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REVIEW ARTICLE OPEN ACCESS

Microbial Bioremediation of Petroleum Contaminated Soil: Structural Complexity, Degradation Dynamics and Advanced Remediation Techniques

Pratik Kakde and Jaigopal Sharma*

Department of Biotechnology, Delhi Technological University, Delhi, India.

Abstract

Soil contamination with petroleum hydrocarbons poses a significant environmental challenge, necessitating effective remediation strategies to mitigate ecological risks. This review paper systematically examines the current state of knowledge regarding soil contamination with petroleum hydrocarbons, focusing on diverse sources and the extent of contamination. The investigation encompasses a range of hydrocarbon compounds, including aliphatic and aromatic fractions, emphasizing the dynamic nature of the contamination scenarios. A thorough review of bioremediation techniques, which have shown promise and sustainability as methods for cleaning up soil contaminated with petroleum hydrocarbons are also involved in order to solve these issues. Each of the three microbial processes, biodegradation, bioaugmentation, and biostimulation, is covered in detail in the paper, along with the complex mechanics underlying each technique. The report also emphasises new developments in genetics and molecular biology that add to our understanding of the metabolic pathways and microbial interactions involved in hydrocarbon breakdown. The effectiveness of plant-assisted bioremediation coupled with bioaugmentation and stimulation, specifically phytoremediation, is also explored, emphasizing the potential of certain plant species to enhance the removal of petroleum hydrocarbons from contaminated soils through rhizosphere interactions and plant-associated microbial activities. Furthermore, the paper evaluates the influence of environmental variables including soil composition, temperature, and moisture content on the effectiveness of bioremediation techniques, offering valuable perspectives on enhancing remediation efficiency through optimal conditions. The possibility for enhancing conventional bioremediation techniques through the incorporation of cutting-edge technology like nano-remediation is also explored.

Keywords: Bioremediation, Microbial Degradation, Total Petroleum Hydrocarbons, Phytoremediation, Soil Contamination

***Correspondence:** sharmajaigopal@dce.ac.in

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INTRODUCTION

As the time passed by and the advancements in industrial revolution led to people having a convenient life, it also brought along the increased concerns of the environmental pollution. The standard of living for people has increased drastically over the past few decades, which in turn has sped up the consumption as well as the accumulation of the raw materials that are used to achieve this lifestyle. Environmental pollution is caused by factors such as population increase, industrialization, urbanization, deforestation, and the advancement of modern technology. The release of the toxic substances like nuclear waste, greenhouse gases, heavy metals and hydrocarbons released by the above factors are of great concern.[1] All these factors have large negative impact on the natural processes taking place in the environment.[2] Exposure of the human or any other species to this polluted water sources, air and soil has led to the death of over 8.4 million people worldwide in the year 2012. Other leading causes of death such as HIV/AIDS are responsible for about 6.9 million less deaths per year worldwide when compared to environmental pollution. The mass majority of this affected population are from Low- and Middle-income countries (LMICs).

Poor countries also suffer the pollution caused by the immigrant industries. The industries empty disproportionate amount of various toxic gases in the atmosphere.[3] Some major contributor to air pollution is seen to be the particulate matter from the power plants and the emission caused by the motor vehicles.

Children's underdeveloped lungs induced with indoor and outdoor air pollution, possess an increased risk of respiratory infections. Asthma and allergic rhinitis have become more common in children and young adults. Ranging from minor inconvenience to death causing diseases air pollution can be responsible for them all. Looking into the WHO reports, we can say that around 2.4 million people succumb to the consequences of air pollution all around the world.[4,5] This issue arises due to the lack of an effective methodology for addressing the problem.

Petroleum contamination of soil

A land oil spill refers to the unintended release of petroleum hydrocarbons into terrestrial environments, often resulting from incidents such as pipeline ruptures, storage tank leaks, or transportation accidents as shown in Figure 1. Most Observed failures that turns out to be major contributors to land oil spills. By the research and data collection, it has been proven that the contamination due to the accumulation of petroleum hydrocarbons (PHCs) is the leading cause of soil contamination. When the oil spills on land, it can infiltrate soil and percolate into groundwater, posing environmental hazards. The spilled oil undergoes physical and chemical transformations, with its components interacting with soil particles, affecting soil structure and permeability. These hydrocarbons can persist in

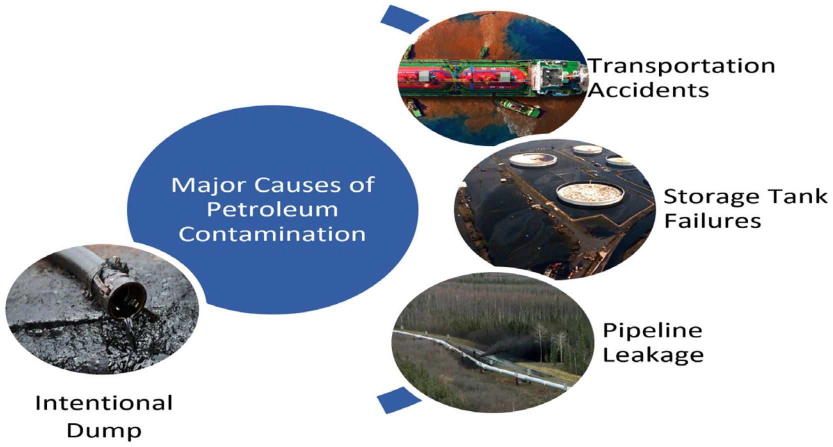

Figure 1. Key Failures Contributing to Major Petroleum Hydrocarbon (PH) Contamination: An Analysis of Predominant Causes in Environmental Incidents

the environment, leading to soil contamination and potential adverse effects on vegetation, microbial communities, and groundwater quality.

Mitigating the impact of land oil spills involves prompt containment, cleanup efforts, and remediation strategies to minimize soil and groundwater contamination, as well as the associated with ecological and human health risk.

