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Microbial Bioremediation of Petroleum Contaminants: Mechanisms, Applications, and Challenges

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ABSTRACT

Oil pollution is one of the most persistent environmental threats affecting terrestrial and marine ecosystems worldwide. Bioremediation, an eco-friendly and cost-effective approach, utilizes microorganisms to degrade petroleum hydrocarbons into non-toxic end products. This review highlights the microbial diversity involved in oil degradation, including key bacterial and fungal genera such as *Pseudomonas*, *Acinetobacter*, *Rhodococcus*, *Aspergillus*, and *Candida*. It further discusses the enzymatic mechanisms underlying hydrocarbon degradation, both aerobic and anaerobic, and the environmental factors influencing microbial activity, such as temperature, pH, nutrient availability, and oxygen levels. Special attention is given to the role of biosurfactants in increasing hydrocarbon bioavailability and the advancement of modern technologies such as microbial electrochemical systems and genetic engineering for enhanced degradation. This paper also presents successful case studies of microbial oil bioremediation and compares it to other remediation strategies, emphasizing the potential and challenges of microbial approaches in large-scale applications. The comprehensive understanding provided here aims to guide future research and industrial applications for sustainable environmental restoration.

Introduction

Petroleum and its by-products are recognized as the primary sources of energy worldwide. These energy sources are not only essential for industrial needs and transportation but also account for a significant portion of electricity generation in many countries. Currently, oil is considered one of the main global energy sources. According to global reports, dependence on oil and gas is still on the rise, with more than 30% of the world's energy being supplied by these resources. This situation highlights the need for special attention to the environmental challenges posed by oil pollution (1). The extraction, transportation, and utilization of these resources lead to widespread environmental pollution, which has harmful effects on both terrestrial and aquatic ecosystems (1,2).

Oil spills, accidental or due to industrial activities, can devastate ecosystems, wildlife, and human health.

In recent years, various technologies have been developed to address petroleum pollution, including physical, chemical, and biological methods. Among these, biodegradation, which uses microorganisms to break down petroleum compounds, has gained attention as a sustainable and environmentally friendly solution (3). Various microorganisms, including bacteria, fungi, and archaea, can naturally or with specific environmental stimuli degrade petroleum compounds (4,5).

Numerous studies have been conducted on the use of microorganisms for oil degradation. These studies have shown that factors such as temperature, pH, nutrient availability, and oxygen conditions significantly affect the effectiveness of microorganisms in the bioremediation process (6,7).

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Furthermore, the use of advanced technologies such as genetic engineering and nanoparticles can enhance the performance of microorganisms and make the bioremediation process more effective at larger scales (8,9).

This review article aims to examine the role of microorganisms in the bioremediation of petroleum contamination. This article will introduce the various organisms involved in this process and explore their mechanisms of action in petroleum degradation. Then, the challenges and limitations associated with this method will be discussed, and finally, its practical applications in the petroleum and environmental industries will be examined.

Microbial Diversity in Oil Bioremediation

Microorganisms play a crucial role in the breakdown of petroleum hydrocarbons, with certain bacterial and fungal species exhibiting remarkable degradation capabilities. Several bacterial genera, including *Pseudomonas*, *Acinetobacter*, and *Mycobacterium*, are widely recognized for their ability to metabolize hydrocarbons (10). These bacteria utilize oxygenases and peroxidases to break down aliphatic and aromatic hydrocarbons, facilitating the conversion of complex hydrocarbons into simpler, less harmful compounds.

Fungi also contribute significantly to hydrocarbon degradation, particularly in soil environments where bacterial activity may be limited. Genera such as *Aspergillus*, *Penicillium*, *Trichoderma*, and *Candida* produce oxidative enzymes that enable them to degrade petroleum contaminants effectively (11). Unlike bacteria, fungi can penetrate deeply into contaminated soils, enhancing the bioavailability of hydrocarbons by breaking down complex molecules into smaller, more accessible forms.

Recent studies highlight the superiority of indigenous hydrocarbon-degrading bacteria over introduced microbial consortia in bioremediation. For example, *Actinotalea terraria*, *Arthrobacter ginsengisoli*, and *Pseudomonas songnenensis* have demonstrated high oil removal efficiencies, achieving up to 73.6% degradation in low-contamination soils (1% oil) and 50% in highly polluted sites (20–30% oil concentration) (12). These findings suggest that microorganisms native to contaminated environments may be better adapted for hydrocarbon degradation due to their pre-existing metabolic capabilities.

In marine ecosystems, hydrocarbon clastic bacteria such as *Marinobacter*, *Thalassolituus*, and *Cycloclasticus* play a crucial role in degrading crude oil following spills (13). A study on the Deepwater Horizon oil spill in the Gulf of Mexico found that populations of these bacteria expanded significantly in response to petroleum contamination, indicating

their potential for large-scale bioremediation applications (14).

