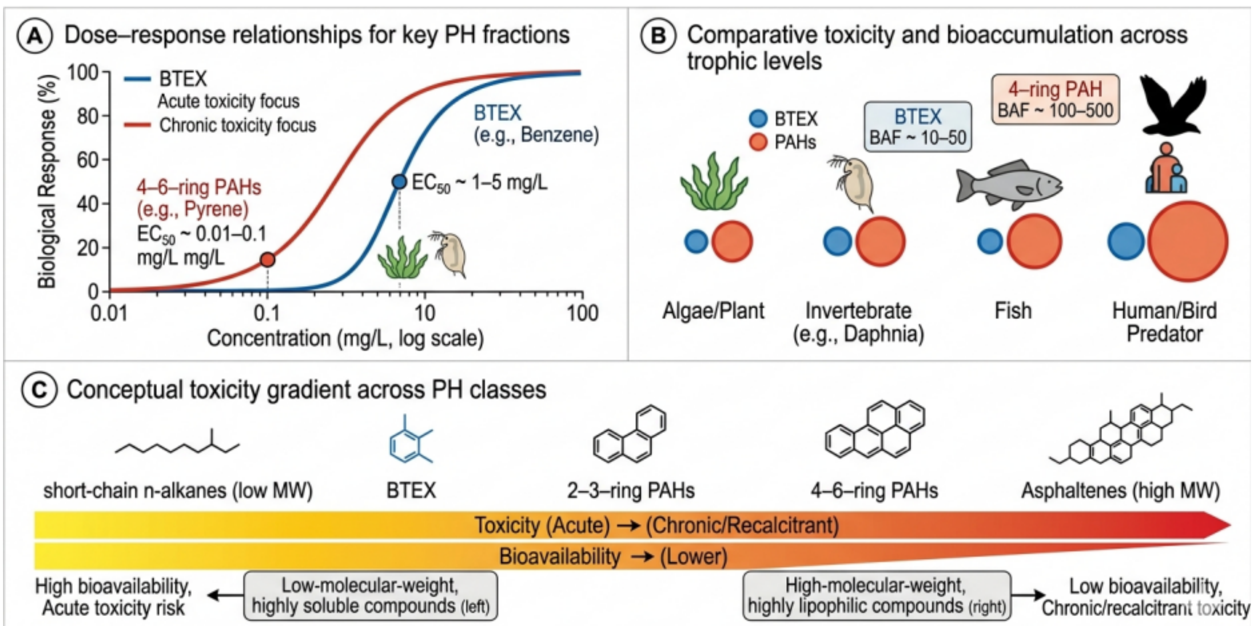

Figure 2. Schematic representation of petroleum hydrocarbon toxicity and bioaccumulation.

Figure 2 provides a data-driven and conceptual overview of the environmental toxicology of Petroleum Hydrocarbons (PHs), illustrating how chemical structure influences bioavailability, lipophilicity, and toxic effects across different ecological receptors. **(A)** Dose response relationships for key PH fractions: A semi-quantitative comparison of effect thresholds (e.g., EC_{50} values) for different PH classes. Mono-aromatics (BTEX, blue line) are shown as more potent contributors to acute toxicity at lower water concentrations (steeper curve, lower EC_{50} value range, e.g., EC_{50} ~1–5 mg/L), typically driving immediate impacts. High-molecular-weight Polycyclic Aromatic Hydrocarbons (4–6 ring PAHs, red line) exhibit lower acute potency (shallower curve, higher EC_{50} value range, e.g., EC_{50} ~0.01–0.1 mg/L) but contribute significantly to chronic, long-term ecological damage due to their environmental persistence. (Units on the y-axis represent biological responses, such as % mortality or growth inhibition). **(B)** Comparative toxicity and bioaccumulation across trophic levels: A conceptual diagram of a food chain (algae/plant → invertebrate → fish → human/bird) illustrating the contrast in bioaccumulation behavior. Blue bars represent BTEX, showing higher bioaccumulation factors (BAFs ~10–50) at lower trophic levels (e.g., invertebrates and small fish). Red bars represent multi-ring PAHs, which, due to their higher lipophilicity, exhibit progressively larger bioaccumulation and biomagnification factors (BAFs ~100–500) through the food web, resulting in the highest concentrations in top predators. **(C)** Conceptual toxicity gradient across PH classes: A horizontal summary relating chemical structure, bioavailability, and resulting environmental risk. A visual continuum is established from low-molecular-weight, highly soluble compounds (e.g., short-chain n-alkanes, BTEX) with high bioavailability and high acute toxicity risk (yellow/orange), to high-molecular-weight, highly lipophilic compounds (e.g., multi-ring PAHs, asphaltenes) with low bioavailability but substantial chronic/recalcitrant toxicity and risk (dark red).

4. Remediation Strategies: Comparative Overview

Given the severity of PH impacts, a spectrum of remediation strategies has been developed, each with distinct mechanisms, efficiency profiles, and sustainability trade-offs. These strategies can be broadly categorized into physicochemical methods (e.g., excavation and disposal, thermal desorption, incineration, chemical oxidation), physical containment (e.g., capping, in situ solidification), and biological approaches (mainly bioremediation, including biostimulation and bioaugmentation). A comparative appraisal of these options is essential to inform site-specific decision-making and to justify the growing emphasis on biologically driven solutions [16].

Physicochemical methods generally offer rapid removal, particularly for localized, high-concentration plumes, but they are often energy-intensive, costly, and generate secondary waste that may require additional treatment or disposal. Incineration and thermal desorption can achieve near-complete destruction of hydrocarbons under controlled conditions, yet their application is typically limited to ex situ scenarios due to safety and regulatory constraints [16,17]. Chemical oxidation using Fenton reagents or persulfate can oxidize BTEX and some PAHs efficiently but may inadvertently generate toxic intermediates or alter soil pH and nutrient availability. In contrast, physical containment strategies such as capping with low-permeability materials prevent further migration of contaminants but leave the original PH load in situ, potentially creating long-term liability and requiring perpetual monitoring [17].

Bioremediation, by contrast, leverages microbial metabolism to transform PHs into less toxic or fully mineralized products, offering a comparatively low-energy, low-cost, and ecologically compatible alternative. In laboratory and pilot-scale studies, biostimulation of indigenous communities has removed 60–90% of TPH within weeks to months, depending on nutrient availability, oxygen supply, temperature, and hydrocarbon composition. Bioaugmentation with selected hydrocarbonoclastic strains or consortia can further enhance degradation rates, particularly for stubborn PAHs and branched alkanes, although field-scale success is highly sensitive to site-specific biogeochemical conditions and microbial competition [17,18].