The severity of the impacts of oil contamination can be well demonstrated by the case study of the Nigerian soil where, groundwater, vegetation, and streams have long been contaminated by land oil spills, with spill extension being the main cause of many difficulties. Because of these, soil restoration is absolutely necessary in the host communities around oil fields and pipelines. The Shell Petroleum Development Company registered 44 land oil spill occurrences, totaling ≥500 bbl between 2011 and 2019, or 53,631 bbl. These accidents were mostly ascribed to 83% of the overall sabotage. The 53,631 bbl of spills were not fully cleaned up from the spill sites, negatively affecting farms, fishponds, rivers, and residential areas. Land oil spills and leakage incidents as bad they are, come in recognition because of the major health effects it can put on the population.[6] The human dependence on petroleum and its byproducts has played a significant role in the occurrence of oil spill pollution. The extraction, transportation, and use of petroleum come with inherent risks that can lead to accidental releases and spills leading to heavy oil deposition on the sea surfaces. The deposition has been increased to such high numbers that the government is obligated to look into the methods to reduce the deposition. One of the methods that were earlier used seemed to be optimal at first but it possessed its own risks. Some of these techniques involved Mechanical containment and recovery technique.[7]

Structural complexity of petroleum hydrocarbons

Global environmental pollution is a result of growing industrialization, mining, oil and gas extraction, and chemical extraction, all of which continuously release significant volumes of contaminants into the environment. Heavy metals and petroleum hydrocarbons (PHCs) are two of the main pollutants that are considered to be a danger to the substantial environment. Of these, pollutants based on polycyclic aromatic hydrocarbons (PHCs) are causing significant harm to ecosystems, flora, and fauna, as well as unfavourable economic outcomes.[8] PHC-based pollutants are also extremely stable, heterogeneous organic molecules with a spectrum of physio-chemical characteristics made up of different carbon and hydrogen atoms organised structurally. Furthermore, some hydrocarbons have a few functional components bonded to them, such as sulphur, oxygen, and nitrogen. The two primary classifications into which it can be further divided are aromatic and aliphatic hydrocarbons.[9] Total petroleum hydrocarbons (TPH) are a term used in analytical methods that designates the quantifiable amount of petroleum hydrocarbons in environmental matrices. It also serves as a regulatory benchmark. Rapid, accurate, on-site, and reasonably priced methodologies are frequently needed for a typical assessment of TPH contamination in soil and sediment in order to support risk assessment procedures and help with site management and remediation. Many approaches of quantifying TPH and its constituent parts have been investigated. Total petroleum hydrocarbon as petrol (TPHg) and total petroleum hydrocarbon as diesel (TPHd), based on the carbon range, are the two forms of TPH that are now analysed in soil and sediment. PHCs are generally composed of around 57% aliphatic hydrocarbons, approximately 29% aromatic hydrocarbons, and approximately 14% asphaltenes with various polar components. Furthermore, aliphatic hydrocarbons exist in both saturated and unsaturated forms as linear or branched-chain hydrocarbons. the increased demand for petroleum oil has resulted in hydrocarbon contamination in soil, particularly agricultural lands, and many other ecosystems worldwide. An example of this is the persistent polycyclic ring seen in aromatic hydrocarbons, such as benzene. Although there are issues with cost-effectiveness, physical and chemical treatments are useful methods for removing large amounts of contamination from small regions.[10]

Petroleum hydrocarbons encompass a vast array of compounds, each with its unique structure. The most primary structures that forms up the PHCs are alkanes (Paraffins), alkynes (Acetylenes) and alkenes (Olefins). Alkanes have a linear or branched structure, consisting of single carbon-carbon bonds and saturated with hydrogen atoms. An example is methane (CH_4), where a single carbon atom is bonded to four hydrogen atoms. Alkenes contain at least one carbon-carbon double bond, leading to a degree of unsaturation in the molecule. Ethene (C_2H_4) serves as a simple example. Alkynes possess at least one carbon-carbon triple bond, resulting in a high degree of unsaturation. Ethyne (C_2H_2) is a representative example. All these simpler structures constitute the Aliphatic group of hydrocarbons. On the other hand, cycloaliphatic hydrocarbons represent a class of organic compounds characterized by the presence of closed-ring structures with aliphatic properties, wherein carbon atoms form a cyclical arrangement. Aromatic hydrocarbons have conjugated double bonds, while cycloaliphatic hydrocarbons are saturated and have single bonds only. Cycloaliphatic hydrocarbons form a wide variety of compounds, such as cycloalkanes, cycloalkenes, and cycloalkynes. Cycloalkanes are cyclic hydrocarbons with no double or triple bonds, cycloalkenes having one or multiple double bonds in the ring, and cycloalkynes having triple bonds between carbon atoms. Cycloaliphatic is a unique class of chemical compounds with distinct physical and chemical properties, and its members are used in multiple industrial applications, such as polymerization, production of solvents, and synthesis of fine chemicals.

Understanding the structures and behaviours of cycloaliphatic hydrocarbons is essential for elucidating their roles in organic chemistry and their utility in industrial applications. Aromatic hydrocarbons constitute a class of organic compounds distinguished by the presence of a stable, planar, and cyclic structure known as an aromatic ring. The prototypical aromatic hydrocarbon is benzene, featuring six carbon atoms connected by alternating single and double bonds within the ring. The conjugated electrons in the aromatic system contribute to its exceptional stability and distinctive resonance structure. The class of aromatic hydrocarbons goes beyond benzene; it includes numerous derived and polyaromatic compounds with multiple benzene rings fused together. Aromatic chemicals have fascinating electrical characteristics that differ significantly from aliphatic chemicals in both stability and reactivity in the last, particularly. However, they are critical to synthetic organic chemistry and serve as primary feed-stocks for many industrial chemicals, drugs, and polymer syntheses. The investigation of the aromatic system is therefore important in leading our attempt to comprehend reactivity in relationships to molecular structure and attributes. The paper makes an attempt to explore and summarize the various bioremediation techniques, emphasizing the role of microbial mechanisms and identifying key factors that influence the success of bioremediation strategies in different environmental contexts as it provides a comprehensive analysis of the effectiveness of different microbial species in degrading various types of petroleum hydrocarbons, highlighting the comparative efficiency of these microorganisms across diverse contamination scenarios.

Literature and data collection

The literature search for the review was conducted using a range of academic databases including PubMed, Scopus, Google Scholar and Web Of Science. The search was limited to peer-reviewed journal articles, conference proceedings, and relevant books published between 2016-2024. Additionally, gray literature, such as reports from environmental agencies and dissertations, was reviewed to capture emerging trends and non-peer-reviewed insights. The keywords used for the literature study include Bioremediation, microbial degradation, total petroleum hydrocarbons, phytoremediation, soil contamination. The review was not limited to any specific geographical region, allowing for a global perspective on bioremediation practices and outcomes. The inclusion criteria also emphasized articles that explored the interactions between different microbial species and their effectiveness in various environmental conditions.