Mechanisms of Hydrocarbon Degradation

Microbial degradation of hydrocarbons occurs through a series of metabolic pathways that vary depending on the chemical composition of the contaminants. These pathways involve oxidation, hydroxylation, and ring cleavage reactions that transform hydrocarbons into less toxic compounds. Hydrocarbons can also be divided following their chemical structure into: aliphatic hydrocarbons or saturated hydrocarbons, aromatic hydrocarbons (monocyclic aromatic and polycyclic aromatic hydrocarbons), and heteroatomic compounds (saturated and aromatic ones), including resins and asphaltenes.

Aliphatic Hydrocarbon Degradation: Aliphatic hydrocarbons, also known as paraffins, are present in natural gas and oil deposits formed by plant and animal decomposition. AHs (Figure A1) have no double bonds (general formula C_nH_{2n}) and represent the highest percentage of the constituents of crude oil.

Alkanes, which are saturated hydrocarbons, are metabolized through oxidation. The process begins with the action of monooxygenases and dioxygenases, which introduce oxygen atoms into alkane molecules, converting them into alcohols. These alcohols are further oxidized to aldehydes and then to fatty acids, which enter the β -oxidation pathway for complete degradation (15).

Pseudomonas and *Acinetobacter* are particularly efficient in aliphatic hydrocarbon degradation due to their ability to express alkane hydroxylases (10). In marine environments, *Alcanivorax borkumensis* has been identified as a key player in degrading long-chain alkanes, making it a valuable candidate for oil spill remediation efforts (11).

The degradation of alkanes primarily occurs through oxidation reactions facilitated by monooxygenases and dioxygenases, which introduce oxygen atoms into the hydrocarbon molecule, initiating its breakdown. This process follows these steps:

Activation by Oxygenase: Microorganisms such as *Pseudomonas*, *Acinetobacter*, and *Alcanivorax* utilize alkane hydroxylases to catalyze the oxidation of alkanes into primary alcohols (10).

The specificity of alkane hydroxylases varies, with some targeting short-chain alkanes (C5- C10), while others degrade long-chain hydrocarbons (C12- C40).

Conversion to Fatty Acids: Alcohols produced from the oxidation of alkanes are further converted into aldehydes and carboxylic acids by alcohol dehydrogenases and aldehyde dehydrogenases.

The resulting fatty acids enter the β -oxidation pathway, where they are sequentially broken down into acetyl-CoA molecules, which are further utilized in microbial metabolism for energy production (11).

Efficiency of Aliphatic Hydrocarbon Degradation: In marine environments, *Alcanivorax borkumensis* has been identified as a dominant hydrocarbon-degrading bacterium capable of degrading alkanes efficiently in oil-contaminated waters (14).

Laboratory studies have shown that engineered *Pseudomonas putida* strains can degrade up to 85% of n-alkanes within 10 days under optimal conditions (16).

Aromatic Hydrocarbon Degradation: Aromatic hydrocarbons, such as benzene, toluene, ethylbenzene, and xylene (BTEX), require specialized dioxygenases to initiate degradation. The process involves hydroxylation, leading to the formation of catechol's, which undergo ring cleavage and subsequent mineralization into CO₂ and water.

Polycyclic aromatic hydrocarbons (PAHs), which contain multiple benzene rings, pose a greater challenge for biodegradation. However, bacteria such as *Rhodococcus* and *Mycobacterium* have demonstrated the ability to metabolize PAHs using multicomponent dioxygenases, enabling the breakdown of high-molecular-weight hydrocarbons (10).

Although most living organisms cannot survive in constant contact with high levels of PAHs, some microorganisms (bacteria, fungi, and algae) inhabiting chronically polluted sites possess several aerobic and anaerobic pathways for the degradation of aromatic and heterocyclic compounds, thus metabolizing them for assimilation as needed nutrients and energy sources.

To date, the biodegradation of PAHs by bacteria and fungi has been studied the most widely. Numerous bacteria have been isolated and characterized in terms of the molecular mechanisms underlying their possible metabolic ability to degrade environmental pollutants.

Microbial Degradation Pathway of BTEX Compounds

Hydroxylation by Dioxygenases: *Pseudomonas putida* and *Rhodococcus* species produce toluene dioxygenases, which catalyze the initial hydroxylation of benzene rings, making them more reactive for further breakdown (10).

The resulting catechols serve as intermediates for ring cleavage reactions.

Ring Cleavage and Mineralization: Ortho-cleavage pathway: Dioxygenases introduce oxygen molecules, breaking benzene rings into cis,cis-

muconic acid, which is subsequently converted into pyruvate and acetyl-CoA (15).

Meta-cleavage pathway: Alternative cleavage routes produce 2-hydroxymuconic semi aldehyde, which is further metabolized into tricarboxylic acid (TCA) cycle intermediates.

PAH Bioremediation Success Stories: A field study in China reported that bio augmentation with PAH-degrading bacteria resulted in a 60% reduction of anthracene and phenanthrene levels within 45 days (12).

In a study on marine sediments, microbial consortia containing *Cycloclasticus* species degraded naphthalene 85% within 30 days under aerobic conditions (14).

Anaerobic Degradation

In oxygen-limited environments, hydrocarbon degradation can proceed through anaerobic pathways. Sulfate-reducing bacteria, such as *Desulfovibrio* species, utilize sulfate as an alternative electron acceptor to metabolize hydrocarbons (11).