Incineration and thermal desorption can achieve >95% TPH removal within days to weeks but at the expense of very high energy and capital costs, substantial emissions, and limited ecological compatibility, making them suitable primarily for small-scale, high-risk, or emergency-type interventions (**Table 2**). In contrast, aerobic bioremediation (via biostimulation or landfarming) typically removes 70–90% of TPH over several weeks to months at a fraction of the cost, with negligible secondary waste and the added benefit of partial soil-property restoration, including improved structure and microbial activity. Bioaugmentation and phytoremediation-based strategies generally offer lower to moderate removal efficiencies but provide long-term, low-impact, and often site-integrated solutions, particularly for large-area, low- to moderate-concentration PH contamination [18]. The comparative perspective underscores why bioremediation is increasingly favored as a core component of large-scale, long-term PH remediation frameworks, even though its performance remains contingent on site-specific environmental and operational constraints that will be examined in Section 5.

The diversity of microbial species and their degradation capabilities presented above underscores the importance of microbial selection in bioremediation strategies. Different microorganisms exhibit varying affinities for specific hydrocarbon fractions, and their effectiveness is often enhanced when functioning within synergistic consortia rather than as isolated strains. Environmental parameters further influence microbial performance, making it necessary to tailor bioremediation approaches to site-specific conditions. These insights reinforce the need for integrated and adaptive remediation systems, which combine microbial efficiency with environmental optimization to achieve sustained pollutant removal.

Table 2. Comparative performance of selected remediation strategies for petroleum hydrocarbon (PH) contaminated matrices.

Remediation Strategy	Typical Treatment Time	Approximate TPH Removal Efficiency	Energy Demand	Capital and Operational Cost	Secondary Waste Generation	Ecological Footprint	References
Excavation & disposal	Days–weeks (excavation); months (landfill)	70–95% (removal, not degradation)	Moderate–high	High	High	High	Xu et al., 2018 [14]
Thermal desorption	Hours–days (ex situ)	85–98%	Very high	Very high	Low–moderate	High	Chen et al., 2020 [9]
Incineration	Minutes–hours	>95%	Very high	Very high	Moderate	Very high	Abu Bakar et al., 2020 [13]
Chemical oxidation	Days–weeks	60–90%	Low–moderate	High	Moderate	Moderate–high	Correa- García et al., 2018 [19]
In situ capping/solidification	Months–years	Negligible (containment only)	Low	Moderate	Low	Moderate	Eliaz et al., 2018 [15]
Biostimulation (aerobic)	Weeks–months	60–90% (often 70–80%)	Low	Low–moderate	Very low	Low	Haritash et al., 2009 [11]
Bioaugmentation + biostimulation	Weeks–months	60–90% (variable)	Low–moderate	Moderate	Low	Low–moderate	Das and Tiwari, 2018 [20]
Landfarming/biopiling	3–12 months	60–85%	Low–moderate	Low–moderate	Very low	Low	Chen et al., 2020 [9]
Phytoremediation	Months–years	30–70%	Very low	Low–moderate	Very low	Low–moderate	Das and Tiwari, 2018 [20]
Hybrid (bio + chemical/electro)	Days–months	70–95%	Low–high	Low–high	Low moderate	Low moderate	Eliaz et al., 2018 [15]

Note: Comparative performance of selected remediation strategies applied to petroleum hydrocarbon contaminated soils and sediments, based on typical field- and pilot-scale data reported in the literature. Treatment times, removal efficiencies, and cost/energy categories are approximate and highly site-dependent. TPH removal efficiency refers to gross reduction of total petroleum hydrocarbons, not necessarily complete mineralization of all fractions. Ecological footprint summarizes typical environmental impact in terms of emissions, habitat disturbance, and long-term land-use implications.

5. Environmental and Biological Factors Affecting Bioremediation

The efficacy of microbial bioremediation is governed by a complex interplay between environmental conditions and biological attributes of the degrading community. Key abiotic factors include temperature, pH, redox potential, moisture content, nutrient availability (especially nitrogen and phosphorus), and oxygen supply, all of which modulate enzyme activity, microbial growth rates, and substrate bioavailability [21]. Optimal temperatures for most hydrocarbonoclastic bacteria fall in the mesophilic range (20–35 °C), with psychrophilic and thermophilic specialists extending degradation capacity to colder or hotter environments, albeit at lower overall rates. Soil pH near neutrality (6–8) generally favors microbial activity, whereas strongly acidic or alkaline conditions may inhibit key enzymes or alter metal speciation and thus influence co-contaminant toxicity [21,22].

Oxygen availability is particularly decisive in aerobic bioremediation, since the initial activation of aliphatic and aromatic hydrocarbons often requires oxygenase-mediated oxidation. In saturated soils and sediments, oxygen diffusion can be severely limited, leading to reliance on anaerobic pathways such as nitrate-, sulfate-, or iron-reducing metabolism, which are typically slower but still capable of degrading BTEX and some PAHs under electron-acceptor-limited conditions [16]. Nutrient amendment, usually in the form of N–P fertilizers, can stimulate indigenous populations by alleviating nutrient limitation and enabling biomass growth; however, excessive fertilization may lead to eutrophication in adjacent water bodies or promote unwanted microbial groups that compete with degraders [23].

Biological factors include microbial diversity, community structure, and functional redundancy, all of which determine the robustness and adaptability of the remediation system. Hydrocarbon-degrading consortia often comprise multiple genera such as *Pseudomonas*, *Rhodococcus*, *Acinetobacter*, *Alcanivorax*, and *Marinobacter* that exhibit complementary substrate preferences, enabling simultaneous degradation of aliphatic, aromatic, and heterocyclic compounds [23]. Co-occurrence of biosurfactant-producing bacteria within these consortia can enhance bioavailability by reducing surface and interfacial tension, thereby mobilizing hydrophobic fractions adhering to soil particles. However, the success of bioaugmentation depends on the ability of introduced strains to compete with indigenous microbiota, withstand environmental stressors, and avoid genetic down-regulation or horizontal gene transfer that may compromise long-term stability [24].

Field-scale variability further complicates predictive modeling of bioremediation outcomes, as heterogeneous soil texture, stratification, and groundwater flow can create microenvironments with divergent redox conditions and contaminant distributions. These spatial gradients necessitate process-oriented monitoring and adaptive management, including periodic re-amendment of nutrients and electron acceptors, and adjusting aeration or mixing regimes to maintain optimal conditions. The next section delves into the mechanistic underpinnings of microbial bioremediation, focusing on the enzymatic pathways and molecular transformations that convert PHs into benign end products [25].