A total of 90 articles were initially identified. After a thorough screening process based on relevance, quality, and the inclusion criteria mentioned above, 48 articles were included in the final review. These selected studies represent the key advances and current understanding in the field of microbial bioremediation of petroleum-contaminated soils.

Literature review

Types of petroleum hydrocarbon contamination

Petroleum hydrocarbons are composed of a variety of substances such as crude oil, refined products, polycyclic aromatic hydrocarbons (PAHs), and chlorinated hydrocarbons which all possess different characteristics and environmental implications. The paper focuses on four major types of petroleum hydrocarbon contamination including crude oil, refined petroleum product contaminants, PAH contaminants and chlorinated hydrocarbon contaminants from the perspective of their specific attributes and impacts. Table 1 below represents the most harmful pollutants in major petroleum categories that have affected various aspects of biodiversity. The soil gets contaminated with crude oil when unrefined petroleum is released into the environment often through oil spillage during extraction, transportation or storage. Crude oil is made up of aliphatic and aromatic hydrocarbons that occurs as a complex mixture containing straight chain alkanes, alkenes, PAHs among others. Spilled oil can modify both the structure and composition of soil resulting in reduced fertility and altered microbial activity. Persistence of some components like PAHs poses long term ecological risks to humans.[11]

Contamination of refined petroleum products includes the discharge of processed petrol and its derivatives, like benzene, gasoline or diesel fuel. Such chemicals have a spectrum of hydrocarbons including paraffins, aromatics as well as additives. Refined petroleum products can enter soil rapidly, altering its physical and chemical characteristics. Vocalization of certain components such as BTEX (benzene, toluene, ethylbenzene and xylene) compounds mainly causes air pollution while any other remnants in the soil may cause persistent pollution even through groundwater.

PAH contamination is associated with polycyclic aromatic hydrocarbons which are made up of several fused aromatic rings. PAHs are often generated by incomplete combustion processes for organic materials e.g. fossil fuels and wood. Their presence in soils permanently and bio-magnification pose risks towards terrestrial ecosystems as well as human health. Carcinogenic properties are known for PAHs that can result to long term health effects when an individual is exposed through contaminated soils.[12]

Chlorinated hydrocarbon contamination involves compounds where chlorine atoms replace hydrogen in hydrocarbons. Common chlorinated hydrocarbons include trichloroethylene (TCE) and perchloroethylene (PCE), often used in industrial processes. These compounds can persist in soil and groundwater, posing significant risks to human health. TCE, for instance, is associated with adverse effects on the nervous system, liver, and kidneys, emphasizing the importance of addressing chlorinated hydrocarbon contamination through rigorous remediation strategies.[13]

Evolution of the remediation techniques

The most frequently used remediation strategies for petroleum contamination include physical and chemical remediation, where physical remediation focuses on stabilization/solidification and pyrolysis and chemical remediation makes use of oxidation and surfactant leaching. Even if pHs can be swiftly removed from soil through physical and chemical remediation, it still needs a large amount of labour and supplies.[14] The above mentioned cleanup methods can hamper land productivity and the organic contents of the soil, which will have a negative impact on soil ecology. Induction of the contaminated soil with Bacterial consortium which are proven to enhance the contaminant degradation is also considered. These techniques include processes like microbial remediation and phytoremediation etc. Unfortunately these bacteria seem to showcase its ability on a reduced scale in a natural environment when compared to its laboratory counterparts. It is commonly assumed that antagonistic relationships with native bacteria and soil heterogeneity are responsible for the long-term and inconsistent results observed in the use of exogenous degrading bacteria (EDB) for microbial remediation. Deep analysis of the contaminated zones helps in selection of the optimal bacterial consortium.

Effects of pHCs on various aspects of environment and human health

The contamination of the soil by petroleum hydrocarbon has varied but related effects on the environment which exert damage to the ecosystem and the services they provide. The introduction of hydrocarbons alters soil physicochemical properties, leading to reduced soil porosity, water retention, and nutrient availability, thus compromising the overall soil quality. The persistence of certain hydrocarbons can impede microbial activity essential for nutrient cycling, disrupting ecological processes. Additionally, the toxicological effects on soil biota, including bacteria, fungi, and higher organisms, may result in reduced biodiversity and ecological imbalances. The mobility of hydrocarbons in the soil matrix facilitates their leaching into groundwater, potentially contaminating drinking water resources and aquatic ecosystems. Furthermore, the phytotoxicity of hydrocarbons may inhibit plant germination and growth, affecting primary productivity and disrupting trophic interactions. Persistent hydrocarbon contamination in soil contributes to long-term ecological stress, challenging the resilience and sustainability of affected ecosystems. These conditions being deteriorating for all the flora and fauna doesn't exempt from damaging the human health in a direct or indirect way.

Petroleum hydrocarbon contamination of soil poses significant risks to human health through various exposure pathways. As depicted in Figure 2**,** Inhalation of volatile organic compounds (VOCs) released from contaminated soil can lead to respiratory irritation, exacerbate pre-existing respiratory conditions, and potentially cause systemic health effects. Direct dermal contact with contaminated soil may result in the absorption of hydrocarbons through the skin, causing skin irritation and, in some cases, dermatological disorders. Moreover, the potential migration of hydrocarbons into groundwater can contaminate drinking water sources, exposing humans to toxic compounds through ingestion. Moreover, certain hydrocarbons, such as benzene, toluene, ethylbenzene, and xylene, are carcinogenic and long-term exposure to them can lead to the development of cancer. Finally, because hydrocarbons in soil are gradually accumulated in the food chain, some crops grown in such land will contain a higher concentration of them, which will further increase the likelihood of human exposure. Thus, effective sources of risk analysis and damage prevention are essential to reduce the hazards of using soil contaminated with petroleum hydrocarbons for human health and protect the people who have been in contact with the substances.

Microbial bioremediation of petroleum hydrocarbons

As society strives for greater industrialization and development, the inadvertent release of pollutants into the environment has led to widespread contamination of air, water, and soil. Traditional methods of pollution control often fall short in effectively addressing these issues, necessitating innovative and sustainable approaches. One such approach that has gained significant attention is microbial bioremediation.