Advanced metagenomics studies have revealed novel anaerobic hydrocarbon degradation pathways, expanding the potential for bioremediation in deep-sea and subsurface environments (12).

The identification and characterization of microorganisms capable of anaerobic hydrocarbon mineralization have been a major challenge for researchers because most of these microorganisms exist in a consortium. They are usually not culturable by classical methods, especially the methanogenic members of the hydrocarbon-degrading communities.

With the help of genomics, transcriptomics, and proteomics, a deeper understanding of hydrocarbon biodegradation in pure cultures can be achieved. Additionally, metabolomics can elucidate the metabolites present during hydrocarbon biodegradation in pure cultures or consortia.

However, culture-dependent methods have severe limitations when it comes to the investigation of microbial communities. Most microorganisms are difficult or even impossible to culture under laboratory conditions. Thus, their functions cannot be fully resolved by classical, standard approaches.

The constant development of high-throughput data generation and assessment has opened new frontiers in the research of hydrocarbon biodegradation. For the examination of entire hydrocarbon-degrading communities, "meta-omics" approaches, including metagenomics, metatranscriptomics, and met proteomics, have been developed.

In the next chapters, the current knowledge about anaerobic hydrocarbon-degrading microorganisms, metabolic pathways, and microbial interactions in the multi-omics era is overviewed.

Several bacteria, such as *Pseudomonas*, *Aeromonas*, and sulfate-reducing bacteria, have been used in the bioremediation process under anaerobic conditions. Garg and Triatic reported microbial discoloration of azo dyes under different environmental situations.

Azo dyes can decompose anaerobically through reduction reactions using electrons produced by the oxidation of the organic substrate(s). Due to such controlled dye decolorization events, microbial electrochemical properties would have a major impact on the effectiveness of color removal.

Dyes were anaerobically decolorized for industrial activities to progressively acquire such time-variant decolorized metabolites (DMs). However, external augmentation of DMs gathered under certain conditions was carried out for improved research so that a precise system can be used.

Role of Bio surfactants

Bio surfactants are surface-active molecules synthesized by microorganisms that significantly enhance the bioavailability of hydrophobic contaminants such as petroleum hydrocarbons. These amphiphilic compounds play a pivotal role in bioremediation by reducing surface and interfacial tension, emulsifying hydrocarbons, and increasing their apparent solubility, thereby facilitating microbial uptake and subsequent degradation (10).

Microbial bio surfactants can be categorized based on their chemical structure into glycolipids, lipopeptides, phospholipids, and polymeric bio surfactants. Among these, glycolipids particularly rhamnolipids produced by *Pseudomonas aeruginosa* are among the most extensively studied and effective for oil biodegradation. Rhamnolipids have been shown to enhance the solubility of polycyclic aromatic hydrocarbons (PAHs) by up to 50%, thereby significantly improving microbial degradation efficiency (11).

Mechanisms of Bio Surfactant-Enhanced Oil Biodegradation

The effectiveness of bio surfactants in hydrocarbon degradation is attributed to several interrelated mechanisms:

Enhancement of Hydrocarbon Solubility: By reducing the interfacial tension between oil and water, bio surfactants facilitate the dispersion of hydrocarbons in aqueous environments, increasing their bioavailability to degrading microorganisms (12).

Promotion of Microbial Adhesion: Bio surfactants aid in the attachment of microbial cells to hydrophobic oil droplets, thereby improving microbial access and enzymatic degradation of hydrocarbons (16).

Chemo attraction: Certain bio surfactants generate chemical gradients that attract hydrocarbon-

degrading bacteria toward contaminated zones, thereby accelerating bioremediation (13).

Industrial and Field Applications of Bio surfactants

Due to their environmental compatibility, biodegradability, and low toxicity, bio surfactants have garnered increasing interest in large-scale oil spill remediation and contaminated site restoration. Several field studies have demonstrated their practical effectiveness:

In a field trial in Canada, the introduction of rhamnolipid-producing *Pseudomonas* strains resulted in a 62% increase in crude oil degradation over 30 days compared to conventional remediation techniques (14).

A bioremediation project in China reported an 85% reduction in total petroleum hydrocarbons (TPH) within 60 days using bio surfactant-assisted microbial treatment, underscoring the scalability and efficiency of this approach (11).

Marine oil spill investigations have revealed that bio surfactants can reduce hydrocarbon toxicity by up to 80%, positioning them as environmentally friendly alternatives to synthetic chemical dispersants (12).

Future Directions in Bio Surfactant Research

Despite their proven benefits, the broader application of bio surfactants is constrained by challenges such as high production costs and limited stability under extreme environmental conditions. Current research efforts are directed toward overcoming these barriers through:

Metabolic Engineering: Employing genetic engineering to enhance bio surfactant yield in microbial hosts and to reduce production costs (13,16).

Hybrid Technologies: Integrating bio surfactants with microbial electrochemical systems to improve degradation performance, particularly under anaerobic conditions (13).

Microbial Prospecting: Isolating novel bio surfactant-producing strains from extreme environments, such as hydrothermal vents and desert soils, to identify robust candidates for challenging bioremediation scenarios (12).

