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Strategies to Enhance Bioremediation of Petroleum Hydrocarbon Degradation in Contaminated Environments: A Review

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ABSTRACT

Background: Petroleum hydrocarbon contamination poses significant environmental and health risks due to its persistence and widespread occurrence. Bioremediation offers a sustainable, cost-effective solution by leveraging microbial degradation mechanisms.
Methods: A literature review was conducted using databases such as PubMed, Web of Science, and Scopus, focusing on peer-reviewed articles published within the last decade. Keywords related to petroleum hydrocarbon bioremediation guided the search, while inclusion/exclusion criteria ensured relevance and quality.
Results: The study revealed a wide array of microbial metabolic pathways capable of degrading hydrocarbons under both aerobic and anaerobic conditions, highlighting enzymatic versatility and genetic adaptability. Innovative enhancement strategies such as bioaugmentation with specialized consortia, biostimulation through nutrient optimization, surfactant-induced bioavailability improvement, and integration with nanotechnology and synthetic biology demonstrated substantial increases in remediation efficiency. Monitoring advancements, including molecular tools and real-time sensors, improved process understanding and control. The emergence of novel contaminants and complex mixtures necessitates adaptive, multidisciplinary approaches.
Conclusion: This review underscores the transformative potential of integrated bioremediation technologies in tackling petroleum pollution. Strategic combinations of microbial engineering, advanced materials, and ecological insights offer scalable solutions. These findings advocate for policy alignment and interdisciplinary collaboration to achieve sustainable environmental restoration.

1. Introduction

1.1 Background on petroleum hydrocarbon contamination

Petroleum hydrocarbons, organic molecules composed of carbon and hydrogen, are ubiquitous environmental pollutants primarily derived from crude oil. These contaminants have become increasingly prevalent due to various human activities; including oil spills, leaking storage tanks, and transportation accidents. The pervasive nature of petroleum hydrocarbon pollution has led to widespread contamination of soil, water, and air, posing significant challenges to environmental management and ecosystem health. The complexity of petroleum hydrocarbon mixtures, which can include aliphatic, aromatic, and heterocyclic compounds, contributes to the persistence and toxicity of these pollutants in the environment. Understanding the sources, distribution, and behavior of petroleum

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hydrocarbons in different environmental matrices is crucial for developing effective remediation strategies and mitigating their long-term impacts on ecosystems and human health (Baghaie, 2022).

1.2 Environmental and health impacts of petroleum pollution

The environmental and health impacts of petroleum pollution are far-reaching and multifaceted, affecting various components of ecosystems and human well-being. In soil environments, petroleum hydrocarbons can significantly alter physical, chemical, and biological properties, leading to reduced fertility, impaired water retention, and disrupted microbial communities. These changes can have significant effects on plant growth, agricultural productivity, and overall ecosystem function. Aquatic ecosystems are particularly vulnerable to petroleum pollution, with oil spills causing immediate and long-term damage to marine and freshwater habitats, biodiversity, and food webs. The persistence of petroleum hydrocarbons in the environment can lead to bioaccumulation and biomagnification in organisms, potentially affecting entire trophic levels. Human health risks associated with petroleum pollution include exposure to carcinogenic compounds, respiratory issues, and other chronic health effects. The contamination of groundwater resources by petroleum hydrocarbons poses a significant threat to drinking water quality and public health, particularly in regions with limited access to alternative water sources. Additionally, the release of volatile organic compounds from petroleum sources contributes to air pollution, exacerbating respiratory problems and contributing to the formation of ground-level ozone (O_3) (Baghaie et al., 2020).

1.3 Overview of bioremediation as a sustainable solution

Bioremediation has emerged as a promising and sustainable approach for addressing petroleum hydrocarbon contamination in various environmental matrices. This eco-friendly technology harnesses the natural ability of microorganisms to degrade complex organic pollutants, transforming them into less harmful or non-toxic substances. Bioremediation offers several advantages over conventional physicochemical remediation methods, including cost-effectiveness, minimal environmental disruption, and the potential for in situ application. The process relies on optimizing environmental conditions to enhance the growth and metabolic activities of indigenous or introduced microorganisms capable of degrading petroleum hydrocarbons. Recent advances in bioremediation techniques have focused on developing integrated approaches that combine biostimulation (enhancing the activity of native microbial populations) and bioaugmentation (introducing specialized microbial strains) to maximize degradation efficiency (Lopes et al., 2022; Muter, 2023). The application of bioremediation extends beyond soil and water systems, with emerging research exploring its potential in addressing air pollution and volatile organic compounds associated with petroleum contamination.

1.4 Objectives and scope of the review

This comprehensive review aims to synthesize and critically evaluate the latest advancements in strategies to enhance bioremediation of petroleum hydrocarbon degradation in contaminated environments. The primary objectives of this review are to: (1) elucidate the fundamental mechanisms underlying microbial degradation of petroleum hydrocarbons; (2) assess the effectiveness of various bioremediation enhancement strategies, including bioaugmentation, biostimulation, and surfactant-enhanced approaches; (3) explore the integration of advanced technologies, such as nanotechnology and genetic engineering, in improving bioremediation efficiency; (4) evaluate monitoring and assessment techniques for tracking bioremediation progress; and (5) identify future research directions and challenges in the field of petroleum hydrocarbon bioremediation. The scope of this review encompasses a wide range of contaminated environments, including soil, groundwater, marine ecosystems, and industrial sites. By providing a comprehensive analysis of current research and emerging trends, this review aims to inform environmental scientists, policymakers, and practitioners about the most effective and innovative approaches for addressing petroleum hydrocarbon contamination through enhanced bioremediation strategies.

2. Materials and Methods

The Materials and Methods section of this review article outlines the systematic approach taken to gather, analyze, and synthesize the current literature on strategies to enhance bioremediation of petroleum hydrocarbon degradation in contaminated environments. This section is crucial for ensuring the reproducibility and reliability of the review process, providing a clear roadmap for readers to understand how the information was collected and evaluated. The subsections are interconnected, with each step building upon the previous one to create a comprehensive and rigorous methodology.