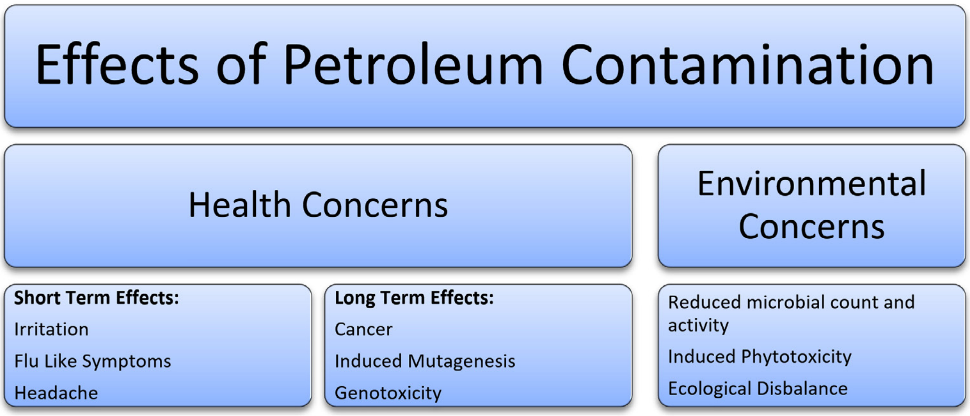

Figure 2. Effects of petroleum hydrocarbon contamination on environment as well as human health adapted from Teklit et al.[43]

Table 1. Characteristics of most common hydrocarbon pollutants and its effects on human health

Type	Structure	Impact	Reference
Aliphatic Hydrocarbons	Open chain Contains C-C, C-H bonds Made of alkanes, alkenes and alkynes	Relatively less toxic but can cause natural attenuation of contamination.	28
Aromatic hydrocarbons	One or more aromatic rings (Typically benzene) Alternating double and Carcinogenic to human and can also single bonds	More persistent. BTEX is toxic to aquatic life. cause hematological and immune system disorders.	36
Chlorinated hydrocarbons	Hydrogen atom substituted with chlorine Aliphatic (Chloroalkanes) and aromatic (Chlorobenzene) Structure	Persistence and accumulation. Highly stable. Liver and kidney damage, reproductive disorders and carcinogenicity.	21
Volatile organic compounds (VOCs)	Diverse group of organic components Low boiling point, High pressure Aliphatic or aromatic HCs coupled with nitrogen, oxygen, sulphur functional group	Contributes to air pollution. Respiratory irritation, Headache. Chronic health effects, including liver and kidney damage.	22
Polycyclic aromatic hydrocarbons (PAHs)	Multiple fused aromatic ring Primary functional group being other aromatic ring	Carcinogenic. Causes developmental and reproductive issues.	37

This cutting-edge field harnesses the power of microorganisms to mitigate and even reverse the impacts of environmental pollution. By capitalizing on the natural abilities of bacteria, fungi, and other microorganisms to break down or transform pollutants, microbial bioremediation offers a promising avenue for restoring ecosystems and safeguarding human and ecological health. In this article, we delve into the diverse methods and applications of microbial bioremediation, exploring how these microscopic heroes are revolutionizing the way we combat pollution and pave the way for a cleaner and healthier planet. An environmentally benign and viable method for cleaning up places affected by petroleum hydrocarbons (PHCs) is microbial remediation. Nowadays, the most popular technique for eliminating dangerous materials from land is biological removal.[15]

Enzymatic mechanism in microbial bioremediation

The bacteria possess enzymes, such as hydroxylases and oxygenases, which initiate the degradation of hydrocarbons by introducing oxygen into their molecular structure. Microbial bioremediation primarily targets the carbon-hydrogen (C-H) bonds present in petroleum hydrocarbons during the degradation process. The initial step in microbial degradation involves the enzymatic activation of these relatively inert C-H bonds, making them more reactive and susceptible to further transformations.[16]

Two key types of enzymes, hydroxylases, and oxygenases, play crucial roles in breaking down these bonds. Hydrolases and oxygenases are enzymatic proteins crucial for microbial bioremediation, particularly in the degradation of petroleum hydrocarbons. Hydrolases play a pivotal role by catalyzing hydrolytic reactions, breaking ester, amide, and other bonds in hydrocarbon molecules. This enzymatic activity results in the introduction of hydroxyl groups, rendering the hydrocarbons more reactive and amenable to subsequent oxidation. On the other hand, oxygenases are instrumental in directly incorporating molecular oxygen into the hydrocarbon structure. Mono-oxygenases and dioxygenases, two major classes of oxygenases, catalyse the introduction of one or two oxygen atoms, respectively, into the hydrocarbon framework. The hydrocarbons' structural classification is summarised in the Table 1 along with their effects on the environment. Although they are generally less hazardous, aliphatic hydrocarbons—which have open-chain structures—can aid in the natural attenuation of pollution. With one or more aromatic rings, aromatic hydrocarbons are more enduring and may be harmful to aquatic life. Chlorinated hydrocarbons are extremely stable compounds that can cause cancer, reproductive problems, liver and kidney damage, and substitution of chlorine atoms for hydrogen atoms. Low boiling point chemicals known as volatile organic compounds (VOCs) can irritate the respiratory tract, give rise to headaches, and have long-term negative health effects. They are also a major source of air pollution. Multiple fused aromatic rings make up polycyclic aromatic hydrocarbons (PAHs), which can cause cancer as well as problems with development and reproduction.

These enzymatic transformations enhance the hydrophilicity of the hydrocarbons, facilitating their degradation by microbial communities. Through the concerted action of hydrolases and oxygenases, microorganisms can initiate the breakdown of recalcitrant C-H bonds in hydrocarbons, paving the way for their eventual mineralization into environmentally benign products such as carbon dioxide and water. Understanding the intricacies of these enzymatic processes is crucial for optimizing microbial bioremediation strategies for hydrocarbon-contaminated sites.

Bioremediation techniques

Bioaugmentation

Bioaugmentation is a remediation technique that involves introducing specific, beneficial microorganisms (such as bacteria, fungi, or algae) into a contaminated environment to enhance the degradation of pollutants. It uses a tailored approach of the degradation of a target pollutant which is dominant in the region. These microorganisms can have the ability to break down or metabolize the pollutants into less harmful substances. Bioaugmentation is commonly used in situations where indigenous microorganisms are insufficient or ineffective at degrading the contaminants. For example, introducing oil-degrading bacteria into an oil spill site to accelerate the breakdown of hydrocarbons. One of the most interesting bioaugmentation techniques is the immobilization of the added augmented microbial strains on a biocarrier, which shows potential future applications for the improved and speedy biological treatment of diverse pollutants. There is a wide range of microorganisms that are able to degrade a variety of pollutants but some pollutants are just resistant to the biodegradation, this resistance may be caused due to the low bioavailability, high stability, high toxicity or low biodegradability of the pollutants.[17]

Although bioaugmentation has been shown to be effective in cleaning up locations that have been contaminated by aromatic chemicals, there are still numerous environmental issues to be solved. The persistence of strains added to soil is one of the most challenging problems. The number of external microbes has been seen to decline quickly after soil inoculation. Numerous studies have demonstrated that biotic and abiotic elements interact to determine how effective bioaugmentation is. For example, *Burkholderia* sp. FDS-1 was used to study how temperature and pH affected the degradation of the nitrophenolic pesticide fenitrothion. It was discovered that the best conditions for bacterial activity were 30°C and a slightly alkaline pH, whereas 10°C and 50°C and highly acidic conditions were inappropriate for pesticide detoxification.[18] To avoid such misconduct bioaugmentation is generally uses genetically engineered or modified bacteria. The process has been tested in labs several times and on various microbial strains.