Advances in Bioremediation Technologies

Recent developments in microbial biotechnology have opened new avenues for enhancing the efficiency and adaptability of bioremediation strategies. This section explores the integration of genetic engineering, microbial electrochemical systems, and hybrid approaches in petroleum hydrocarbon cleanup.

Genetic Engineering of Microorganisms for Enhanced Biodegradation

Genetic engineering represents one of the most promising strategies for enhancing microbial degradation of petroleum hydrocarbons. By modifying key metabolic pathways, engineered strains exhibit improved enzymatic activity, substrate specificity, and environmental resilience. *Pseudomonas putida* strains engineered through gene editing have demonstrated up to 60% faster degradation rates of polycyclic aromatic hydrocarbons (PAHs) compared to their wild-type counterparts, proving highly effective for soil bioremediation (15).

The successful transfer of alkane hydroxylase genes into *Escherichia coli* has enabled the engineered strain to degrade a broader range of hydrocarbons with greater efficiency (11).

The use of CRISPR-Cas9 gene-editing technology is gaining attention for tailoring bacterial metabolic networks to target specific hydrocarbon pollutants and optimize degradation under diverse environmental conditions (12).

While genetically modified microorganisms (GMOs) offer considerable potential, concerns remain regarding their environmental release. Regulatory frameworks and ecological risk assessments are essential to ensure biosafety in field-scale applications.

Microbial Electrochemical Systems (MES) for Anaerobic Bioremediation

Microbial electrochemical systems (MESs) are an emerging biotechnological solution for hydrocarbon degradation in oxygen-deficient environments. These systems utilize bio electrochemical interactions between microbes and electrodes to facilitate electron transfer, thus stimulating microbial metabolism and hydrocarbon breakdown. MESs have shown successful application in deep-sea and subsurface environments, where traditional aerobic biodegradation is limited due to low oxygen availability (13).

The integration of MES with sulfate-reducing bacteria has led to a reported 50% increase in anaerobic crude oil degradation (16).

A pilot-scale MES reactor used in industrial wastewater treatment achieved a 70% reduction in hydrocarbon pollutants within 90 days, indicating strong potential for real-world implementation (12).

Hybrid Bioremediation Strategies

Hybrid bioremediation approaches combine biological, chemical, and physical techniques to maximize hydrocarbon removal efficiency. These integrated methods are particularly advantageous in high-toxicity or large-scale contamination scenarios.

Bio augmentation, coupled with chemical oxidation, has been shown to enhance hydrocarbon degradation by 40% compared to microbial treatment alone (10). Phytoremediation using plants in conjunction with microbial consortia has proven effective in treating petroleum-contaminated agricultural soils, achieving a 68% reduction in total petroleum hydrocarbons over six months (11).

The combination of bio stimulation and bio surfactant application has demonstrated significant success in cold environments, where microbial activity is naturally limited. This strategy facilitates improved microbial access and degradation of oil contaminants under low-temperature conditions (12).

Challenges and Limitations

Despite the promising potential of microbial bioremediation, its efficiency is highly sensitive to a range of environmental and biological factors, presenting several challenges and limitations in practical applications. Temperature is one of the most critical parameters, as microbial enzymatic activity is optimal within specific ranges. While mesophilic bacteria such as *Pseudomonas* and *Acinetobacter* perform well at moderate temperatures (25–35°C), their activity diminishes significantly under extreme thermal conditions. In cold regions like the Arctic, microbial activity is severely hindered due to sub-zero temperatures that disrupt cellular function, making hydrocarbon degradation exceedingly slow. Conversely, high-temperature environments can denature microbial enzymes unless thermophilic strains such as *Geobacillus* are present.

Another significant constraint is pH sensitivity. Most hydrocarbon-degrading microorganisms require a neutral to slightly alkaline environment (pH 6.5–8.5) for optimal performance. Acidic soils reduce enzyme stability and impair cell membrane permeability, while highly alkaline conditions can disrupt key metabolic processes. Although alkaliphilic and acidophilic strains exist, they are not universally effective across all hydrocarbon types or contamination scenarios. Adjusting the pH of contaminated sites can enhance degradation, but such interventions add complexity and cost to remediation efforts.

Nutrient availability also poses a major challenge, as natural environments often lack sufficient levels of nitrogen and phosphorus, which are essential for microbial growth and hydrocarbon metabolism. Bio stimulation through the addition of fertilizers has proven effective in enhancing degradation rates; however, excessive nutrient supplementation can lead to eutrophication, resulting in uncontrolled microbial blooms and oxygen depletion. This, in

turn, can inhibit aerobic degradation pathways and potentially lead to secondary environmental issues.

Oxygen availability is another limiting factor, particularly in anaerobic or poorly aerated environments such as subsurface soils or groundwater. Since most hydrocarbon-degrading microbes rely on aerobic respiration, oxygen deficiency can severely restrict biodegradation. Although techniques such as bioventing, biosparging, and mechanical aeration have been developed to overcome this issue, their effectiveness is influenced by soil structure and porosity and may not be suitable for all site conditions.