2.1 Literature search strategy

The literature search strategy employed in this review is designed to be comprehensive. Multiple electronic databases are utilized to ensure a wide coverage of relevant publications, including PubMed, Web of Science, Scopus, and specialized environmental science databases. The search terms are carefully selected to encompass various aspects of petroleum hydrocarbon bioremediation, including but not limited to "petroleum hydrocarbons," "bioremediation," "microbial degradation," "contaminated environments," and "enhancement strategies." Boolean operators (and, or) and truncation symbols are used to refine and expand the search as needed. The search is limited to peer-reviewed articles published in the last ten years (from January 2014 to December 2024) to focus on the most recent advancements in the field. Additionally, the reference lists of key articles and relevant review papers are manually screened to identify any additional studies that may have been missed in the initial database search. This multi-faceted approach ensures a thorough and up-to-date collection of literature on the topic (Figure 1).

2.2 Inclusion and exclusion criteria

To maintain the focus and relevance of the review, clear inclusion and exclusion criteria are established. Studies are included if they meet the following criteria: (1) focus on bioremediation of petroleum hydrocarbons in contaminated environments; (2) present original research, review articles, or meta-analyses; (3) published in peer-reviewed journals within the last ten years; (4) written in English; and (5) address strategies or techniques to enhance bioremediation efficiency. Exclusion criteria include: (1) studies focusing solely on non-biological remediation methods; (2) articles not directly related to petroleum hydrocarbon degradation; (3) conference abstracts or proceedings without full-text availability; (4) non-peer-reviewed publications; and (5) studies with insufficient methodological details. These criteria ensure that only high-quality, relevant studies are included in the review, providing a solid foundation for the analysis and synthesis of current knowledge in the field.

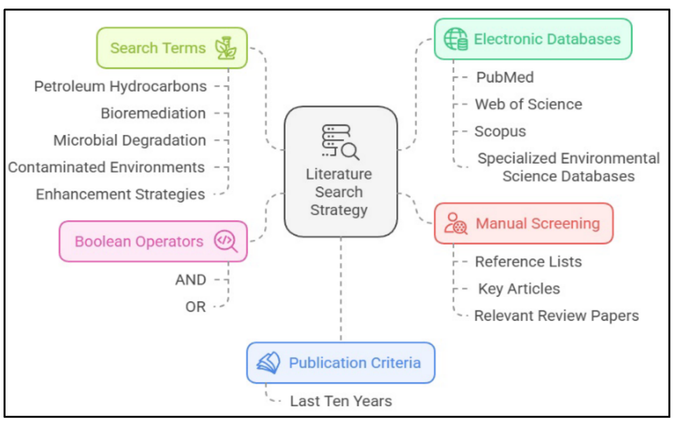

Figure 1. Comprehensive literature search strategy for bioremediation

3. Results and Discussion

3.1 Fundamental Mechanisms of Petroleum Hydrocarbon Biodegradation

The biodegradation of petroleum hydrocarbons is a complex process involving intricate metabolic pathways, specialized enzymes, and genetic adaptations (Tables 1 and 2). Understanding these fundamental mechanisms is crucial for developing effective bioremediation strategies and enhancing the natural degradation processes (Baghaie et al., 2024). This section explores the microbial metabolic pathways, key enzymes, and genetic basis underlying the degradation of petroleum hydrocarbons, providing insights into potential targets for optimization and engineering of more efficient bioremediation approaches.

3.1.1 Microbial metabolic pathways for hydrocarbon degradation

Microbial metabolic pathways for hydrocarbon degradation are diverse and highly specialized, reflecting the complex nature of petroleum hydrocarbons. These pathways can be broadly categorized into aerobic and anaerobic processes, each employing distinct enzymatic mechanisms. In aerobic degradation, oxygen serves as both a reactant for initial hydrocarbon activation and a terminal electron acceptor. The most common aerobic pathway involves the initial oxidation of alkanes by monooxygenases, followed by sequential oxidation to fatty acids, which are then processed through β-oxidation. Aromatic hydrocarbons are typically degraded through ring-cleaving dioxygenases, leading to the formation of catechols or protocatechuates. These intermediates undergo further ring cleavage and subsequent metabolism. Anaerobic degradation pathways, while less understood, have gained significant attention in recent years. These pathways often involve initial activation through fumarate addition, carboxylation, or hydroxylation, followed by β-oxidation-like reactions. The diversity of these pathways reflects the adaptability of microorganisms to various environmental conditions and substrate types (Azizi et al., 2024). Recent studies have revealed novel metabolic pathways and gene arrangements involved in hydrocarbon degradation. For instance, metagenomics analyses of oil reservoirs have uncovered unique functional metabolic pathways for aromatic compound biodegradation. These pathways often comprise genes belonging to different known degradation routes, showcasing novel gene arrangements and potentially new degradation mechanisms (Mohapatra & Phale, 2021). This genetic diversity highlights the adaptive capabilities of microbial communities in petroleum-contaminated environments and suggests the existence of yet undiscovered degradation pathways (Figure 2).

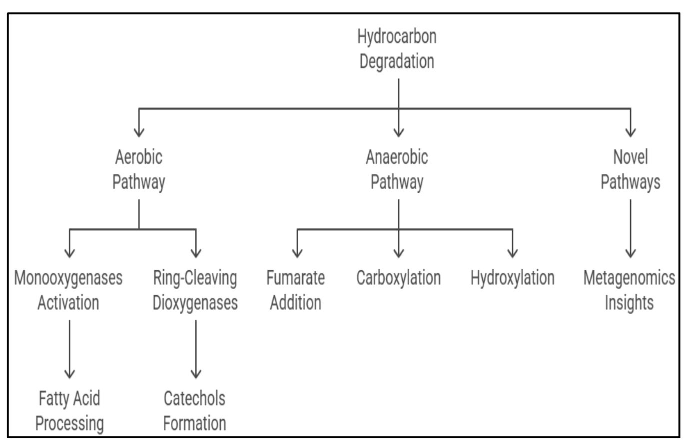

Figure 2. Microbial Hydrocarbon Degradation pathway

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Table 1. Bioremediation of Petroleum hydrocarbon-polluted soil by microorganisms (Mekonnen et al., 2024)