Bioaugmentation, as a concept within environmental biotechnology, is currently in the phase of evolving from theoretical applications to practical implementation in real-life scenarios. While the widespread deployment of bioaugmentation techniques may not have

Table 2. Degradation of different components of petroleum hydrocarbon with various microbial preparations and comparison of their effects

	Type of HC contamination	Method of Bioremediation	Efficiency	Reference
Rhodococcus sps.[1]	TPH[2]	Bioaugmentation	Effective in wide range of temperature, salinity, pH, and other variables.	38
Bacillus methylotrophicus	TPH	Biostimulation	80.24% aged TPH removed.	16
Indigenous consortium+ Sophorolipids	TPH	Biostimlation	100% degradation is achievable in 6 days	26
Athrobacter globiformis+ rhamnolipids+ mixed surfactants	PAH[3]+ DDT[4]	Biostimulation	60.7% PAH and 29.3% DDT degradation higher than control	39
Pseudomonas brassicacearum MPDS	Napthlene+ PAH	Bioaugmentation	31.1% napthlene removed and inceased PAH degradation.	40
Seretia marcescenes+ *Bacillus megaterium*+ *Aeromonas hydrophilia*	PAH (aceneph-thalene, Fluorine)	Bioaugmentation	98.92% fluorine degradation and 98.16% acenephthalene degradation was obtained.	29
Panicum virgatum+ Bacterial strains	TPH	Phytoremediation	Increased degradation ofTPH. Most efficient in saturated anaerobic environment.	41
Acetinomycetales+ *Melilotus officinalis*	TPH+PAH	Phyto+ microbial bioremediation	Increased TPH degradation by 32% and the degradation of other HCs were found.	42
Rhizosphere	TPH+ Aliphatic HCs[5]	Bioaugmentation	Improved TPH degradation by 32% and the degradation of other HCs were found.	10
Rhodococcus sp. *Sejongia* sps.	PAH	Bioaugmentation+ Biostimulation	Increased PAH degradation	12

[1]Species; [2]Total Petroleum Hydrocarbons; [3]Polycyclic Aromatic Hydrocarbons; [4] dichloro-diphenyl-trichloroethane; [5]HCs- Hydrocarbons

reached full fruition, there is considerable potential for its utilization in various fields. Laboratory tests have demonstrated successful outcomes with various strains of microorganisms, showcasing the feasibility of the approach in controlled settings. Various studies have been done which deals with degradation of petroleum hydrocarbons by addition of required microbial concussions to enhance the degradation. Bioaugmentation procedures are mostly coupled with biostimulation processes to get better outcomes like in the study aimed to assess the efficacy of permanganate pretreatment in tandem with subsequent bioaugmentation for the degradation of petroleum hydrocarbons in unsaturated soil, marking a significant departure from conventional approaches. Diesel-contaminated unsaturated soil underwent pretreatment with 0.5-pore-volume (5%) potassium permanganate using both solution pouring and foam spraying techniques, the latter incorporating a surfactant. Total petroleum hydrocarbon (TPH) removal efficiencies were measured at 37% and 72.1% for solution pouring and foam spraying, respectively. The subsequent application of permanganate-bioaugmentation foam resulted in a final TPH concentration of 438 mg/kg, indicating an impressive 92.1% total reduction. Notably, the experiment avoided soil mixing or disturbance. This led to the findings like the PP foam, in conjunction with bioaugmentation foam, showcased a remarkable TPH removal efficiency, attributing its success to increased soil pH and effective remediation agent infiltration facilitated by foaming. The application of PP foam elevated the soil pH in acidic conditions, fostering enhanced microbial activity and contributing to a first-order biodegradation rate of 0.068 d-1 post-PP oxidation. Chromatogram peak analysis verified the removal of 94% of relatively persistent hydrocarbons (C18-C22) through PP-bioaugmentation. Physicochemical parameter evaluations demonstrated that PP, when combined with bioaugmentation foam, significantly improved TPH degradation and biodegradation rates in unsaturated diesel-contaminated soil.[19]

However, the translation of these successes to large-scale, real-life projects remains a challenge that demands further attention. The prospect of enhancing specific biological processes by introducing carefully selected microorganisms into targeted environments, such as wastewater treatment plants, holds promise. Although challenges and research gaps exist, the successful completion of laboratory tests with various strains provides a foundation for optimism. Bioaugmentation is still in its early stages of development, and more research is needed to address scaling up issues and optimize methods for real-world use. It seems possible to include bioaugmentation into large-scale projects with sustained effort, which could lead to a paradigm change in biological and environmental engineering. The benefits of bioaugmentation in addressing environmental issues and increasing process efficiency are becoming more and more possible as scientists and engineers tackle current obstacles. The reviewed research has shown that TPH pollution (up to 380,000 mg kg^{-1}) in clay, silt, and sandy soils can be effectively treated with composting technique. With a maximum of 99%, the majority of these investigations recorded removal efficiencies of greater than 70%. TPHs are used as carbon and energy sources by the microorganisms throughout the composting process.[20]

Table 2 includes a list of microbes and microbial consortium, which work together to give varied efficiency in degradation of the hydrocarbon pollutants processes like biostimulation when applied in conjunction to phytoremediation have also been shown to have positive effects in degradation of the contaminant.