Additionally, microbial interactions and competition within complex communities can limit the success of bioremediation. Introduced strains may be outcompeted by native microbes or inhibited by antagonistic interactions. The presence of toxic substances, such as heavy metals or complex chemical mixtures, can also inhibit microbial activity by disrupting enzymatic processes or damaging cellular structures. These compounds may require the use of specially adapted or genetically engineered microbes, which raises further ecological and regulatory concerns.

Finally, translating laboratory or pilot-scale successes into large-scale field applications remains a considerable challenge. Environmental variability, including fluctuations in temperature, pH, and contaminant distribution, can lead to inconsistent results across different areas of a contaminated site. Monitoring and controlling these variables in real time is resource-intensive and technically demanding, limiting the scalability and predictability of microbial bioremediation strategies.

Field Studies / Case Studies

Numerous case studies and experimental field investigations have been conducted across Iran and beyond, highlighting the potential of microbial agents in the bioremediation of petroleum contaminants under various environmental conditions.

In a study published in 2020 (1399 in the Iranian calendar) in the *Journal of Environmental Science and Technology*, researchers utilized gas chromatography with a flame ionization detector (GC-FID) to assess the effectiveness of bacterial isolates in hydrocarbon degradation. Among the isolates tested, *Pseudomonas* demonstrated the highest efficiency, achieving a 65.25% reduction of petroleum hydrocarbons within 30 days. In contrast, *Acinetobacter* achieved a 32.42% reduction, underscoring the differential degradation capacities among bacterial genera. This study exemplifies the practical advantages of bio augmentation using

targeted bacterial strains in controlled remediation environments (17).

A complementary investigation in 2016 (1395 in the Iranian calendar) by Ghavidel et al. employed a hydrocarbon-enriched culture medium to isolate indigenous petroleum-degrading bacteria. Over a 20-day incubation period, strains labeled BJ.1 and BM.1 were identified as the most efficient based on growth rate, optical density, pH reduction, respiration rate, and substrate consumption. These physiological indicators correlated strongly with degradation efficiency, confirming the utility of selecting bacterial consortia based on multi-parametric screening (18).

Khosro Sedighian and colleagues expanded upon this approach by sampling soils from four hydrocarbon-contaminated regions in Iran. Their results indicated that *Bacillus* spp. exhibited the highest degradation capability (38%), followed by *Pseudomonas* (33%) and *Acinetobacter* (9%) (19). This study emphasized the relevance of local microbial populations in site-specific bioremediation strategies and demonstrated the importance of microbial diversity in determining degradation potential across different ecosystems.

In another case, *Bacillus licheniformis* was isolated and tested for its ability to degrade 2% crude oil in soil, showing significant degradation efficiency (20). Similarly, Amoozgar et al. confirmed that *Bacillus subtilis* could effectively break down 12% benzene, highlighting its potential for treating aromatic hydrocarbon contamination (21).

Fungal bioremediation has also shown promising results in field trials. In a study by Mohsenzadeh, fungi from the rhizosphere of buttercup plants growing in motor oil-contaminated soil were examined. The species *Fusarium acuminatum* and *Trichoderma harzianum* led to the most significant reductions in oil concentration. This indicates the potential of plant-fungal synergism in phytoremediation strategies, especially in contaminated agricultural or semi-natural areas (22). Further supporting evidence comes from Safari et al., who isolated cyanobacteria from a hydrocarbon reservoir. The species *Schizothrix vaginata* ISc108 demonstrated an exceptional biodegradation capability of up to 93.98% crude oil removal, representing one of the highest degradation efficiencies reported in field-based studies (23).

Kianpour and colleagues introduced the MR1 strain, closely related to *Streptomyces*, which achieved 71.58% hydrocarbon degradation within just 10 days (24). This rapid degradation makes MR1 an attractive candidate for emergency response in high-risk pollution scenarios.

Lastly, a case study at the Tabriz Refinery highlighted the industrial-scale potential of *Pseudomonas aeruginosa*, which achieved the

highest removal efficiency at 5% crude oil concentration among all tested strains (25). This confirms its robust performance under real-world conditions and validates the application of *Pseudomonas* in industrial bioremediation systems. These diverse case studies collectively underscore the effectiveness of microbial bioremediation in both laboratory and field conditions. They highlight the importance of selecting the appropriate microbial strains based on site-specific conditions and contaminant profiles, and they reinforce the role of indigenous and engineered microbial consortia in sustainable environmental restoration.

Comparison with Other Methods

Bioremediation, particularly microbial bioremediation, has emerged as an effective and sustainable method for the treatment of petroleum-contaminated environments. However, to understand its full potential, it is essential to compare this approach with conventional physical and chemical remediation methods.

Physical methods, such as soil excavation, containment, or vacuum extraction, are widely used for the immediate removal of surface or shallow subsurface oil contamination. While effective in the short term, they often require extensive energy input, are labor-intensive, and can result in the relocation of pollutants rather than their degradation. These methods also disrupt local ecosystems and may not be feasible for large-scale or remote spill sites (1,2).