Microorganism	PHCs-degrading species Extraction/Isolation site	Substrate	Experiment Condition	% Of degradation
Proteobacteria groups	Diesel-degrading bacteria were isolated from soil samples contaminated by leaking diesel storage tanks.	TPH	TPH cons.: 36,328 mg kg-1, Period: 60 days, incubation temperature: 28°C	99
Bacillus sotensis	The strains were isolated from oily polluted soil	PAH	CN/P: 00/10/1, period: 160 days, temperature: 37°C, surfactant tween 80 (0.12% (v/v)), moisture: 10%, ppm	32.4
Fisrobacterium johnsoniae and Shewanella baltica	The bacteria isolate (BS1 and BS2) is used in the experiment	PAH	Temperature: 30°C, pH: 7	93.32
Bacillus subtilis (M16K, and M19F)	Isolated from Petroleum contaminated sediments and industrial wastewater and cultivated in Luria Berrain (LB) broth modified with 1% metal solutions	Crude oil Phenanthrene	Temp: 30 (±3) °C, for 28 days. Conc. 1% crude oil. Conc. 100 mg/L phenanthrene	>94.0
Pseudomonas aeruginosa (NCIM 5514)	Extracted from the soil of oil and natural gas sites	Crude oil PAHs	Temp: 37°C, Conc. 3% v/v, 180 rpm for 60 days,	61.0
Gordonia strain (Q8)	Isolated from water and crude oil using mineral salt medium (MM) at an adjusted pH of 7.0 and enrichment with 2 g/L crude oil incubated at 40°C	Naphthalene Phenanthrene Anthracene, Pyrene	Temp: 40°C for 7 days under aerobic conditions and 150 rpm	100
Proteobacteria Bacteroidetes and Firmicutes groups	Occurred from aged PAH-contaminated landfill soil	PAH	PAH content: 7,350 mg/kg-1, Soil pH: 6.5, Incubation period: 102 days, C: N ratio of pea straw: >22.1; Temperature: 28°C	66.6

Table 2. Bioremediation of Petroleum hydrocarbon-polluted soil by microorganisms (Mekonnen et al., 2024)

Microorganism	PHCs-degrading species Extraction/ Isolation site	Substrate	Experiment Condition	% Of degradation
P. ostreatus	Fungi species were isolated from Soil samples collected from Tamil Nadu, India	TPH	fungi were incubated in a room temperature, period of degradation; 21 days	87
Aspergillus sp	The hydrocarbon-degrading strains were isolated from the soil	n-alkanes	agitation speed of 150 rpm, Room temperature, 16 days	50.61
Paecilomyces formosus	Decay wood samples were collected, and antibiotics (0.01% of ampicillin and streptomycin) were used as selective media for the isolation of fungal strains	TPH	agitation at 120 rpm, degradation period: 60 days, incubation temperature at 30 °C	92 ± 2.35
Aspergillus niger Fusarium solani Curvularia lunata Trichoderma harzianum	Fungi were isolated from rhizospheric soil samples from the root of *L. aegyptiaca* using a serial dilution technique	kerosene diesel spent engine oil Crude oil	Incubation Temperature: 28°C; 40 days	78 70 83 77
Polyporus sp. S133	Extracted from a petroleum-polluted soil site, species are cultured by an enrichment method containing malt extract agar, glucose, polypeptide, and agar at 4°C using sterile woodmeal	Crude oil	Conc. 3 g of crude oil, 25°C, for 30 and 60 days	93
Aspergillus sp. RFC-1	The strain was extracted from crude Petroleum soil	Alkane (hexadecane)	Conc. 1% nhexadecane, 30°C, 130 rpm for 10 days	86.3

3.1.2 Key enzymes involved in petroleum hydrocarbon catabolism

The degradation of petroleum hydrocarbons relies on a suite of specialized enzymes that catalyze the initial activation and subsequent breakdown of these recalcitrant compounds. Alkane monooxygenases (AlkB) play a crucial role in the initial oxidation of alkanes, introducing oxygen atoms to form primary or secondary alcohols. These enzymes are often accompanied by electron transfer proteins such as rubredoxin (AlkG) and rubredoxin reductase (AlkT), which facilitate the electron flow necessary for the oxidation reaction. For aromatic hydrocarbons, ring-hydroxylating dioxygenases are key players, catalyzing the initial dihydroxylation of the aromatic ring. Subsequent steps involve ring-cleaving dioxygenases, which open the aromatic ring structure (Faisal, 2019). In anaerobic conditions, enzymes such as alkylsuccinate synthases catalyze the addition of fumarate to alkanes, initiating their degradation. Recent studies have also identified novel enzymes and electron transfer systems, such as disulfide isomerases with iron-binding cysteine motifs, which may play crucial roles in the alkane degradation pathways of certain bacterial species (Zhao et al., 2019). The diversity and specificity of these enzymes are essential for developing targeted bioremediation strategies and engineering more efficient degradation pathways. Metatranscriptomic analyses have revealed the expression of numerous enzymes involved in hydrocarbon degradation under various environmental conditions, providing insights into the functional changes within microbial communities in response to oil contamination (Song et al., 2021).

3.1.3 Genetic and molecular basis of hydrocarbon degradation

The genetic and molecular basis of hydrocarbon degradation is complex and involves a wide array of genes and regulatory systems. Many of the genes encoding key catabolic enzymes are organized in operons, allowing for coordinated expression in response to the presence of hydrocarbon substrates. Regulatory genes, such as those encoding transcriptional activators, play crucial roles in sensing environmental cues and initiating the expression of degradation pathways. Recent metagenomic studies have revealed novel gene arrangements and previously unknown catabolic clusters, highlighting the diversity of genetic strategies employed by microorganisms for hydrocarbon degradation (Zakaria et al., 2021). The horizontal transfer of catabolic genes between different bacterial species has been identified as a significant mechanism for the spread of degradation capabilities within microbial communities. This genetic plasticity allows for rapid adaptation to changing environmental conditions and the presence of new substrates. Furthermore, the application of synthetic biology and genetic engineering techniques, such as CRISPR-Cas systems, has opened new avenues for enhancing the degradation capabilities of microorganisms through targeted genetic modifications. Recent advances in environmental genomics and metabolomics have provided deeper insights into the microbial strategies for hydrocarbon degradation. These approaches have revealed a diverse array of catabolic pathways and enzymatic systems, allowing for the identification of unique structural, functional, and metabolic pathways used by microbial communities for contaminant degradation. The combination of metabolic engineering with synthetic biology, systems biology, and evolutionary engineering approaches has the potential to develop highly efficient microbial strains for target-dependent bioprocessing and degradation of petroleum hydrocarbons (Choi et al., 2019).