Biostimulation

The approaches most commonly employed for in situ bioremediation of unintentional spills and persistently contaminated sites worldwide involve the addition of pre-grown microbial cultures in order to speed the breakdown of undesirable compounds (bioaugmentation) and/or the introduction of nutrients and other additional materials to the native microbial population to encourage faster propagation (biostimulation). In bioremediation, biostimulation is a technique used to support the natural processes in contaminated environments—like soil or groundwater—that aid in the disintegration or transformation of pollutants. Unlike bioaugmentation, which involves the introduction of specific microorganisms, biostimulation focuses on optimizing the conditions for the native microorganisms already residing in the contaminated site. The primary objective of biostimulation is to stimulate the growth and metabolic activity of these indigenous microorganisms, enabling them to more effectively break down or convert contaminants into less harmful substances.[21] In accordance with several reports, specific types of microorganisms have the metabolic pathways needed to break down contaminated substances. Some of the most investigated microbial species for their capability for bioremediation include *Pseudomonas* species, *Mycobacterium* species, *Haemophilus* species, *Rhodococcus* species, *Paenibacillus* species, and *Ralstonia* species.[22] These strains can break down petroleum hydrocarbons, aromatic hydrocarbons like benzene, toluene, ethylbenzene, and xylene, as well as polyaromatic hydrocarbons like naphthalene, phenanthrene, anthracene, pyrene, and the extremely carcinogenic benzo [alpha] pyrene (*Pseudomonas*, *Haemophilus*, and *Mycobacterium*). Biostimulation mostly depends on supplying the optimal conditions to this native bacterium for faster and better decontamination of the polluted sites. This factor can be the presence of water, ph of the soil, utilizable nitrogen, phosphorus source etc. The rate of decontamination is accelerated corresponding to biostimulation as the system's ability to degrade contaminants is improved by the addition of one or more rate-limiting nutrients.

Various techniques have been employed to make the environment optimal for the microbial species that would be responsible for the biodegradation of the contaminants. nutrients such as carbon, phosphorus, nitrogen which can stimulate the microbial growth. Co-substrate addition is another such technique. For some microbes, certain pollutants may act as co-substrates. These co-substrates can promote the development of microorganisms that can biodegrade the intended contaminants. It should be kept in mind that the added contaminant is not harmful and can be controlled when the work is done.[23]

Biostimulation is employed across a diverse range of environmental scenarios where the objective is to heighten the natural processes carried out by microorganisms for the degradation or transformation of contaminants in polluted environments. The effect of biostimulation was effectively studied by Omar *et al*. The study showed that the contamination of soil by residual automotive oil is a pressing global environmental issue, and biostimulation stands out as a highly suitable technology for effectively addressing this problem. The biostimulation study made the use of enzymes, surfactants, and vermicompost. The study employed a factorial design with various factors, including time, pH, temperature, and biostimulation strategies involving enzymes and additives. Specifically, enzymes derived from *Ricinus communis* L. seeds, along with commercial vermicompost and Triton X-100 surfactant, were utilized. The results revealed that the most remarkable removal efficiency, reaching an impressive 99.9%, was achieved after a 49-day period, under conditions of pH 4.5, a temperature of 37°C, and biostimulation involving enzymes and vermicompost (3% w/v-5% w/w). Surprisingly, the addition of the surfactant did not significantly contribute to the enhancement of removal efficiency.[24] Particularly useful bioremediation method in this study was proven to be vermicompost. There have been studies that make both use of biostimulation and bioaugmentation to control the contamination in a particular zone. A study performed by Yang *et al.* Showed that the simultaneous introduction of a TCC-degrading *Ochrobactrum* sp. TCC-2 and electrode into the TCC and PAHs co-contaminated sediments resulted in the proposal of a novel electro-biostimulation and bioaugmentation combination remediation system. According to the results, the combined system's PAHs and TCC degradation efficiencies were 2.9-3.0 and 4.6 times, respectively, greater than those of the control group (which received neither electro-biostimulation nor bioaugmentation treatments). The enhanced electroactive bacteria and PAHs degraders (such as *Desulfobulbus, Clostridium*, and *Paenarthrobacter*) and the introduced strain TCC-2 worked together to accelerate the breakdown of TCC and PAHs. By raising the functional gene abundances associated with different metabolic activities, the preferential elimination of the TCC inhibitory impact with bioaugmentation treatment could restore microbial functioning.[25] Coupling various bioremediation process have been proven of value, as shown in Table 2.

Coupling of phytoremediation with bio-augmentation

Phytoremediation is a sustainable and eco-friendly approach used to clean up environmental pollutants from soil, water, or air by harnessing the natural abilities of certain plant species. Phytoremediation started to become a research area in the early 1980s by emerging studies on metal uptake by hyper-accumulating plants and the reports of toxicity caused by the pesticides in crops came out. The exposure to heavy metals may occur through polluted soil, phytotoxicity-induced reduction in the quality of food crops, decreased land production due to agricultural practices leading to food insecurity, and a variety of other land issues. Given that plants cover the majority of the planet's surface, it makes sense that they have a significant impact on the fate and movement of chemicals and xenobiotic substances. Numerous neutral hydrophobic compounds in soil underwent phytoremediation, primarily by bacteria in the rhizosphere rather than within the plant. The root zone provided a favourable environment for microbes, who seemed to benefit from exudates, dissolved oxygen, and secondary metabolites released by plants.[19-21] Exudates functioned as supplementary substrates for the catabolism of aromatic compounds, such as long-chain alkanes in petroleum hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), and benzene/ toluene/ ethylbenzene/ xylenes (BTEX).[26]

Given that exudates were a highly accessible and degradable carbon source, they occasionally inhibited metabolic degraders, causing diauxic or catabolic suppression in the breakdown of target molecules. Rhizosphere bacteria have a major role in TPH phytoremediation, however plant uptake and BTEX chemical transformation may also play a role. Among the most prevalent families are Actinobacteria, Proteobacteria, and Bacteriodetes. It has been effectively proved that petroleum hydrocarbons can be permanently removed from soil at former tank farm sites by phytoremediation.[27] While plant absorption and BTEX chemical transformation may contribute to TPH phytoremediation, rhizosphere bacteria are primarily responsible for the process. Actinobacteria, Proteobacteria, and Bacteriodetes are among the dominant families. It has been effectively proved that petroleum hydrocarbons can be permanently removed from soil at former tank farm sites by phytoremediation.