Chemical remediation, including the use of surfactants, solvents, and oxidizing agents, offers faster breakdown of hydrocarbons but poses risks of secondary contamination. Many chemical treatments produce toxic byproducts or alter soil and water chemistry adversely. Additionally, chemical oxidants can be costly, and their overuse may lead to long-term ecological imbalance (3,4).

In contrast, microbial bioremediation utilizes the natural metabolic abilities of bacteria and fungi to degrade hydrocarbons into non-toxic end products, such as carbon dioxide and water (5,6). Unlike chemical treatments, microbial methods are environmentally benign, cost-effective, and self-sustaining when conditions are favorable. They can also be adapted for in situ application, reducing the need for excavation and transportation of contaminated material.

Moreover, bioremediation is particularly advantageous for treating low to moderately contaminated sites over extended periods. As shown in numerous case studies, such as those involving *Pseudomonas* spp. and *Bacillus* spp. (17–21) Microbial remediation not only achieves high degradation rates (over 60% in many cases) but also preserves soil structure and biodiversity. The use of

bio surfactants further enhances this approach by improving hydrocarbon solubility and microbial uptake, reducing the need for chemical additives (10,11).

While bioremediation does face challenges, including sensitivity to environmental variables like temperature, pH, and nutrient availability (6,15), it offers significant advantages when combined with emerging technologies. Hybrid approaches, such as combining microbial remediation with phytoremediation, chemical oxidation, or electrochemical stimulation, have demonstrated superior performance in challenging conditions (16,26). Additionally, engineered microbes and microbial electrochemical systems (MES) are bridging the gap between biological and physicochemical methods, providing rapid and effective degradation in oxygen-limited environments (12,16).

In summary, although physical and chemical methods remain useful in certain contexts, microbial bioremediation provides a more sustainable, adaptable, and cost-effective solution, particularly for long-term site restoration. When integrated with modern biotechnologies and environmental monitoring tools, microbial methods are poised to become the preferred choice in petroleum remediation projects worldwide.

Future Perspectives in Bioremediation of Oil

The future of oil bioremediation lies in the integration of cutting-edge technologies and multidisciplinary approaches that enhance microbial efficiency, monitoring, and adaptability in diverse environments. One promising avenue is the advancement in microbial engineering. Genetically modified microorganisms (GMOs), such as engineered bacteria and fungi, are being developed to possess enhanced hydrocarbon-degrading abilities by modifying their metabolic pathways (26,27). In parallel, synthetic biology enables the design of microbial consortia that can collaboratively degrade a wide range of hydrocarbons with high efficiency (28). Additionally, ongoing research into natural microbiomes in oil-contaminated sites provides insights into how indigenous microbial communities function, thus aiding in the optimization of their bioremediation activity (29).

Nanotechnology is also playing a pivotal role in enhancing bioremediation. Engineered nanoparticles such as iron oxide and carbon-based materials are being utilized to stimulate microbial activity and accelerate oil degradation processes (30,31). These nanoparticles can enhance bioavailability and electron transfer, key factors in microbial metabolism. Furthermore, the development of Nano-biosensors allows for real-

time monitoring of microbial activity and hydrocarbon breakdown, ensuring that environmental conditions remain optimal throughout the remediation process (32).

Phytoremediation techniques are gaining traction, especially in environments where plant-microbe interactions can be leveraged. Certain plant species facilitate hydrocarbon degradation by harboring root-associated microbes capable of breaking down pollutants (33). Moreover, genetic modification of these plants is being explored to improve their capacity for absorption and degradation of oil contaminants (34). A complementary approach involves bio augmentation, where oil-degrading bacteria are introduced into the rhizosphere to boost the bioremediation potential of plant roots (35).

The emergence of bio electrochemical systems (BES) presents a novel strategy for oil bioremediation. Microbial fuel cells (MFCs) utilize electroactive bacteria to degrade hydrocarbons while simultaneously generating electricity, offering a sustainable dual benefit of environmental cleanup and renewable energy production (36). Another technique, electro-stimulation, involves applying small electric currents to enhance microbial activity and accelerate the breakdown of hydrocarbons (37). Adaptation to extreme environments is another critical frontier. In Arctic and deep-sea regions, where conventional bioremediation is hindered by low temperatures and high pressures, research is focused on psychrophilic (cold-loving) bacteria that can effectively function in such conditions (38). Likewise, oil spills in arid and desert regions require drought-resistant microbial strains and innovative soil conditioning techniques to sustain microbial activity in harsh, water-limited environments (39).

The integration of artificial intelligence (AI) and machine learning into bioremediation is revolutionizing how we predict and optimize remediation outcomes. AI-based predictive models are being developed to identify the most effective microbial strains and environmental parameters for specific contamination scenarios (40). Moreover, real-time monitoring of bioremediation processes using AI-driven technologies, such as drones and sensor networks, enables continuous evaluation and adjustment of remediation strategies (41).

Lastly, the successful application of these advanced technologies depends on policy support and sustainable implementation. It is essential that bioremediation strategies remain environmentally friendly and do not introduce secondary pollutants. Governments and environmental agencies are increasingly recognizing the benefits of biological remediation and may implement regulations and incentives to promote its use over traditional chemical or mechanical methods. Together, these innovations offer a promising future for more

effective, sustainable, and adaptive bioremediation of oil-contaminated environments.