3.2 Strategies to Enhance Bioremediation Efficiency

Enhancing bioremediation efficiency is crucial for effectively addressing petroleum hydrocarbon contamination in various environments. This section explores diverse strategies that have been developed and refined over the past decade to optimize the natural degradation processes. These approaches include bioaugmentation, biostimulation, surfactant-enhanced bioremediation, and phytoremediation. Each strategy targets specific aspects of the bioremediation process, from introducing specialized microbial communities to modifying environmental conditions to enhance degradation rates. The interplay between these strategies often leads to synergistic effects, resulting in more effective and efficient remediation outcomes (Anza et al., 2019) (Figure 3).

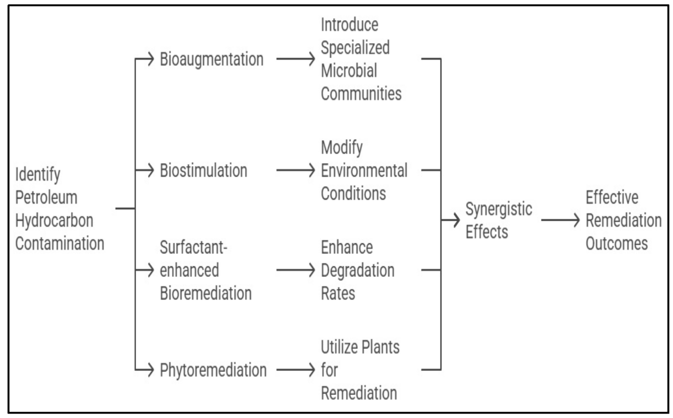

Figure 3. Strategies for enhancing phytoremediation efficiency

3.2.1 Bioaugmentation techniques

Bioaugmentation involves the introduction of specialized microbial strains or consortia to enhance the degradation of petroleum hydrocarbons in contaminated environments. This technique has gained significant attention in recent years due to its potential to accelerate the bioremediation process, especially in environments with limited indigenous microbial populations or those lacking specific degradative capabilities. Recent studies have focused on isolating and screening bacterial strains from natural hydrocarbon springs, which have shown promising results in enhancing in-situ bioremediation. These naturally adapted microorganisms often possess superior degradative abilities and can withstand the harsh conditions associated with hydrocarbon-contaminated sites (Kuppusamy et al., 2016). The selection of appropriate microbial strains for bioaugmentation is critical and often involves a combination of culture-dependent and molecular techniques. Next-generation sequencing has revealed the dominance of species from the *Proteobacteria* phylum in hydrocarbon-degrading communities, but other phyla such as *Flavobacterium* and *Gordonia* also play important roles. The use of microbial consortia, rather than single strains, has shown increased effectiveness due to the synergistic interactions between different species. These consortia can degrade a wider range of hydrocarbon compounds and adapt more readily to changing environmental conditions (Ghosh et al., 2016). Recent advancements in bioaugmentation techniques include the development of encapsulated microbial cells, which provide protection against environmental stressors and allow for the controlled release of microorganisms. Additionally, the integration of genetic engineering approaches has led to the creation of enhanced microbial strains with improved degradative capabilities (Liu et al., 2019). However, the application of genetically modified organisms in field conditions remains a topic of debate due to ecological and regulatory concerns.

3.2.2 Biostimulation methods

Biostimulation involves the modification of environmental conditions to enhance the growth and activity of indigenous hydrocarbon-degrading microorganisms. This approach typically focuses on optimizing nutrient availability, oxygen levels, pH, and temperature to create favorable conditions for microbial metabolism. Recent research has highlighted the importance of balanced nutrient supplementation, particularly nitrogen and phosphorus, in stimulating hydrocarbon degradation. Advanced biostimulation techniques have emerged in recent years, including the use of slow-release fertilizers and oxygen-releasing compounds. These innovations allow for sustained nutrient and oxygen delivery, overcoming limitations associated with rapid depletion in highly contaminated environments. Additionally, the application of organic amendments, such as compost and biochar, has shown promise in enhancing soil structure and microbial activity, leading to improved biodegradation rates. The optimization of environmental conditions through biostimulation often involves a delicate balance, as excessive nutrient addition can lead to eutrophication or inhibition of microbial activity. Recent studies have focused on developing site-specific biostimulation strategies based on comprehensive characterization of soil properties and indigenous microbial communities. This tailored approach has resulted in more effective and environmentally sustainable remediation outcomes (Kumar & Raut, 2024).

3.2.3 Surfactant-enhanced bioremediation

Surfactant-enhanced bioremediation has emerged as a promising strategy to address the limited bioavailability of hydrophobic petroleum compounds. This approach involves the use of surfactants to increase the solubility and mobility of hydrocarbons, making them more accessible to degrading microorganisms. Recent research has focused on the development and application of both synthetic and biosurfactants to enhance bioremediation efficiency. Biosurfactants, produced by microorganisms, have gained particular attention due to their biodegradability and lower toxicity compared to synthetic alternatives. Studies have shown that certain bacterial strains, particularly those isolated from hydrocarbon-contaminated environments, can produce highly effective biosurfactants (Pacwa-Płociniczak et al., 2016; Zahed et al., 2022). These compounds not only enhance hydrocarbon solubilization but also stimulate microbial growth and activity. The integration of surfactant-enhanced techniques with other bioremediation strategies has shown synergistic effects. For instance, the combination of biosurfactant application with bioaugmentation has resulted in significantly improved degradation rates in both soil and aquatic environments. However, the selection of appropriate surfactants and their optimal concentrations remains crucial, as excessive amounts can potentially inhibit microbial activity or lead to contaminant mobilization beyond the treatment zone (Tiwari & Tripathy, 2023).