The simultaneous combination of phytoremediation and bioaugmentation in environmental remediation results in a synergistic fusion, which utilizes the innate abilities of plants and microbial communities to achieve a more effective purification of polluted ecosystems. Phytoremediation, based on the phyto-extraction potential of plants, can bind metals and certain organic poisons. This botanical skill is improved when it is integrated with biological augmentation, a biotechnological process where some microbes are introduced knowingly into an environment so that they can metabolize stubborn pollutants. The deliberate interaction between plant roots and introduced microorganisms in the rhizosphere (i.e., the area around roots) raises contaminant translocation rates as well as microbial activities. This merger increases the range of rehabilitation since phytoremediation focuses on metal absorption while bioaugmentation targets organic pollutants; thus, multiple types of contaminants can be addressed. Furthermore, nutrient availability is enhanced through such introduced microbial communities leading to increased plant healthiness and strength. This combined approach's flexibility is demonstrated by its ability to customize the composition of the microbial community to site-specific soil conditions, ensuring a customized and effective remediation plan. The combination of these techniques results in faster breakdown of contaminants since bacteria and plants have a strong symbiotic relationship that works to speed up the cleanup process.

Moreover, the long-term effectiveness of this combination is demonstrated by the creation of a long-lasting, sustainable solution, whereby bioaugmentation maintains microbial activity in the rhizosphere and phytoremediation offers persistent plant-driven contaminant uptake. When combined, phytoremediation and bioaugmentation show promise as a flexible and long-term way to reduce environmental contamination in a variety of ecological environments, as well as a scientifically nuanced and complex approach. Subsequent investigations into the amalgamation of phytoremediation and bioaugmentation are anticipated to concentrate on enhancing and perfecting the mutually beneficial relationships between microbial communities and plants. A deeper comprehension of the precise mechanisms regulating plants' cohabitation with introduced microbes in the rhizosphere is required,[28] with a focus on improving the efficiency of pollutant breakdown. Furthermore, scientists could investigate the creation of innovative plant-microbe alliances designed to tackle new pollutants and various soil circumstances. Plants and microorganisms with improved remediation capacities may be engineered through the use of cutting-edge molecular and genetic approaches.[29] Furthermore, studies may delve into the long-term ecological impacts and sustainability of integrated approaches, considering factors such as the potential for unintended consequences and the establishment of resilient ecosystems post-remediation. Overall, future work in this field is poised to advance the scientific underpinnings and practical applications of integrated phytoremediation and bioaugmentation strategies for sustainable and effective environmental cleanup.[30]

Physical and biological factors affecting biodegradation

The bioremediation of petroleum hydrocarbons is influenced by a combination of physical and biological factors that collectively dictate the success and efficiency of the remediation process.[31] The flowchart (Figure 3) adapted from Teklit et al. Shows a list of various physical and biological factors that determines the pace of biodegradation under unaltered natural conditions.

One of the diverse affecting factors of petroleum hydrocarbons involve the oxygen management for better breathing of the microbial culture in the contaminated environment. Techniques like aeration can help us to make oxygen available to the microbes. Aeration systems can be used in wastewater treatment plants or bioremediation systems to maintain aerobic conditions. While in other cases creating anaerobic conditions by limiting oxygen availability can promote the growth of anaerobic microorganisms that are effective in biodegrading certain pollutants. These conditions are regulated depending on the microbes that are to be enhanced for better biodegradation.

In addition to the oxygen management, pH control is another important factor to be considered. pH control is a critical aspect of biostimulation and various other processes in microbiology, chemistry, and environmental science. It involves the regulation and maintenance of the acidity or alkalinity of a solution or environment to create optimal conditions for desired biological or chemical reactions. pH significantly impacts the activity of microorganisms. Different microorganisms thrive in specific pH ranges, so controlling pH is crucial for creating an environment where desired microorganisms can flourish. Many chemical reactions are pH-dependent. Altering pH can affect reaction rates, solubility, and the availability of ions and molecules. In bioremediation, for example, adjusting pH can influence the rate of contaminant degradation. This adjustment helps in biostimulation but are dependent on the species of interest. Several methods have been employed to manage the pH of the contaminated environments

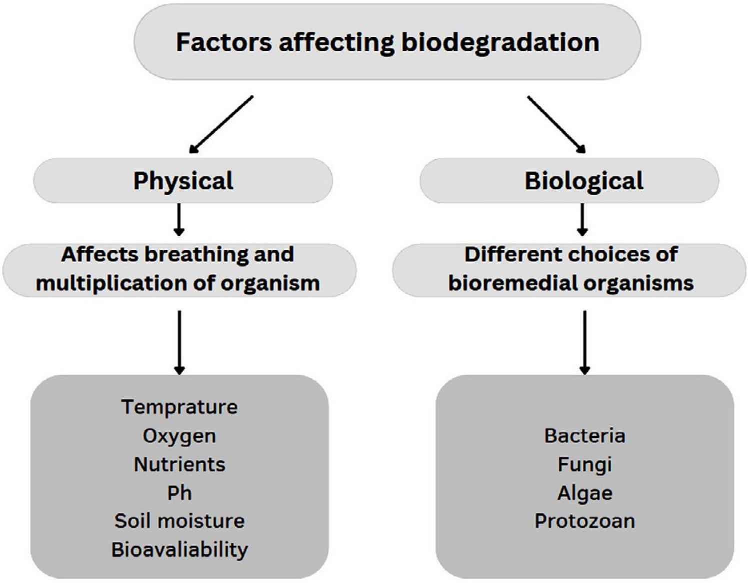

Figure 3. Physical factors and bioremedial choices that affects the biodegradation of petroleum hydrocarbons adapted from Teklit et al.[43]

including acid/base addition. The most common method for pH control involves adding acids or bases. Common acids include sulfuric acid and hydrochloric acid, while sodium hydroxide and calcium carbonate are typical bases used for pH adjustment. Another method to manipulate the pH is the use of the buffer systems. Buffer solutions consist of weak acids and their corresponding conjugate bases, which resist changes in pH when small amounts of acid or base are added. Some indirect methods of pH regulation include CO_2 control as the concentration of carbon dioxide (CO_2) in the environment can influence pH.

Temperature control is also a critical aspect of many scientific, industrial, and biological processes. It involves regulating and maintaining the temperature of a system or environment within a specific range to achieve desired outcomes. Temperature profoundly influences biological processes, including enzyme activity, microbial growth, and metabolic rates. Many organisms have specific temperature optima at which they function most efficiently. Temperature affects the rate of chemical reactions. In most cases, higher temperatures increase reaction rates. Temperature control systems typically involve heating and cooling elements. Heating is achieved through electrical or gas heaters, while cooling can be accomplished using refrigeration systems, cooling coils, or heat exchangers. Temperature control devices, such as thermostats and temperature controllers, monitor the current temperature and activate heating or cooling mechanisms to maintain the desired set-point. Many systems also make use of feedback control loops to adjust heating or cooling in response to temperature fluctuations. Proportional-Integral-Derivative (PID) controllers are commonly used for this purpose. Managing all the factors to be in Favor of the degradation of the contaminant helps in fastening the process of biodegradation and getting the natural balance of the contaminated zones back.