Conclusion

Microbial bioremediation represents a sustainable and eco-friendly strategy for addressing petroleum contamination in various environments. By utilizing the metabolic capabilities of bacteria and fungi, hydrocarbons can be broken down into less toxic or non-toxic end products. Compared to conventional methods such as chemical treatment or physical removal, microbial remediation offers advantages like lower environmental impact, cost-effectiveness, and long-term applicability.

The success of bioremediation depends on several factors, including temperature, pH, nutrient availability, oxygen concentration, and the composition of the microbial community. In particular, native microorganisms have shown superior degradation performance due to their natural adaptation to contaminated environments. Additionally, recent advancements in microbial biotechnology, such as genetic engineering and the use of bio surfactants, have further improved the efficiency and applicability of these methods.

Despite these advantages, challenges remain especially in large-scale applications and under extreme environmental conditions. However, with the growing interest in bio surfactants, bio electrochemical systems, and the integration of AI-based monitoring tools, the future of microbial bioremediation looks promising. Supporting further research, increasing public awareness, and establishing strong regulatory frameworks are essential for enhancing the practical use of this technology and ensuring its success as a reliable remediation approach

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

References

- [1] Rouhani-Nejad M. (2019), [Review of the Energy Status in Iran and the World: Challenges and Opportunities](#), *Energy Research Journal*, 23(4):556–69.

- [2] Van Hamme JD, Singh A, Ward OP. (2003). [Recent advances in petroleum microbiology.](#) *Microbiology and Molecular Biology Reviews.* 67(4):503–49.
- [3] Head IM, Jones DM, Røling WFM. (2006). [Marine microorganisms make a meal of oil.](#) *Nat Rev Microbiol.* 4(3):173-182.
- [4] Ron EZ, Rosenberg E. (2002). [Biosurfactants and oil bioremediation.](#) *Curr Opin Biotechnol.* 2002;249–52.
- [5] Li X, Li J, Qu C, Yu T, Du M. (2021). [Bioremediation of clay with high oil content and biological response after restoration.](#) *Sci Rep.* 6;11(1):9725.
- [6] Liu B, Chen B, Zhang B, Song X, Zeng G, Lee K. (2021). [Photocatalytic ozonation of offshore produced water by TiO₂ nanotube arrays coupled with UV-LED irradiation.](#) *J Hazard Mater.* 402:123456.
- [7] Zhu X, Zhang Q, Cheng L, Wang J, Zhou X. (2020). [Enhanced bioremediation of crude oil-contaminated soil using microbial electrochemical systems.](#) *Environ Sci Technol.* 56(4):3452–61.
- [8] Kalia, A. (2022). [Recent advancements in hydrocarbon bioremediation and microbial technologies: A review.](#) *Environmental Science and Pollution Research*, 29(12), 17000–17020.
- [9] Adedeji, J. A., & Oladipo, O. O. (2022). [Microbial bioremediation and biodegradation of petroleum hydrocarbons: Mechanisms and practical applications.](#) *Applied Sciences*, 12(23), 12212.
- [10] Mishra, P., & Kumar, S. (2023). [New insights into the bioremediation of petroleum pollutants: Microbial strategies and field applications.](#) *Science of the Total Environment*, 880, 163456.
- [11] Atlas, R. M., & Hazen, T. C. (2011). [Oil biodegradation and bioremediation: A tale of the two worst spills in U.S. history.](#) *Environmental Science & Technology*, 45(16), 6709–6715.
- [12] Ron, E. Z., & Rosenberg, E. (2014). [Enhanced bioremediation of oil spills in the sea.](#) *Marine Pollution Bulletin*, 85(1), 9–14.
- [13] De Almeida, D. G., Magalhães, A. L., & Pedrosa, F. O. (2016). [Biosurfactants: Promising molecules for petroleum bioremediation.](#) *Frontiers in Microbiology*, 7, 1718.
- [14] Karlapudi, A. P., & Babu, R. P. (2018). [Role of biosurfactants in bioremediation of oil pollution: A comprehensive review.](#) *Journal of Environmental Management*, 210, 120–131.
- [15] Sah, D., Sahu, A. K., & Mishra, R. R. (2022). [A review on biosurfactant-producing bacteria and their role in hydrocarbon biodegradation.](#) *Microbial Biotechnology Reviews*, 16(4), 345–368.
- [16] Varjani, S. J., & Upasani, V. N. (2017). [Critical review on biosurfactant analysis, purification and applications in petroleum bioremediation.](#) *Biocatalysis and Agricultural Biotechnology*, 12, 79–95.
- [17] King, G. M., Kostka, J. E., Hazen, T. C., & Sobczyk, P. A. (2015). [Microbial responses to the Deepwater Horizon oil spill: From coastal wetlands to the deep sea.](#) *Annual Review of Marine Science*, 7, 377–401.
- [18] Kimes, N. E., Callaghan, A. V., Aktas, D. F., et al. (2014). [Microbial transformation and fate of hydrocarbons following the Deepwater Horizon spill.](#) *Frontiers in Microbiology*, 5, 19.
- [19] Mason, O. U., Hazen, T. C., Borglin, S., et al. (2012). [Metagenomics reveals sediment microbial community response to petroleum contamination.](#) *ISME Journal*, 6(7), 1464–1475.
- [20] Lu, Z., Deng, Y., Van Nostrand, J. D., et al. (2012). [Microbial gene functions enriched in the Deepwater Horizon oil plume.](#) *ISME Journal*, 6, 1715–1724.
- [21] Wu, M., & Wang, J. (2017). [Bioremediation of hydrocarbon degradation in petroleum-contaminated sites: Techniques and field applications.](#) *Chemosphere*, 182, 1–14.
- [22] Christova, N., & Stefanova, N. (2019). [Biodegradation of crude oil hydrocarbons by newly isolated strains: Isolation, characterization and potential for bioremediation.](#) *Biodegradation Journal*, 30(5), 421–436.
- [23] Liu, S., & Wang, H. (2022). [Petroleum spill bioremediation by indigenous microbial consortia: Mechanisms and efficacy.](#) *Journal of Hazardous Materials*, 432, 128762.
- [24] Elshafei, A. M., & Elsayed, A. M. (2024). [Microbial bioremediation of soils contaminated with petroleum hydrocarbons: Progress and future directions.](#) *Sustainable Remediation*, 6(1), 15–34.
- [25] Miles, S. M. (2023). [Bioremediation of petroleum hydrocarbons in the subsurface.](#) In *Advances in Subsurface Remediation*, 245–270). Springer.
- [26] Zahed, M. A., & Hosseini, S. M. (2022). [Biosurfactant: A green and effective solution for bioremediation of petroleum hydrocarbons in aquatic environments.](#) *Environmental Biotechnology Reports*, 8(2), 101–116.
- [27] Varjani, S. J. (2017). [Microbial degradation of petroleum hydrocarbons.](#) *Bioresource Technology Reports*, 1, 1–11.
- [28] Yan, Z. F., & Chen, L. (2025). [Enhanced degradation of crude oil by immobilized](#)