3.2.4 Phytoremediation and rhizoremediation approaches

Phytoremediation and rhizoremediation have gained increasing attention as sustainable and cost-effective strategies for petroleum hydrocarbon remediation, particularly in soil environments. These plant-based approaches leverage the symbiotic relationships between plants and their associated microorganisms to enhance contaminant degradation and uptake (Alotaibi et al., 2021). Advancements in rhizoremediation techniques have highlighted the importance of plant-microbe interactions in enhancing biodegradation rates. The rhizosphere provides a favorable environment for microbial growth and activity, with plant root exudates serving as additional carbon sources and stimulating the expression of hydrocarbon-degrading genes. Recent research has explored the potential of genetically modified plants with enhanced exudate production or the ability to harbor specific microbial communities, although field application of such approaches remains limited due to regulatory constraints (Upadhyay et al., 2019). The integration of phytoremediation with other bioremediation strategies, such as bioaugmentation and biostimulation, has shown promising results in field trials. These combined approaches leverage the strengths of each technique, resulting in more comprehensive and efficient remediation of petroleum-contaminated sites. However, the success of phytoremediation and rhizoremediation approaches depends heavily on site-specific factors, including soil properties, contaminant characteristics, and climatic conditions, necessitating careful planning and ongoing monitoring for optimal results (Nwankwegu et al., 2022).

3.3 Advanced Technologies for Bioremediation Enhancement

The field of bioremediation has seen significant advancements in recent years, with the integration of cutting-edge technologies to enhance the efficiency and effectiveness of petroleum hydrocarbon degradation (Karishma et al., 2024). This section explores three key areas of technological innovation: nanotechnology-based approaches, genetic engineering and synthetic biology applications, and the integration of bioremediation with other remediation technologies. These advanced technologies offer promising solutions to overcome limitations in traditional bioremediation methods and provide new avenues for addressing complex contamination scenarios.

3.3.1 Nanotechnology-based approaches

Nanotechnology has emerged as a powerful tool in enhancing bioremediation processes for petroleum hydrocarbon contamination. The unique properties of nanomaterials, such as high surface area-to-volume ratio and enhanced reactivity, make them ideal candidates for improving the efficiency of microbial degradation (Kumari & Singh, 2016). Nanoparticles can serve as carriers for microbial delivery, providing protection and support for degrading microorganisms in harsh environmental conditions (Baghaie & Keshavarzi, 2018). For instance, iron oxide nanoparticles have been shown to enhance the growth and activity of hydrocarbon-degrading bacteria by serving as electron acceptors and improving oxygen availability in contaminated soils. Additionally, nano-enhanced bioremediation systems have been developed, incorporating nanomaterials such as carbon nanotubes and graphene oxide to improve the bioavailability of hydrophobic contaminants and stimulate microbial activity. These systems have demonstrated increased degradation rates and a broader spectrum of hydrocarbon removal compared to conventional bioremediation approaches (Nandini et al., 2023) (Figure 4).

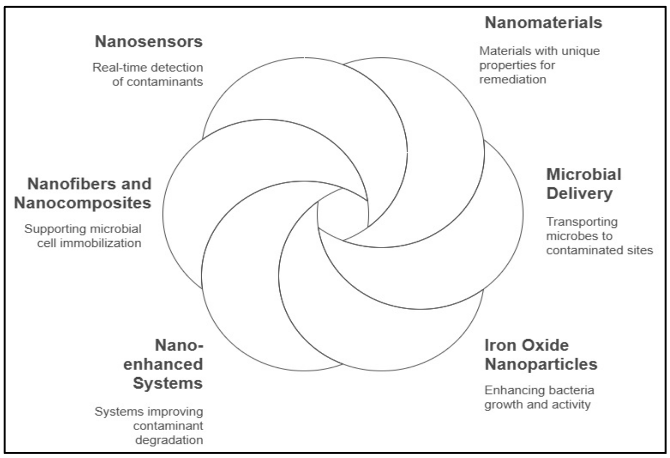

Figure 4. Enhancing bioremediation with nanotechnology

Recent studies have also explored the use of nanofibers and nanocomposites as support materials for immobilizing microbial cells, enhancing their survival and metabolic activity in contaminated environments. The integration of nanotechnology with bioremediation not only improves degradation efficiency but also allows for better control and monitoring of the remediation process through the development of nanosensors for real-time detection of contaminants and microbial activity (Gordegir et al., 2019).

3.3.2 Genetic engineering and synthetic biology applications

Genetic engineering and synthetic biology have opened new frontiers in bioremediation, allowing for the development of enhanced microbial strains with improved degradation capabilities. CRISPR-Cas9 technology has emerged as a powerful tool for metabolic pathway optimization in hydrocarbon-degrading microorganisms (Maqsood et al., 2024). This precise gene-editing technique enables the modification of key enzymes involved in hydrocarbon catabolism, leading to increased substrate specificity and degradation rates (Figure 5).

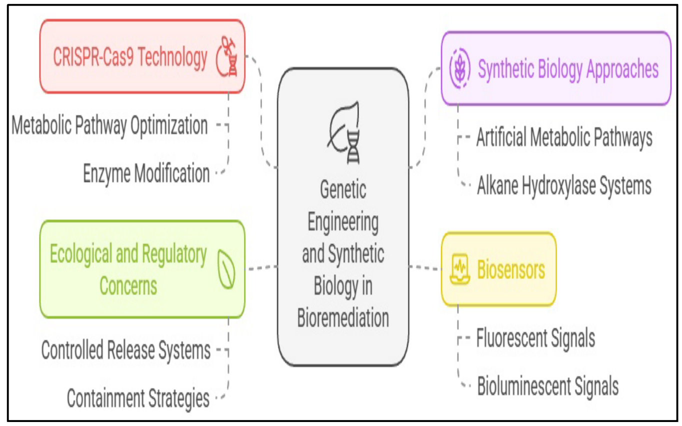

Figure 5. Advancements in bioremediation through genetic engineering

Synthetic biology approaches have been employed to design and construct artificial metabolic pathways in microorganisms, enabling them to degrade recalcitrant compounds that are resistant to natural biodegradation processes. For example, engineered bacteria with enhanced alkane hydroxylase systems have shown remarkable efficiency in degrading long-chain alkanes, which are typically challenging to remediate (Chunyan et al., 2023). Furthermore, the development of biosensors using genetically modified microorganisms has facilitated real-time monitoring of contaminant levels and biodegradation progress in situ. These biosensors can be designed to produce fluorescent or bioluminescent signals in response to specific hydrocarbons, providing valuable data for optimizing remediation strategies. While the application of genetically modified organisms in field conditions remains a topic of debate due to ecological and regulatory concerns, controlled release systems and containment strategies are being developed to address these issues. The integration of genetic engineering and synthetic biology with traditional bioremediation approaches holds great promise for addressing complex contamination scenarios and improving the overall efficiency of petroleum hydrocarbon remediation (Alaidaroos, 2023).