6. Microbial Bioremediation Mechanisms

Microbial degradation of petroleum hydrocarbons (PHs) follows a tightly regulated sequence of enzyme-catalyzed transformations that convert structurally diverse molecules into central intermediates such as acetyl-CoA, pyruvate, and succinate, which then feed into the tricarboxylic acid (TCA) cycle for energy generation and biomass production. The specific pathways invoked depend on hydrocarbon class (aliphatic, aromatic, heterocyclic, asphaltene-associated), redox conditions, and the genetic repertoire of the degrading organism, with aerobic and anaerobic routes often operating in parallel or sequentially across heterogeneous matrices [25,26].

6.1. Aliphatic Hydrocarbon Oxidation

Aerobic degradation of aliphatic hydrocarbons typically initiates via terminal or subterminal monooxygenation, mediated by membrane-associated or soluble alkane hydroxylases (e.g., AlkB, CYP153, or related P450 systems). In Pseudomonas and Acinetobacter strains, AlkB-type enzymes catalyze O_2- and NADH-dependent hydroxylation of n-alkanes at C_1 or C_2, producing primary alcohols that are oxidized to aldehydes and then carboxylic acids by alcohol and aldehyde dehydrogenases [26]. The resulting fatty acids enter β-oxidation, a conserved pathway where the acyl-CoA dehydrogenase–hydratase–dehydrogenase–thiolase quartet shortens each chain by two carbons per cycle, generating acetyl-CoA, NADH, and $FADH_2$; this process is highly efficient for low- to mid-molecular-weight n-alkanes (C_8–C_{25}), with reported mineralization efficiencies exceeding 80% over days–weeks under optimized aeration and nutrient supply [26,27].

Branched alkanes and cycloalkanes are attacked through analogous oxygenase-driven routes, but steric hindrance at branch points and ring structures slows catalysis, reducing first-order rate constants by 30–70% compared with linear analogues. For example, Rhodococcus spp. employ cytochrome P450–mediated hydroxylation of isoalkanes, yielding tertiary alcohols that are less amenable to β-oxidation and may accumulate as persistent intermediates unless processed by auxiliary dehydrogenases or ring-opening enzymes [27].

6.2. Aromatic and PAH Ring-Cleavage Pathways

Aromatic hydrocarbons, including mono- and polycyclic aromatics (PAHs), undergo extradiol or intradiol dioxygenase-mediated ring hydroxylation, forming arene cis-dihydrodiols that are dehydrogenated to catechols. Naphthalene dioxygenase (NDO), biphenyl dioxygenase (BphA), and related multi-component Fe^{2+}-dependent enzymes introduce two hydroxyl groups ortho to each other, enabling subsequent ring cleavage via ortho- (intradiol) or meta- (extradiol) fission by catechol 1,2- or 2,3-dioxygenases [28].

Ortho-cleavage of catechols yields 3-carboxy-cis,-cis-muconate, which is processed through a series of decarboxylation and isomerization steps into pyruvate and acetyl-CoA, whereas meta-cleavage generates 2-hydroxymuconic semialdehyde, which is further converted to pyruvate and acetaldehyde or acetyl-CoA. For multi-ring PAHs such as phenanthrene, successive attacks by ring-specific dioxygenases and ring-cleavage enzymes progressively reduce ring number, often yielding quinone-type intermediates that can act as redox-active species or generate reactive oxygen species unless efficiently reduced by quinone reductases or downstream TCA-cycle flux [28]. Field studies on phenanthrene-contaminated coastal soils report half-lives ranging from 30 to 180 days depending on oxygen availability and microbial community composition, illustrating that pathway branching critically modulates degradation velocity and intermediate toxicity [29].

6.3. Anaerobic Activation and Electron-Acceptor-Linked Degradation

Under anaerobic or low-oxygen conditions, PHs are activated via alternative routes that couple hydrocarbon oxidation to the reduction of nitrate, sulfate, ferric iron, or even carbonate as terminal electron acceptors. Anaerobic degradation of aliphatic and aromatic compounds often begins with fumarate addition (yielding benzylsuccinate-type adducts) or carboxylation, reactions catalyzed by glycyl-radical enzymes in sulfate- and iron-reducing bacteria such as Desulfobacterota spp. and certain Firmicutes [29].

For example, in nitrate-reducing enrichments from petroleum-contaminated aquifers, benzene degradation proceeds via fumarate-derived intermediates that are further oxidized to succinyl-CoA and fed into reversal of the TCA cycle or fermentation-associated pathways, yielding acetate, CO_2, and biomass. Anaerobic pathways generally exhibit lower reaction velocities and longer half-lives (weeks–months) compared with aerobic oxidation, but they remain thermodynamically feasible and ecologically relevant in saturated soils and sediments where oxygen diffusion is limited [24]. Lab-scale column experiments on BTEX-contaminated sand show that nitrate- and sulfate-based bioremediation can achieve 60–80% TPH removal over 60–120 days, albeit with higher sensitivity to electron-acceptor replenishment and pH buffering than aerobic systems. These mechanistic insights reveal that PH mineralization is governed by the thermodynamic and kinetic constraints of initial activation, the regulatory architecture of catabolic operons, and the metabolic integration of intermediates into central carbon and energy metabolism. In the next section, these principles are translated into operational bioremediation techniques, where pathway engineering and process control are used to maximize flux through the most efficient catabolic routes [30,31].

Figure 3 provides a visual synthesis of the catabolic logic underlying microbial petroleum hydrocarbon (PH) degradation, illustrating how structurally diverse aliphatic, aromatic, and anaerobic substrates converge on common central intermediates such as acetyl-CoA, pyruvate, and succinate through distinct enzymatic routes. The branching architecture of the pathways reflects a fundamental trade-off between degradation speed and thermodynamic feasibility: aerobic aliphatic oxidation and aromatic ring-cleavage reactions proceed rapidly under oxygen-replete conditions, maximizing energy yield, whereas anaerobic activation routes such as carboxylation and fumarate addition enable persistent but slower degradation in oxygen-limited environments [31]. This mechanistic framework directly informs the design and operationalization of bioremediation techniques discussed in the following section, where strategies such as biostimulation, bioaugmentation, landfarming, and bioelectrochemical systems are tailored to optimize oxygen supply, nutrient availability, and electron-acceptor conditions so as to channel PHs through these convergent catabolic pathways toward complete mineralization [32]. The degradation pathways depicted above highlight the enzymatic and metabolic complexity involved in the breakdown of petroleum hydrocarbons. Microbial degradation proceeds through a series of oxidation and transformation steps, converting complex hydrocarbons into simpler intermediates that can be assimilated into central metabolic pathways. The efficiency of these processes depends on multiple factors, including microbial diversity, enzyme specificity, and environmental conditions such as oxygen availability and nutrient levels. Understanding these mechanisms is essential for optimizing bioremediation systems, as it allows for targeted enhancement of microbial activity and improved degradation efficiency in contaminated environments.