Challenges and limitations

In recent years, Microbial remediation has emerged as the preferred method for the degradation of petroleum hydrocarbons, which is experiencing rapid development driven by advancements in molecular biology.[32] Although the process is advancing at a steady pace, still many challenges are faced in deciding the most optimal microbial mechanisms to get the best possible degradation of the contaminant. This can be observed due to factors like complexity and bioavailability of the contaminant. Pollutants such as petroleum hydrocarbons are frequently complex combinations with different chemical structures. Degrading particular components requires different microbial species, therefore a single solution is rarely possible. Certain pollutants are extremely poisonous, which can either kill the microbes meant to break down the contaminants or impede microbial activity. On the other hand, Microbes may have reduced access to contaminants if they are entrapped in soil particles or exist in non-aqueous phases.[33] This restricts bioremediation's efficacy. Particularly in places with low water content or dense soils, microorganisms' capacity to travel across the contaminated environment and reach the contaminants is frequently restricted.

Multiple environmental variables may have a substantial impact on microbial activity, including pH, temperature, oxygen availability, and moisture content. Creating bioremediation techniques that work in all environments is difficult due to these conditions' significant variations. The natural microbial populations can compete with the introduced bacteria, either outcompeting them or preventing their growth. To avoid this, choice of a microbial group which can work synergistically with the indigenous population is mandatory.[34] This requires metagenomic studies of the indigenous species as well as comparing the data with the microbial population of interest. In multiple cases scaling of this laboratory findings to an industry level are far too expensive given laboratory settings always do not translate into success in ground applications as achieving consistent and predictable results in diverse environments can be challenging.

In several cases it is unclear how different microbial species interact with one another and with their surroundings. This restricts the potential for bioremediation process optimization. Numerous bacteria have genetic and metabolic capacities that are still being uncovered; bioremediation may benefit from unrealized potential.[35]

Future prospect

In the evolving landscape of microbial bioremediation for petroleum hydrocarbon-contaminated soil, future research endeavours could delve into the development and optimization of innovative strategies that capitalize on the synergy between various remediation techniques. Exploring the potential of synthetic biology and genetic engineering to design microbial consortia with enhanced hydrocarbon-degrading capabilities could revolutionize bioremediation approaches. Engineered microbes could be tailored to exhibit increased metabolic efficiency, broader substrate specificity, and resilience to environmental stressors, thereby elevating the overall efficacy of remediation efforts. Additionally, there is a growing need for a more nuanced understanding of the complex interactions within microbial communities during bioremediation processes. The dynamics of microbial consortia in response to different hydrocarbon compositions and environmental conditions may be revealed by metagenomic and metatranscriptomic analyses, which could lead to the development of strategies to manipulate microbial diversity for the best remediation outcomes as well as insights into community-level adaptations. Furthermore, future studies may examine the effects of shifting environmental conditions on the effectiveness of microbial bioremediation and look for microbial strains that can flourish in different climatic scenarios as climate change continues to affect global ecosystems. The integration of genetic engineering, sophisticated molecular analyses, and climate resilience studies in this all-encompassing approach has the potential to propel the field of microbial bioremediation forward and pave the way for future generations of environmentally friendly and cost-efficient solutions to soil contamination caused by petroleum hydrocarbons.

CONCLUSION

The removal of petroleum hydrocarbons using microbial species is getting lot of attention in recent years. Methods based on these microbes have several advantages over the traditional physical and chemical methods. This can be accredited to the capabilities of microbes to degrade the hydrocarbons into least toxic components, well within a specific time limit with less labour-intensive work. The review provided a comprehensive examination of the multifaceted aspects of PHC contamination, including the structural complexity of hydrocarbons, their diverse contamination scenarios, and the evolution of remediation techniques. We also explored the varied effects of Petroleum hydrocarbon contamination human health as well as the environmental systems, thus underscoring the need for more effective intervention strategies. We observed that the selection of different bioremediation techniques and microbial communities affects the efficiency of the degradation process in distinct ways. The selection of the process and the microbes in highly dependent on the structural complexity of the contaminants and the environmental factors like pH, Temperature, moisture and nutrient content of the soil. The key enzyme groups that are found to be participating in degradation of the contaminants involves hydroxylases and oxygenases whose optimal performance is based on the above-mentioned environmental factors.

The synergistic interactions between various bacterial species and their association with plant systems has demonstrated significant potential for advancing bioremediation of petroleum hydrocarbons. It enhances the degradation of complex contaminants by utilizing diverse metabolic capabilities of microbial consortia and the beneficial effects of plant-associated mechanisms, such as enhanced bioavailability and transformation of contaminants. This innovative integration of phytoremediation and bioaugmentation represents a significant breakthrough, using the mutually beneficial relationship between plants and microbial consortium to improve the cleanup of locations contaminated with petroleum hydrocarbons. The review also highlighted the influence of physical and biological factors on biodegradation, emphasizing the importance of tailored strategies to optimize remediation outcomes. The comparison of different microbial preparations underscores the potential for more targeted and efficient bioremediation processes.

However, one of the biggest hindrances with these bioremediation strategies is our limited knowledge on how indigenous microbial species degrade contaminants in their varied environmental niches as abilities to perform whole-genome build functional predictions lag far behind. This lack of knowledge hinders the efficient optimization of bioremediation strategies. In conclusion, as powerful and scalable of a solution bioremediation is in theory — overcoming the challenges will be critical to unlock its potential. This suggests that further research and innovation are required to exploit the potential of microbial communities for developing resilient Methodologies towards sustainable environmental restoration.

To overcome these challenges further research should be done to better understand the mechanisms underlying microbial interactions in intricate soil conditions. Furthermore, it is essential to take a considerate approach that takes into account the socioeconomic factors and public acceptance of microbial bioremediation techniques. In the effort to lessen the negative effects of petroleum contamination on the environment, sustainable and environmentally friendly restoration techniques ought to take centre stage. As the area develops, the incorporation of state-of-the-art technologies, such as omics-based analyses, clarifies the dynamics of microbial communities and provide guidance for customized and successful remediation procedures. However, we can assume the evolving landscape of microbial bioremediation holds immense potential for mitigating the complex challenges posed by petroleum hydrocarbon contamination, requiring ongoing interdisciplinary collaboration and a commitment to sustainable environmental stewardship.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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