3.3.3 Integration of bioremediation with other remediation technologies

The integration of bioremediation with other remediation technologies has emerged as a promising approach to address the limitations of individual techniques and achieve more comprehensive and efficient cleanup of petroleum-contaminated sites. Electrobioremediation, which combines electrokinetic remediation with bioremediation, has shown significant potential in enhancing the degradation of hydrocarbons in low-permeability soils (Cameselle & Reddy, 2022). This technique uses an electric field to mobilize contaminants and stimulate microbial activity, resulting in improved biodegradation rates. Recent studies have demonstrated the effectiveness of electrobioremediation in treating soils contaminated with a wide range of petroleum hydrocarbons, including recalcitrant polycyclic aromatic compounds. Photocatalytic-assisted bioremediation is another innovative approach that combines the oxidative power of photocatalysis with microbial degradation. This technique utilizes photocatalysts, such as titanium dioxide nanoparticles, to generate reactive oxygen species that partially degrade complex hydrocarbons into more bioavailable intermediates (Brindhadevi et al., 2024). These intermediates are then more readily metabolized by indigenous microorganisms, leading to enhanced overall degradation efficiency. The integration of phytoremediation with microbial bioremediation has also shown promising results, particularly in the treatment of shallow soil contamination. This combined approach leverages the synergistic effects of plant-microbe interactions, where plant root exudates stimulate microbial activity and enhance contaminant bioavailability. Additionally, the integration of bioremediation with chemical oxidation techniques, such as Fenton's reagent or persulfate activation, has been explored as a means to address recalcitrant compounds and accelerate the overall remediation process (Zhou et al., 2019). These integrated approaches offer the potential for more rapid and complete remediation of petroleum-contaminated sites, particularly in complex environmental matrices where traditional single-technology approaches may be limited.

3.4 Monitoring and Assessment of Bioremediation Progress

Effective monitoring and assessment of bioremediation progress are crucial for optimizing petroleum hydrocarbon degradation strategies and ensuring successful remediation outcomes. This section explores three key aspects of monitoring and assessment: advanced analytical techniques for contaminant detection, molecular tools for microbial community analysis, and ecotoxicological assessments and biomarkers. These approaches provide comprehensive insights into the effectiveness of bioremediation processes, allowing for real-time adjustments and informed decision-making throughout the remediation process.

3.4.1 Advanced analytical techniques for contaminant detection

Advanced analytical techniques have revolutionized the detection and quantification of petroleum hydrocarbons in contaminated environments, enabling more accurate and sensitive monitoring of bioremediation progress. Gas chromatography-mass spectrometry (GC-MS) remains a cornerstone technique, offering high-resolution separation and identification of individual hydrocarbon compounds. Recent advancements in GC-MS technology, such as two-dimensional GC-MS (GC×GC-MS), have further enhanced the ability to resolve complex hydrocarbon mixtures and detect trace-level contaminants (Ranjan Maji et al., 2023). Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) has emerged as a powerful tool for characterizing the molecular composition of petroleum hydrocarbons, providing unprecedented insights into the transformation of these compounds during bioremediation. In situ sensing technologies, including fiber optic chemical sensors and laser-induced fluorescence spectroscopy, have been developed for real-time monitoring of hydrocarbon concentrations in soil and groundwater (Ghandehari et al., 2018). These techniques allow for continuous, non-invasive measurements, facilitating rapid assessment of bioremediation effectiveness. Additionally, the integration of portable and field-deployable analytical instruments, such as handheld X-ray fluorescence (XRF) spectrometers and portable GC-MS systems, has enabled on-site contaminant analysis, reducing the need for time-consuming laboratory testing and allowing for more responsive remediation strategies.

3.4.2 Molecular tools for microbial community analysis

Molecular tools for microbial community analysis have become indispensable for understanding the dynamics of hydrocarbon-degrading populations during bioremediation. Next-generation sequencing technologies, particularly amplicon sequencing of 16S rRNA genes and metagenomic sequencing, provide comprehensive profiles of microbial community composition and functional potential. These approaches have revealed the complex interplay between different microbial taxa in hydrocarbon degradation processes and have identified key players in various bioremediation scenarios (Adetitun & Tomilayo, 2023). Quantitative PCR (qPCR) techniques targeting specific functional genes, such as alkane monooxygenases and aromatic ring-cleaving dioxygenases, allow for the quantification of degradation potential within microbial communities. Recent developments in single-cell genomics and metatranscriptomics have enabled the exploration of gene expression patterns in situ, providing insights into the active metabolic pathways during bioremediation. Stable isotope probing (SIP) techniques, combined with high-throughput sequencing, have been employed to directly link specific microbial taxa to the degradation of particular hydrocarbon compounds (Kim et al., 2023). These molecular approaches not only facilitate the monitoring of bioremediation progress but also inform the development of more targeted and effective remediation strategies.