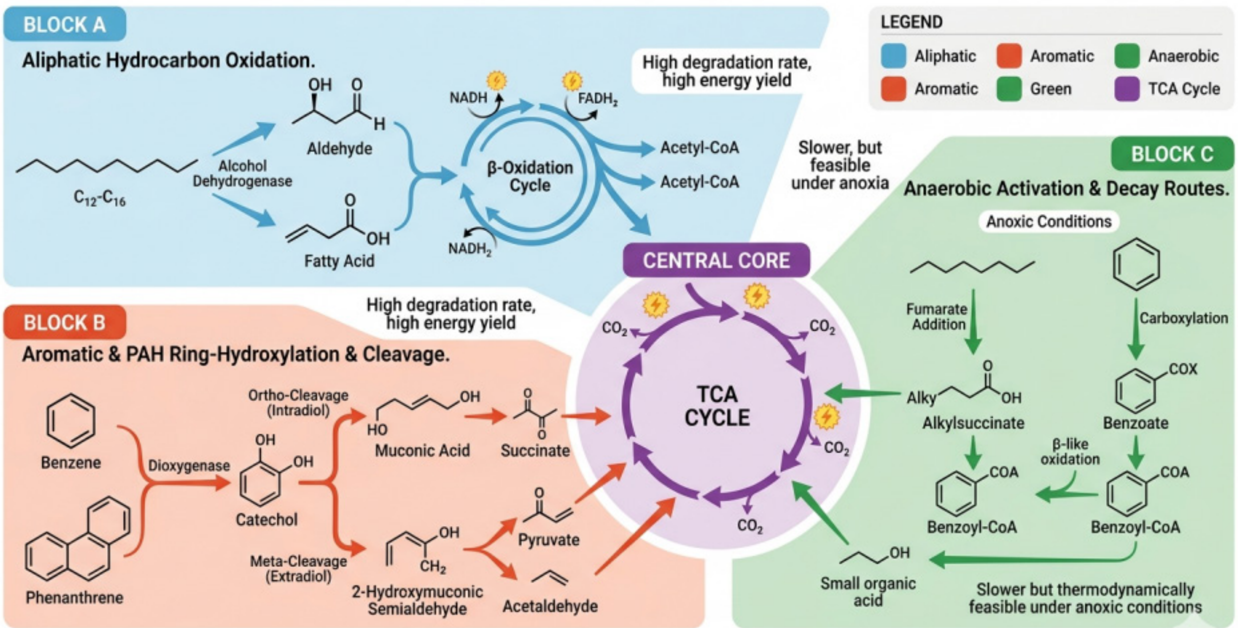

Figure 3. Schematic overview of microbial petroleum hydrocarbon degradation pathways.

Figure 3 illustrates the primary enzymatic strategies and catabolic logic employed by microorganisms to metabolize diverse Petroleum Hydrocarbons (PHs), emphasizing the differences in activation mechanisms, degradation speed, and thermodynamic feasibility. Arrows indicate the direction of metabolic flow, converging on common central intermediates. **Block A (Blue):** Aliphatic Alkane Oxidation. This block visualizes the aerobic activation of an n-alkane (C_{12}-C_{16}). A specific alkane monooxygenase enzyme introduces oxygen to yield a primary alcohol, followed by oxidation to an aldehyde and then a fatty acid (e.g., palmitic acid). The fatty acid enters the β-oxidation spiral (where NADH and $FADH_2$ are generated), cleaving two-carbon units in each cycle to produce multiple molecules of Acetyl-CoA for energy. Annotation: "High degradation rate, high energy yield". **Block B (Orange):** Aromatic & PAH Ring Hydroxylation & Cleavage. This block shows how complex cyclic structures are activated aerobically. Simple aromatics (Benzene/BTEX) and Polycyclic Aromatic Hydrocarbons (Phenanthrene, 3 rings) are initially hydroxylated by specialized dioxygenase enzymes to form dihydrodiols, which are further dehydrogenated to converge on catechol. Two common branching pathways emerge from catechol, yielding different central intermediates for the TCA cycle: 1. Ortho-Cleavage (Intradiol): Ring cleavage between hydroxyl groups, leading to the production of Succinate and Acetaldehyde. 2. Meta-Cleavage (Extradiol): Ring cleavage adjacent to a hydroxyl group, yielding Pyruvate and Acetaldehyde. Annotation: "High degradation rate, high energy yield". **Block C (Green):** Anaerobic

Activation & Decay Routes. This block contrasts the aerobic pathways, visualizing the catabolic strategies used by specialized microbes under strictly anoxic conditions (e.g., nitrate-, sulfate-, or methanogenic-reducing environments). Distinct activation mechanisms, such as Fumarate Addition (alkanes/BTEX) or Carboxylation (aromatics), are highlighted as initial, often slow, activation steps. These unique activation products are subsequently funneled into specialized anoxic β-like oxidation paths to produce Benzoyl-CoA and, ultimately, simpler inputs for the central core. Annotation: "Slower but thermodynamically feasible under anoxic conditions". **Central Core (Purple):** TCA CYCLE (Central Metabolic Hub). This prioritized diagram emphasizes that all specialized peripheral pathways visually converge on a universal central metabolic loop. The TCA cycle (accepting Acetyl-CoA, Succinate, and Pyruvate from the various PH degradation routes) serves as the ultimate engine for generating significant energy and cellular precursor molecules while releasing CO_2 as a byproduct of respiration.

7. Bioremediation Techniques

The mechanistic principles of microbial bioremediation are implemented through a range of techniques, each tailored to site-specific contamination levels, matrix characteristics, and logistical constraints. These techniques can be broadly grouped into in situ and ex situ approaches, with the former emphasizing minimal disturbance and lower cost, and the latter enabling more intensive control of environmental conditions at the expense of excavation and transport. Within this framework, biostimulation, bioaugmentation, landfarming, biopiling, bioslurping, and in situ bioreactors represent the most widely deployed strategies for PH-contaminated soils and sediments [32,33].

Biostimulation involves enriching indigenous microbial communities by manipulating nutrient supply, electron acceptors, and physicochemical conditions without introducing exogenous strains. In contaminated soils, nitrogen and phosphorus are commonly limiting, and their amendment (often at C:N:P ratios of 100:10:1) has been shown to stimulate hydrocarbonoclastic populations and increase TPH removal by 50–80% over several months in field trials [33]. Aeration, achieved through tilling, mechanical mixing, or air injection, further enhances aerobic degradation by improving oxygen diffusion and disrupting hydrophobic layers that impede microbial access.In groundwater systems, in situ bioremediation is often combined with pump-and-treat systems or direct push injection to deliver oxygen, nutrients, and electron acceptors into the subsurface [34].

