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Robust Bioremediation of Petroleum Hydrocarbon contaminated soil by microorganisms: A Review

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ABSTRACT:

Contamination of soil by oil and hydrocarbons is a significant environmental issue with widespread consequences. Traditional methods for remediation often lack efficiency and sustainability. Bioremediation, which harnesses the metabolic capabilities of microorganisms, particularly bacteria, has emerged as a promising and environmentally friendly approach for restoring soil. This article examines recent advancements in the use of bacterial-mediated bioremediation techniques for soils contaminated with oil and hydrocarbons. It explores various mechanisms employed by bacteria to degrade and transform hydrocarbons, such as the production of extracellular enzymes and metabolic pathways involved in hydrocarbon catabolism. The article also addresses challenges and limitations associated with bacterial bioremediation, including the impact of co-contaminants and the need for long-term monitoring. The abstract concludes by underscoring the sustainable and cost-effective nature of bacterial bioremediation as a viable strategy for mitigating the environmental impact of oil and hydrocarbon-contaminated soils. As the field evolves, further research and technological innovations are crucial to optimize bacterial bioremediation processes and ensure their widespread applicability in environmental restoration efforts.

Introduction

Hydrocarbons have dual origins, arising from both the biosynthetic activity of microorganisms and plants through the enzymatic reduction of fatty acid molecules, and from gradual geochemical processes acting on biological compounds at high temperatures and pressures over extended geological periods. However, the escalating demand for oil products driven by industrialization has significantly elevated the baseline exposure of the environment to various hydrocarbons[13]. Activities across the oil industry, encompassing exploration, extraction, transportation, refining, and management of oily waste, serve as potential sources of environmental pollution. The discharge of hydrocarbons into soil and air occurs through the effluents of petroleum wells, oil refinery operations, industrial wastewaters, fuel consumption, wood-processing activities, as well as the use of detergents, pesticides, paints, and other chemicals [11].

Crude oils comprise a blend of diverse aliphatic hydrocarbons with both low and high molecular weights, along with various monocyclic and polycyclic aromatic compounds. These compounds may have carbon atoms in their backbone substituted by nitrogen, sulfur, or oxygen. Notably, a soap molecule is considered a hydrocarbon, featuring a lengthy hydrocarbon chain containing a carboxylic acid group at the end, and it is the potassium or sodium salt of such a fatty acid [9.] The length and characteristics of hydrocarbons vary in oil-derived compounds, ranging from C1–C10 in gasoline, C9–C16 in jet fuel and kerosene, to C10–C50 in waste oil. The persistence of hydrocarbons, owing to their low water solubility, makes them highly resistant to degradation, resulting in their prolonged presence in the environment.

Petroleum oil stands as a crucial strategic asset, sparking intense competition among nations. The flourishing petrochemical industry, driven by anthropogenic reliance on oil to meet energy needs, also brings about environmental degradation[21]. Throughout the various stages of petroleum production, including storage, transportation, refining, and processing, incidents like blowouts during oilfield development, leaks from pipelines and storage tanks, accidents involving oil tankers and tankers, waxing in oil wells, and refinery and petrochemical equipment overhauls often lead to spills and discharges of petroleum hydrocarbons [17]. Efforts to address large spills aim at recycling or

eliminating the spilled materials to the greatest extent possible. However, in certain cases, recovering the spilled substances proves challenging, leaving them in the affected areas and posing persistent environmental risks. This perpetual threat of contamination accompanies oil exploitation, particularly in environments with limited capabilities to manage oil-contaminated surroundings. This challenge is particularly pronounced in extreme or unique settings such as polar regions, deep-sea areas, deserts, and wetlands [21].