3.4.3 Ecotoxicological assessments and biomarkers

Ecotoxicological assessments and biomarkers play a crucial role in evaluating the environmental impact of petroleum hydrocarbons and the effectiveness of bioremediation efforts. Standardized ecotoxicity tests using model organisms from different trophic levels, such as bacteria, algae, invertebrates, and plants, provide valuable information on the overall toxicity reduction during the bioremediation process (Wang et al., 2024). Recent advancements in high-throughput ecotoxicity screening methods, including microbial biosensors and cell-based assays, allow for rapid assessment of contaminant toxicity and biodegradation progress. Biomarkers, both at the molecular and organismal levels, have been developed to assess the exposure and effects of petroleum hydrocarbons on biota. Molecular biomarkers, such as the expression of cytochrome P450 enzymes and heat shock proteins, provide early warning signs of contaminant stress in organisms (Han et al., 2017). Physiological and behavioral biomarkers, including growth inhibition, reproductive impairment, and avoidance responses, offer insights into the long-term ecological impacts of hydrocarbon contamination. The integration of multiple biomarker responses through the development of integrated biomarker indices has improved the robustness and interpretability of ecotoxicological assessments (Schoenaers et al., 2016).

3.5 Future Perspectives and Challenges

The field of petroleum hydrocarbon bioremediation is at a critical juncture, facing both unprecedented challenges and exciting opportunities. As we delve deeper into the complexities of environmental contamination and microbial ecology, new frontiers emerge that demand innovative approaches and interdisciplinary collaboration. This section explores four key areas that will shape the future of bioremediation: emerging contaminants and complex petroleum mixtures; climate change impacts; scaling up laboratory findings, and regulatory and policy considerations. These interconnected topics highlight the multifaceted nature of bioremediation research and the need for holistic strategies to address increasingly complex environmental issues. By examining these future perspectives and challenges, we can better prepare for the evolving landscape of petroleum hydrocarbon remediation and develop more effective, sustainable solutions for environmental restoration.

3.5.1 Emerging contaminants and complex petroleum mixtures

The landscape of petroleum contamination is becoming increasingly complex, presenting new challenges for bioremediation strategies. Emerging contaminants, such as oxygenated additives, nanoparticles used in enhanced oil recovery, and novel refinery byproducts, are altering the traditional understanding of petroleum hydrocarbon remediation (Erfani et al., 2024). These compounds often exhibit unique physicochemical properties that can affect their bioavailability, toxicity, and degradation pathways. For instance, the increasing use of biofuels and fuel oxygenates has introduced compounds like ethanol and methyl tert-butyl ether (MTBE) into environmental matrices, which can alter the behavior and degradation of co-occurring hydrocarbons. Recent research has focused on identifying microbial consortia capable of degrading these complex mixtures, with particular emphasis on polycyclic aromatic hydrocarbons (PAHs) and their oxygenated derivatives (Cao et al., 2009). Metagenomic studies have revealed novel degradation pathways and enzymes that may be exploited for enhanced bioremediation of these recalcitrant compounds. For example, the discovery of novel ring-cleaving dioxygenases in bacteria isolated from PAH-contaminated soils has opened new avenues for engineering more efficient degradation pathways. Additionally, the interaction between petroleum hydrocarbons and other environmental contaminants, such as heavy metals and microplastics, is becoming an area of increasing concern (Khalid et al., 2021). These interactions can alter the bioavailability and toxicity of hydrocarbons, necessitating the development of integrated remediation approaches that address multiple contaminant classes simultaneously. Recent studies have explored the use of multi-functional microbial consortia that can simultaneously degrade hydrocarbons and immobilize heavy metals, offering promising solutions for complex contamination scenarios. The development of advanced analytical techniques, such as two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS), is enabling more comprehensive characterization of complex petroleum mixtures and their transformation products during bioremediation. This improved understanding of contaminant complexity is driving the development of more targeted and effective bioremediation strategies tailored to specific site conditions and contaminant profiles.

3.5.2 Climate change impacts on bioremediation efficiency

Climate change is poised to have profound impacts on the efficiency and effectiveness of bioremediation processes for petroleum hydrocarbons. Rising global temperatures are expected to alter microbial community structures, metabolic rates, and contaminant behavior in ways that could significantly affect remediation outcomes. On one hand, increased temperatures may accelerate microbial metabolism and enhance the bioavailability of certain hydrocarbon compounds, potentially leading to faster degradation rates. Studies have shown that temperature increases of just a few degrees can result in significant improvements in hydrocarbon mineralization rates in both soil and aquatic environments. However, these potential benefits are likely to be offset by a range of climate-related challenges. Altered precipitation patterns and increased frequency of extreme weather events can lead to changes in soil moisture content, oxygen availability, and nutrient dynamics, all of which play critical roles in microbial degradation processes. For instance, prolonged drought conditions can severely limit microbial activity and contaminant bioavailability, while increased flooding can lead to the remobilization of sequestered hydrocarbons and the creation of anaerobic conditions that may favor slower degradation pathways (Chen et al., 2024). Rising sea levels and increased coastal erosion present additional challenges, potentially leading to the release of previously sequestered hydrocarbons from marine sediments and creating new contamination scenarios in coastal environments. Researchers are increasingly focusing on developing climate-resilient bioremediation strategies that can adapt to changing environmental conditions (Dash et al., 2024). This includes the identification and engineering of thermotolerant microbial strains capable of maintaining high degradation efficiencies across a broader temperature range. Recent studies have explored the potential of extremophilic bacteria and archaea as sources of robust hydrocarbon-degrading enzymes that could be used to enhance bioremediation under extreme conditions. Additionally, the development of adaptive biostimulation approaches that can maintain optimal degradation rates under variable moisture and temperature regimes is gaining attention. These may include the use of slow-release nutrient formulations and moisture-retaining amendments to buffer against environmental fluctuations. The integration of climate models with bioremediation planning is becoming increasingly important, allowing for the development of long-term remediation strategies that account for projected environmental changes (Baskaran & Byun, 2024). As climate change continues to alter ecosystem dynamics, there is a growing need for interdisciplinary research that combines microbial ecology, environmental chemistry, and climate science to develop holistic and resilient bioremediation solutions.