Bioaugmentation supplements biostimulation with the introduction of selected microbial cultures or consortia known to degrade target PH fractions. For example, consortia containing *Pseudomonas*, *Rhodococcus*, and *Acinetobacter* have been applied to PAH-contaminated soils, achieving 60–90% removal of 3–4-ring PAHs within 8–12 weeks under controlled conditions. However, field-scale outcomes are highly variable, with success depending on pre-adaptation of inocula, compatibility with indigenous communities, and avoidance of starvation or predation [35].

Landfarming and biopiling are ex situ techniques that involve spreading contaminated soil in thin layers (landfarming) or stacked windrows (biopile) to facilitate aeration, nutrient mixing, and microbial activity. Under optimized conditions, landfarming can remove 60–80% of TPH within 6–12 months, although heavy-crude or asphaltene-rich matrices may require longer treatment or complementary physicochemical pretreatment. Biopiling similarly enhances degradation rates by concentrating heat and organic matter, often achieving >70% TPH reduction within weeks to months, depending on matrix size and climate [36].

8. Phytoremediation and Plant–Microbe Interactions

Phytoremediation represents a synergistic strategy that couples plant-based technologies with microbial degradation to enhance the removal, transformation, and immobilization of petroleum hydrocarbons (PHs) in contaminated soils and wetlands. In this approach, plants act as more than passive filters; they modify the rhizosphere through root exudation, oxygenation, and hydraulic redistribution, thereby creating microhabitats that favor hydrocarbon-degrading bacterial and fungal communities [9]. The resulting plant–microbe interactions amplify bioavailability, stimulate microbial activity, and extend the effective treatment volume beyond the immediate root zone, making phytoremediation a particularly attractive option for large-area, low-to-moderate PH contamination [37].

Three primary phytoremediation mechanisms operate in concert: phytostabilization, phytodegradation, and phytovolatilization. Phytostabilization involves the immobilization of PHs on root surfaces or in the rhizosphere through adsorption, complexation, and sequestration, thereby reducing leaching and bioavailability while allowing gradual microbial attenuation. Phytodegradation relies on plant enzymes (e.g., peroxidases, laccases, and cytochrome P450s) that can transform hydrocarbons into polar, less toxic metabolites, which may then be mineralized by rhizosphere microbes or sequestered in plant tissues [38]. Phytovolatilization, often under-emphasized in many reviews, refers to the uptake of volatile PHs such as BTEX through roots, translocation to shoots, and release into the atmosphere via stomatal emission. Although this process can reduce soil concentrations, it must be carefully managed due to the risk of atmospheric dispersion and secondary air-pollution concerns, particularly in urban settings or near sensitive receptors [38].

The rhizosphere is a hotspot of microbial activity, where low-molecular-weight root exudates (e.g., sugars, organic acids, amino acids) serve as readily utilizable carbon sources that support a diverse microbiome enriched in hydrocarbon-degrading taxa. Genus-level groups such as *Pseudomonas*, *Rhizobium*, *Burkholderia*, and *Plantibacter* are frequently enriched in the rhizosphere of oil-tolerant plants like *Phragmites australis*, *Spartina alterniflora*, and selected grasses, where they degrade aliphatic and aromatic PHs via pathways analogous to those occurring in bulk soil [39]. In some cases, rhizosphere communities have been shown to increase TPH removal by 20–40% compared to unplanted controls, highlighting the catalytic role of plant-derived substrates and micro-oxygenation in sustaining aerobic metabolism even in partially saturated soils [40].

The diversity of plant–microbe interactions outlined above reflects the adaptive capacity of phytoremediation systems to function across a wide range of contaminated environments. Variations in total petroleum hydrocarbon removal efficiency are influenced not only by plant species and associated microbial consortia but also by site-specific factors such as soil composition, contamination level, and environmental conditions. While many systems demonstrate promising degradation efficiencies, persistent uncertainties remain regarding long-term stability, intermediate metabolite accumulation, and ecological safety. These limitations emphasize the importance of integrating phytoremediation with complementary approaches and optimizing system design through controlled environmental management. Building on these insights, the next section examines emerging technologies that combine biological systems with advanced materials and genetic tools to enhance remediation efficiency and predictability. Plant–microbe combinations such as *Phragmites australis*, *Pseudomonas* and *Spartina alterniflora Alcanivorax* can achieve 60–80% TPH reduction within one to two growing seasons, significantly outperforming unplanted controls and demonstrating the catalytic role of the rhizosphere in sustaining hydrocarbonoclastic activity (**Table 3**). However, the table also highlights several research gaps, including limited long-term data on performance under extreme climatic stress (e.g., prolonged drought, flooding, or salinity fluctuations), incomplete characterization of partially oxidized intermediates such as PAH-derived quinones in the rhizosphere, and insufficient quantification of phytovolatilization-mediated exposure risks to adjacent terrestrial and aquatic ecosystems [41]. These unresolved issues underscore the need for standardized multi-season field trials, non-invasive monitoring of volatile emissions, and improved integration of phytoremediation outcomes into broader ecological and human-health risk-assessment frameworks that will be revisited in the Discussion and Future Perspectives sections.

The next section extends this framework to emerging technologies that integrate phytoremediation with advanced materials, genetic engineering, and systems-level "omics," thereby broadening the scope and robustness of PH bioremediation.

Table 3. Plant-microbe combinations used in phytoremediation of petroleum hydrocarbon (PH) contaminated soils and wetlands.