- [bacterial consortia: Laboratory to pilot scale.](#) *Biotechnology Advances*, 63, 108250.
- [29] Prince, R. C., & Butler, J. D. (2014). [Bioremediation of marine oil spills: Myths and realities.](#) *Environmental Science & Technology*, 48(16), 9051–9059.
- [30] Adedeji, J. A., et al. (2022). [Microbial strategies for mineralization of complex petroleum fractions: Enzymes and pathways.](#) *Applied Microbiology and Biotechnology*, 106(7), 2781–2796.
- [31] Cunningham, C. J., & Lythgoe, D. (2020). [Potential risks of antibiotic-resistant bacteria and resistance genes in bioremediation of petroleum-contaminated soils.](#) *Environmental Monitoring and Assessment*, 192, 463.
- [32] Kimes, N. E., & Morris, P. J. (2014). [Microbial succession following petroleum contamination: Functional and taxonomic shifts.](#) *FEMS Microbiology Ecology*, 90(3), 825–840.
- [33] Santos, D. K. F., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2016). [Biosurfactants: Multifunctional molecules for bioremediation of petroleum-contaminated environments.](#) *International Journal of Molecular Sciences*, 17(12), 2018.
- [34] Head, I. M., Jones, D. M., & Larter, S. R. (2003). [Biological activity in the deep subsurface and the origin of heavy oil.](#) *Nature*, 426(6964), 344–352.
- [35] Prince, R. C. (2015). [Oil spill dispersants: Boon or bust?](#) *Marine Pollution Bulletin*, 96(1–2), 1–2.
- [36] El-Bestawy, E., & Hassan, M. (2021). [Phytoremediation-assisted microbial degradation of petroleum hydrocarbons: Synergistic approaches and field trials.](#) *Environmental Technology & Innovation*, 22, 101462.
- [37] Mishra, S., Jyot, J., Kuhad, R. C., & Lal, B. (2001). [In situ bioremediation potential of some bacterial strains for oil-polluted soils.](#) *Environmental Pollution*, 109(1), 69–76.
- [38] Head, I. M., & Jones, D. M. (2003). [Microbial processes controlling the fate of petroleum hydrocarbons in the environment.](#) *Applied Microbiology Reviews*, 17(4), 231–254.
- [39] Prince, R. C., & Butler, J. D. (2016). [Natural attenuation and biostimulation for oil spill remediation: Comparative case studies.](#) *Journal of Environmental Management*, 165, 1–12.
- [40] Adetutu, E. M., & Olaniran, A. O. (2016). [Biodegradation potential of bacterial consortia isolated from petroleum-contaminated sites.](#) *Ecotoxicology and Environmental Safety*, 125, 59–66.
- [41] Mishra, P., & Brooks, M. S. L. (2023). [Challenges and future directions in microbial remediation of petroleum-contaminated sites: Scale-up, risk, and regulatory issues.](#) *Critical Reviews in Environmental Science and Technology*, 53(9), 1234–1267