3.5.3 Scaling up laboratory findings to field applications

The translation of laboratory-scale bioremediation findings to successful field applications remains one of the most significant challenges in the petroleum hydrocarbon remediation field. While controlled laboratory experiments often demonstrate high degradation efficiencies and provide valuable insights into degradation mechanisms, the complex and heterogeneous nature of real-world contaminated sites can lead to reduced effectiveness when scaling up. Factors such as soil heterogeneity, contaminant aging, bioavailability limitations, and the presence of diverse indigenous microbial communities can all influence the success of bioremediation strategies in the field. Recent research has focused on developing more realistic laboratory models that better simulate field conditions, including the use of soil columns, mesocosm experiments, and pilot-scale studies (Brisson et al., 2024). These approaches allow for the evaluation of bioremediation strategies under more complex conditions, providing valuable insights into potential challenges and optimization opportunities before full-scale implementation. For example, the use of large-scale lysimeters has enabled researchers to study the long-term behavior of contaminants and microbial communities under varying environmental conditions, bridging the gap between laboratory and field studies. Advances in in situ monitoring technologies are playing a crucial role in improving our ability to assess and optimize bioremediation progress in field settings. Real-time sensors for key parameters such as oxygen levels, nutrient concentrations, and microbial activity are enabling more responsive and adaptive remediation strategies. Remote sensing techniques, including hyperspectral imaging and LiDAR, are being increasingly employed to monitor large-scale bioremediation projects, providing valuable data on vegetation health, soil properties, and contaminant distribution. The integration of these monitoring technologies with predictive modeling approaches is enhancing our ability to design and optimize bioremediation strategies for specific site conditions. Machine learning algorithms are being developed to analyze complex environmental datasets and predict bioremediation outcomes under various scenarios, allowing for more informed decision-making in the field. Additionally, the concept of "adaptive bioremediation" is gaining traction, where treatment strategies are continuously adjusted based on real-time monitoring data and model predictions (Oladosu et al., 2024). This approach allows for more efficient resource allocation and improved remediation outcomes in heterogeneous and dynamic field environments. As we continue to bridge the gap between laboratory findings and field applications, there is a growing recognition of the importance of site-specific optimization and the need for flexible, adaptive remediation approaches that can respond to the unique challenges of each contaminated site.

3.5.4 Regulatory and policy considerations for bioremediation implementation

The implementation of bioremediation strategies for petroleum hydrocarbon contamination is subject to a complex and evolving regulatory landscape. As the field of bioremediation advances, there is a growing need for regulatory frameworks that can keep pace with technological innovations while ensuring environmental and public health protection. Key regulatory challenges include the development of standardized protocols for assessing bioremediation effectiveness, establishing appropriate end-point criteria for remediation projects, and addressing concerns related to the introduction of non-native or genetically modified microorganisms into the environment. Recent policy discussions have focused on the need for risk-based approaches to remediation that consider both the potential benefits and risks of bioremediation technologies. This shift towards risk-based decision-making is particularly important in the context of emerging contaminants and complex mixtures, where traditional cleanup standards may not adequately address the full spectrum of environmental and health risks (Geng et al., 2024). The development of site-specific cleanup goals that take into account intended land use, ecological receptors, and long-term environmental impacts is becoming increasingly common. Additionally, there is growing recognition of the importance of life cycle assessment (LCA) in evaluating the overall sustainability of different remediation options, including bioremediation. LCA approaches can help policymakers and practitioners make more informed decisions by considering factors such as energy consumption, greenhouse gas emissions, and resource use associated with different remediation strategies. The regulatory landscape is also evolving to address the challenges posed by climate change and its potential impacts on bioremediation effectiveness. Some jurisdictions are beginning to require climate change vulnerability assessments as part of remediation planning, ensuring that long-term remediation strategies are resilient to projected environmental changes. The use of adaptive management approaches in bioremediation projects is gaining regulatory acceptance, allowing for more flexible and responsive remediation strategies that can be adjusted based on monitoring data and changing site conditions (Yang et al., 2024). As the field of bioremediation continues to advance, there is an increasing need for harmonization of regulatory approaches across different regions and countries. International collaborations and knowledge-sharing initiatives are playing a crucial role in developing best practices and standardized methodologies for bioremediation implementation and assessment. The involvement of multiple stakeholders, including regulatory agencies, industry, academia, and affected communities, in the development of bioremediation policies is becoming increasingly important. This collaborative approach helps ensure that regulatory frameworks are scientifically sound, practically implementable, and responsive to societal needs and concerns.

4. Conclusion

The persistent challenge of petroleum hydrocarbon contamination necessitates innovative and effective remediation strategies to protect ecosystems and human health. This review highlights the critical role of bioremediation as a sustainable solution for addressing the complex environmental issues posed by petroleum hydrocarbons. The intricate mechanisms of microbial degradation, including the diverse metabolic pathways and specialized enzymes involved, are essential for understanding how to optimize these natural processes. Bioremediation techniques, particularly biostimulation and bioaugmentation, have shown promise in enhancing the degradation efficiency of petroleum hydrocarbons across various contaminated environments. By tailoring environmental conditions to support microbial growth and activity, these methods can significantly reduce the concentration of harmful pollutants in soil and water systems. Furthermore, the integration of advanced technologies such as nanotechnology and genetic engineering presents exciting opportunities to improve bioremediation outcomes, making it possible to tackle more challenging contamination scenarios. Despite the progress made in this field, several challenges remain. The variability in microbial community compositions across different environments can affect the predictability of bioremediation outcomes. Additionally, the presence of recalcitrant compounds within petroleum mixtures often hinders complete degradation. Future research should focus on elucidating the genetic and metabolic diversity of microbial populations involved in hydrocarbon degradation, as well as developing more refined approaches for monitoring bioremediation progress. In conclusion, this review underscores the importance of continued investment in bioremediation research and development. By fostering collaboration among environmental scientists, policymakers, and industry stakeholders, we can enhance our collective ability to mitigate the impacts of petroleum hydrocarbon pollution. The insights gained from this review not only contribute to the scientific understanding of bioremediation processes but also serve as a foundation for implementing effective strategies that promote environmental sustainability and public health. As we move forward, it is imperative to embrace innovative solutions that harness nature's capabilities while addressing the pressing challenges posed by petroleum contamination in our ecosystems.

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The authors declare that there is no conflict of interest.

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There were no ethical considerations to be considered in this research.

Using artificial intelligence

During the preparation of figures, the authors used AI in order to improving its quality.

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