Plant Species/Type	Associated Microbial Partners (Examples)	Typical TPH Removal Efficiency (%)	Treatment Duration	Key Observations and Research Gaps	References
Phragmites australis (common reed)	*Pseudomonas* spp., *Rhizobium* spp., *Burkholderia* spp.	60–80%	6–12 months (one growing season)	Strong rhizosphere stimulation; limited data under extreme salinity/drought and long-term performance	Chen et al., 2020 [9], Das and Tiwari, 2018 [20]
Spartina alterniflora (cordgrass)	*Alcanivorax* spp., *Marinobacter* spp., *Pseudomonas* spp.	55–75%	1–2 growing seasons	Effective in saline environments; unclear fate of partially oxidized PAHs	Chen et al., 2020 [9]
Zea mays (maize)	*Pseudomonas* spp., *Bacillus* spp., *Rhizobium* spp.	40–65%	3–6 months	Suitable for moderate contamination; phytovolatilization of BTEX insufficiently quantified	Abu Bakar et al., 2020 [13]
Medicago sativa (alfalfa)	*Sinorhizobium* spp., *Pseudomonas* spp., *Arthrobacter* spp.	50–70%	4–8 months	Nitrogen fixation enhances degradation; long-term microbiome effects poorly studied	Chen et al., 2020 [9], Xu et al., 2018 [14]
Avena sativa (oat)	*Pseudomonas* spp., *Sphingomonas* spp.	35–60%	3–6 months	Fast-growing; limited data under high-PAH stress and repeated exposure cycles	Abu Bakar et al., 2021 [13], Xu et al., 2018 [14]
Brassica napus (rapeseed)	*Pseudomonas* spp., *Rhodococcus* spp., *Bacillus* spp.	45–65%	2–4 months	High biomass; potential accumulation of toxic intermediates in plant tissues	Correa- García et al., 2018 [19]
Eichhornia crassipes (water hyacinth)	*Pseudomonas* spp., *Acinetobacter* spp., *Bacillus* spp.	40–60%	1–3 months	Effective in aquatic systems; risk of phytovolatilization exposure	Wilkes et al., 2016 [18]
Typha latifolia (cattail)	*Pseudomonas* spp., *Rhizobium* spp., *Actinobacteria* spp.	50–75%	1–2 growing seasons	Wetland adaptability; limited data on persistence of PAH intermediates	Haritash et al., 2009 [11]
Lolium perenne (ryegrass)	*Pseudomonas* spp., *Rhizobium* spp., *Streptomyces* spp.	45–65%	6–12 months	Widely used; incomplete degradation products sometimes detected	Xu et al., 2018 [14]
Grass mixtures (naturalized)	Mixed rhizosphere communities (*Pseudomonas*, *Rhizobium*, *Micrococcus*)	40–70% (variable)	1–2 years	Cost-effective; performance under extreme climates poorly documented	Correa- García et al., 2018 [19]

Note: Representative plant-microbe combinations used in phytoremediation of petroleum hydrocarbon contaminated soils and wetlands, compiled from field, mesocosm, and pot-scale studies. TPH removal efficiency refers to the reported reduction in total petroleum hydrocarbons over the stated treatment duration, with ranges reflecting variability across sites and experimental conditions. The "key observations and research gaps" column highlights documented performance trends and outstanding uncertainties regarding long-term resilience, intermediate metabolite persistence, and potential phytovolatilization-mediated exposure.

9. Emerging Technologies in Bioremediation

Recent advances in biotechnology are transforming petroleum-hydrocarbon remediation from a largely empirical practice into a more predictable, systems-oriented discipline. Emerging technologies ranging from nanobioremediation and genetically engineered microorganisms (GEMs) to high-throughput "omics" and bioelectrochemical systems (BES) offer the potential to accelerate degradation, extend substrate range, and enable real-time monitoring and adaptive control [42].

9.1. Nanobioremediation

Nanobioremediation exploits engineered nanoparticles (e.g., zero-valent iron [nZVI], titanium dioxide [TiO_2], graphene oxide, and metal-organic frameworks) to enhance microbial activity, facilitate pollutant oxidation, or immobilize hydrophobic fractions. For instance, nZVI functions both as a reductant and a carrier for hydrocarbonoclastic bacteria, increasing TPH removal by 20–40% compared with microbial treatment alone in heavily contaminated soils [28,42]. In TiO_2-based photocatalytic systems, UV irradiation generates reactive oxygen species (ROS) that oxidize BTEX and certain PAHs, with mesocosm-scale studies reporting 60–80% removal of low-ring PAHs (e.g., naphthalene and phenanthrene) within 7–14 days under controlled light intensity [43].

However, the environmental implications of nanoparticle persistence and toxicity remain unresolved. Chronic exposure experiments with earthworms and soil microbes show that concentrations above 100 mg kg^{-1} of nZVI or TiO_2 can reduce microbial biomass by 25–50% and alter community composition, raising concerns about long-term ecological impacts and secondary contamination. Regulatory frameworks for nanomaterial release in remediation are still evolving, necessitating life-cycle assessments and site-specific toxicity testing before large-scale deployment [44].

9.2. Genetically Engineered Microorganisms (GEMs) and Synthetic Biology

Genetically engineered microorganisms (GEMs) represent a powerful frontier, in which hydrocarbon-catabolic pathways are optimized, broadened, or integrated into robust chassis strains to enhance degradation kinetics and substrate specificity [45]. Engineered Pseudomonas putida and Acinetobacter strains expressing additional alkane hydroxylases or PAH dioxygenases have been reported to degrade multi-ring PAHs and even some asphaltene-associated fractions under laboratory conditions, with 2–3-fold higher degradation rates than wild-type counterparts. In one mesocosm trial, an engineered *P. putida* strain expressing a hybrid naphthalene dioxygenase-encoding plasmid removed 75% of phenanthrene from artificially contaminated soil within 4 weeks, versus 45% in unplanted, non-augmented controls [46,47].

Despite these promising results, regulatory and public-acceptance barriers limit the field-scale use of GEMs, particularly due to concerns about unintended release, horizontal gene transfer, and co-selection of antibiotic resistance genes. Field-scale trials with GEMs are rare, and where they occur, containment strategies (e.g., suicide genes, nutrient-dependent promoters) are essential to prevent stable colonization [47]. Future directions include the development of non-living biocatalysts (cell-free enzyme systems) and genetically recorded organisms with reduced capacity for gene transfer, which may reconcile high performance with biosafety requirements.

9.3. High-Throughput Omics and Predictive Microbiome Engineering

High-throughput "omics" technologies, such as metagenomics, metatranscriptomics, metaproteomics, and metabolomics, are providing unprecedented resolution of microbial community structure and function in PH-impacted ecosystems. By identifying key degraders, regulatory networks, and metabolic bottlenecks, these approaches enable the rational design of microbial consortia, nutrient regimes, and process conditions that maximize degradation efficiency [48]. For example, metagenomic surveys of oil-spill-affected coastal soils have revealed enrichment of hydrocarbonoclastic genera such as Alcanivorax, Marinobacter, and Thalassolituus, all carrying alkB and nah gene clusters, which can be leveraged to guide bioaugmentation strategy [49].

Machine-learning-assisted analyses of metagenomic time-series have begun to predict TPH removal trajectories with >80% accuracy in controlled mesocosms, by correlating shifts in alkB- and nah-carrier abundance with observed degradation rates. Nevertheless, integrating omics-driven insights into operational monitoring systems remains a challenge due to variability in sequencing depth, bioinformatics pipelines, and metadata reporting [50]. Future work will need to standardize sampling protocols, develop open-source data platforms, and couple omics outputs with physicochemical models to create predictive, decision-support tools for site managers [51].

9.4. Bioelectrochemical Systems (BES) and Hybrid Configurations

Bioelectrochemical systems (BES), including microbial fuel cells (MFCs) and bioelectrochemical reactors, exploit electroactive microbial communities to oxidize PHs and generate electrical current or valuable metabolites simultaneously. In such systems, exoelectrogenic bacteria transfer electrons derived from hydrocarbon oxidation to an anode via cytochrome-based electron-shuttling systems or conductive nanowires, enabling energy recovery under controlled potential or current conditions [52]. Laboratory-scale BES treating diesel-contaminated sand have demonstrated 70–80% TPH removal within 40–60 days, with concurrent power densities of 20–80 mW m^{-2}, depending on electrode material and hydraulic retention time [53].

Hybrid configurations that couple BES with biostimulation or phytoremediation are particularly promising. For example, a BES–phytoremediation setup tested in a greenhouse trial on gasoline-impacted soil achieved 85% TPH removal over 12 weeks, with the plant-root micro-aeration and MFC anode jointly enhancing microbial activity near the anode root interface [54]. However, scaling BES to field applications is constrained by electrode cost, reactor design, and the need for continuous monitoring and maintenance, suggesting that modular, low-cost BES units integrated into conventional bioremediation workflows will be more practical than stand-alone installations [55]. These emerging technologies collectively signal a shift toward data-driven, adaptive, and possibly energy-positive remediation systems, in which microbial degradation is not only a terminal treatment step but also a component of integrated resource-recovery landscapes [53].

10. Limitations and Challenges

Despite notable advances, microbial and plant-based bioremediation of petroleum hydrocarbons faces several persistent limitations and systemic challenges that constrain its predictability, scalability, and long-term sustainability. Chief among these is the problem of incomplete degradation, particularly for high-molecular-weight PAHs, branched alkanes, and asphaltenic fractions, which often leave behind partially oxidized intermediates that may be more toxic or recalcitrant than the parent compounds [56]. In some cases, these intermediates accumulate in soil or sediments, necessitating secondary treatment or long-term monitoring to prevent downstream ecological and health impacts [57].

Environmental variability is another major obstacle, as fluctuations in temperature, moisture, pH, redox conditions, and nutrient availability can significantly alter microbial community composition and metabolic activity across seasons and spatial gradients [58]. Heterogeneous soil structure, subsurface stratification, and preferential flow paths further complicate the uniform distribution of nutrients, electron acceptors, and inocula, often resulting in patchy degradation patterns and "hot spots" of residual contamination. Field-scale trials have repeatedly shown that laboratory-optimized conditions rarely translate directly to complex real-world systems, underscoring the need for adaptive monitoring and dynamic process control [59].

From a biological standpoint, the introduction of exogenous microbial strains or consortia through bioaugmentation is fraught with uncertainties related to competition with indigenous communities, predator-prey interactions, and genetic instability. Some engineered or non-native strains may fail to establish, or may experience down-regulation of key catabolic genes once environmental conditions diverge from laboratory benchmarks [60]. Moreover, exposure to contaminated matrices can promote co-selection of antibiotic resistance genes (ARGs), as hydrocarbon stress and associated heavy-metal co-contamination create selective pressure favoring resistant genotypes. The spread of ARGs through horizontal gene transfer poses non-trivial public-health risks and complicates the regulatory approval of GEMs and bioaugmentation products [61,62].

Regulatory and socio-economic constraints further impede the deployment of advanced bioremediation technologies, particularly nanobioremediation and GEMs. Stringent biosafety regulations, limited insurance coverage, and low public trust in genetic engineering often delay or prevent field trials, while unclear liability frameworks complicate long-term stewardship of remediated sites [28]. Economically, although bioremediation is generally cheaper than physicochemical alternatives, the upfront investment in monitoring, infrastructure, and skilled personnel can be prohibitive for low- and middle-income regions, even where PH contamination is most severe [11].

These limitations underscore the need for integrated, multi-process remediation strategies that combine the strengths of conventional methods, microbial bioremediation, phytoremediation, and emerging technologies under a unified risk-assessment and governance framework. The next section outlines future perspectives that could guide research, policy, and practice in the coming decade

11. Future Perspectives

The future of petroleum-hydrocarbon bioremediation lies in the integration of systems biology, digital monitoring, and circular-economy principles into holistic, site-adaptive remediation frameworks. Advances in high-throughput "omics" and machine-learning models are expected to enable the development of predictive tools that can forecast microbial community responses, degradation trajectories, and risk profiles for diverse PH-contaminated environments, thereby reducing reliance on trial-and-error approaches. Such models could be calibrated with field-scale data from long-term monitoring networks, providing decision-support systems for regulators, engineers, and communities [63].

Hybrid remediation schemes that combine bioremediation with phytoremediation, bioelectrochemical systems, and carefully designed nanomaterials offer a promising pathway toward more robust and scalable solutions. For example, plant-based systems can be integrated with BES to simultaneously remove PHs and generate renewable energy, while nanomaterials can be deployed as targeted delivery systems for microbes or nutrients in deep-rooted or subsurface contamination zones [64]. These hybrid configurations will require standardized protocols for performance evaluation, lifecycle analysis, and risk assessment, as well as clear guidelines for nanomaterial fate and impact.

Policy and governance frameworks must evolve to keep pace with technological innovation, ensuring that regulatory scrutiny is proportionate, science-based, and responsive to emerging evidence [65,66]. International collaborations could facilitate the harmonization of risk-assessment criteria, monitoring protocols, and liability mechanisms, particularly for transboundary contamination and offshore oil-spill scenarios. At the local level, participatory approaches that involve affected communities in monitoring, decision-making, and stewardship can enhance social acceptance and long-term sustainability of remediation projects [67].

Finally, training the next generation of environmental engineers, microbiologists, data scientists, and policy specialists in interdisciplinary problem-solving will be essential to bridge the gap between laboratory-scale discoveries and real-world implementation [68]. By aligning fundamental research with practical needs and ethical considerations, the field of microbial bioremediation can mature from a reactive clean-up tool into a proactive, predictive, and preventive pillar of global environmental management. With this foundation in place, Sections 12 and 13 (Discussion and Conclusion) will provide a critical synthesis and concise forward outlook, deliberately avoiding repetition while emphasizing actionable insights.

12. Discussion

The evidence synthesized in this review reveals that microbial bioremediation of petroleum hydrocarbons (PHs) is a context-dependent, systems-level intervention rather than a plug-and-play technology, with success contingent on the interplay between microbial ecology, geochemistry, engineering design, and governance [5,13]. Across laboratory, pilot, and field studies, bioremediation consistently achieves 60–90% removal of total petroleum hydrocarbons (TPH) over weeks to months, particularly for aliphatic and low- to mid-molecular-weight aromatic fractions, provided that nutrient supply, oxygen availability, and community structure are appropriately managed [17,25]. However, this performance envelope is highly heterogeneous: some PH-impacted coastal soils report TPH reductions of 85–95% within 3–6 months under optimized biostimulation, whereas similar strategies in heavy-crude-contaminated industrial soils may remove only 40–60% over 12–18 months, highlighting the influence of matrix composition and climatic variability [30,32].

Critical Synthesis of Bioremediation Performance and Limitations

A key insight emerging from recent work is that microbial communities in contaminated sites are highly plastic, with rapid acquisition and reconfiguration of catabolic traits through plasmid-borne gene clusters, integrative elements, and horizontal gene transfer [39,44]. This plasticity enhances adaptive potential and underpins the success of spontaneous natural attenuation at many oil-spill sites, where Alcanivorax, *Marinobacter*, and *Thalassolituus* populations expand within days of hydrocarbon exposure and drive initial TPH declines [50,52]. However, the same mechanisms that confer resilience also create vulnerabilities, as PH-contaminated soils and sediments often show co-enrichment of hydrocarbon-degradation genes and antibiotic resistance genes (ARGs), potentially amplifying selective pressure for resistant genotypes upon fertilization or bioaugmentation [55]. Metagenomic surveys from PAH- and oil-impacted sites report up to 3–10-fold enrichment of ARGs encoding efflux pumps and β-lactamases relative to uncontaminated controls, underscoring the need for precautionary risk assessment when deploying nutrient amendment or GEMs [58,61].

From a comparative standpoint, bioremediation outperforms conventional physicochemical methods in terms of energy efficiency, cost, and ecological footprint but tends to lag in predictability and speed, especially for recalcitrant fractions such as four- to six-ring PAHs and asphaltene-rich matrices. In aerobic systems, where oxygenase-driven pathways dominate, biodegradation rates decline sharply beyond C_{20} n-alkanes and highly substituted PAHs, while anaerobic routes, though thermodynamically feasible, often proceed at slower rates and may yield incomplete mineralization [64,69]. This mechanistic limitation implies that bioremediation is most effective when treated as a component within a hybrid remediation framework, where biostimulation, biopiling, and phytoremediation are staged with physicochemical pretreatment, such as chemical oxidation or thermal desorption of the most recalcitrant fractions [67]. Multi-process field trials conducted at refinery sites in the USA and Europe report that such hybrid schemes can increase overall TPH removal by 15–30% relative to standalone bioremediation, while shortening treatment time and reducing residual hot spots.

13. Conclusion

Microbial bioremediation of petroleum hydrocarbon pollutants represents a scientifically mature yet still evolving field that offers a sustainable, cost-effective alternative to conventional physicochemical remediation methods [6,13]. By harnessing the metabolic versatility of indigenous and engineered bacteria, along with synergistic plant microbe interactions, bioremediation can achieve substantial degradation of aliphatic and aromatic hydrocarbons in diverse environments, typically removing 60–90% of total petroleum hydrocarbons within weeks to months under optimized conditions [16,24]. However, incomplete degradation of high-molecular-weight PAHs and asphaltenes, environmental variability, and the potential for co-selection of antibiotic resistance genes remain significant limitations that constrain reliability and predictability at the field scale [29,31].

Practically, the most promising pathway forward lies in the integration of bioremediation with phytoremediation, nanobioremediation, bioelectrochemical systems, and other advanced technologies under unified, risk-informed frameworks. Such hybrid approaches can broaden substrate range, accelerate degradation kinetics, and reduce secondary waste, while simultaneously generating renewable energy or valuable byproducts in some configurations [38,42]. To realize this potential, stakeholders must invest in long-term field trials, standardized monitoring protocols, and accessible data platforms that bridge the gap between laboratory-scale discoveries and real-world implementation [50,53].

Looking ahead, microbial bioremediation will increasingly converge with systems biology, digital monitoring, and circular-economy thinking, enabling more predictive, adaptive, and socially responsible environmental management [58,60]. By aligning scientific innovation with robust governance, public engagement, and equitable access, the field can evolve from a reactive clean-up tool into a proactive strategy for safeguarding soil, water, and human health in the era of persistent petroleum-derived pollution [67].

Abbreviations

ARGs—Antibiotic Resistance Genes

ATP—Adenosine Triphosphate

CO_2—Carbon Dioxide

DNA—Deoxyribonucleic Acid

DNA-seq—DNA Sequencing

EPS—Extracellular Polymeric Substance

FAD—Flavin Adenine Dinucleotide

$FADH_2$—Flavin Adenine Dinucleotide (Reduced Form)

GC—Gas Chromatography

GD–MS—Gas Chromatography–Mass Spectrometry

GEM—Genetically Engineered Microbe

GEMs—Genetically Engineered Microorganisms

H_2O—Water

HPLC—High-Performance Liquid Chromatography

LC–MS—Liquid Chromatography–Mass Spectrometry

mg/L—Milligrams per Liter

NAD^+—Nicotinamide Adenine Dinucleotide (Oxidized Form)

NADH—Nicotinamide Adenine Dinucleotide (Reduced Form)

NMR—Nuclear Magnetic Resonance

OMICS—Genomics, Transcriptomics, Proteomics and Metabolomics (collectively)

PAH—Polycyclic Aromatic Hydrocarbon

PAHs—Polycyclic Aromatic Hydrocarbons

PCR—Polymerase Chain Reaction

PGPR—Plant Growth-Promoting Rhizobacteria

PGPRs—Plant Growth-Promoting Rhizobacteria (plural)

PHs—Petroleum Hydrocarbons

ppm—Parts Per Million
PWHCs—Petroleum Waste Hydrocarbons
qPCR—Quantitative Polymerase Chain Reaction
qRT–PCR—Quantitative Reverse Transcription Polymerase Chain Reaction
rpm—Revolutions Per Minute
SIP—Stable Isotope Probing
TPH—Total Petroleum Hydrocarbon
TDS—Total Dissolved Solids
USEPA—United States Environmental Protection Agency
UV—Ultraviolet
UVI–Vis—Ultraviolet–Visible Spectroscopy
VOC—Volatile Organic Compound
VOCs—Volatile Organic Compounds
WHO—World Health Organization
°C—Degree Celsius
3D—Three-Dimensional

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The data used in this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest regarding this publication.

AI Use Statement

The authors declare that no artificial intelligence (AI) tools were used in the preparation of this manuscript.

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