Emerging Biodegradation Technologies

Petroleum hydrocarbons exert harmful effects on both plants and animals, posing environmental contamination challenges that prompt collaborative efforts from academic and industrial experts to devise mutually agreed-upon remediation strategies, mitigating the impact on petroleum-contaminated media. Notably, approximately 60–90% of the chemical composition of petroleum is biodegradable, driving research toward innovative degradation technologies, particularly focusing on the bioremediation of petroleum hydrocarbons in both soil and water [2]. Under aerobic conditions, most organic pollutants undergo rapid and complete degradation. The initial intracellular attack on organic pollutants involves oxidation and activation, with oxygen playing a crucial role in enzymatic stimulation through peroxides and oxygenates. Peripheral degradation pathways transform organic pollutants into intermediate stages of the central intermediate metabolism, such as the tricarboxylic acid cycle [14]. Cell biomass metabolism is generated by metabolites derived from major precursors like acetyl-CoA, pyruvate, and succinate. Synthesis of saccharides necessary for various biosynthetic processes and growth occurs through gluconeogenesis. Specific enzyme systems may facilitate pH reduction, and additional processes, including microbial cell adhesion to substrates and the production of biosurfactants, are also involved. pH values can be preferentially metabolized by either a single strain of microorganism or a consortium of microbial strains from the same or different genera. It has been demonstrated that a consortium offers more diverse approaches than individual cultures in metabolizing or degrading petroleum hydrocarbons (PHs) [1]

Bioremediation

The process of breaking down hydrocarbon compounds into smaller organic and inorganic components through the influence of biological agents such as microorganisms, plants, or plant residues, with the ultimate goal of restoring soil or water to its original state, is commonly referred to as Bioremediation. This technique leverages the inherent capability of native microorganisms to address pollutants, simultaneously creating conditions that promote an increased rate of biodegradation [16]. Often, biological processes serve as a viable alternative to chemical or physical methods for cleaning up oil spills, as bioremediation typically requires less equipment and labor than other approaches. Bioremediation techniques are categorized based on their location, with in-situ (on-site where the pollution occurred) and ex-situ (outside the pollution site) bioremediation being the two main classifications. In-situ bioremediation allows microorganisms to function efficiently, benefiting from the local environment without requiring an adaptation phase [12]. In contrast, ex-situ bioremediation methods primarily involve the physical removal of pollutants without direct microbial participation in the remediation process. Notably, in-situ bioremediation is preferred due to its cost-effectiveness compared to transporting contaminated soil off-site, and it is more effective for remediating large areas. This section provides an overview of recent bioremediation technologies and the operational factors that contribute to the success of each technology [15].

Degradation of hydrocarbons

More than 17,000 organic compounds have been identified in crude oil and categorized into four primary classes: saturates, aromatics, asphaltenes, and resins.The susceptibility of hydrocarbons to microbial degradation can generally be ranked as follows: linear alkanes > branched alkanes > small aromatics > cyclic alkanes [8]. Bioremediation efforts following the Exxon Valdez oil spill have revealed that lighter alkanes are depleted first, while certain compounds, such as high-molecular-weight polycyclic aromatic hydrocarbons (PAHs), may exhibit limited or no degradation [3].

Numerous environmental factors influence the breakdown of hydrocarbons by microorganisms. In marine environments, low levels of phosphorous and nitrogen may restrict the growth of oil-degrading microorganisms, consequently slowing down oil consumption. Efforts have been made to optimize the C:N:P balance to 100:10:1 (referred to as biostimulation) by 'fertilizing' oil-spill areas. In open-sea environments, the rapid dilution of soluble nutrients has led to the consideration of insoluble or hydrophobic (oil-soluble) fertilizers to enhance biostimulation effectiveness. Biosurfactants, seen as another promising form of biostimulation, increase the oil-surface area, thereby making more oil available for bacterial attack [10]. When oil reaches beaches and becomes sequestered in sediments, its bioavailability can be significantly reduced, impeding or even preventing biodegradation. Depending on local circumstances, beach tilling may be employed to expose sequestered oil. However, tilling

itself can be disruptive to coastal plants and animals, potentially causing more harm than benefit. Local conditions, including temperature, wave action (mixing), oxygen availability, and other factors, play a crucial role in determining the efficiency of oil breakdown[4].

Anaerobic Hydrocarbon Biodegradation

The microbial degradation of various substrates, particularly resilient hydrocarbons, faces significant limitations under anaerobic conditions. This constraint arises from the necessity for molecular oxygen as the final electron acceptor, slowing down these biological processes. Despite this, numerous studies on microorganisms and the G0 values of anaerobic degradation reactions indicate the energetic feasibility of anaerobic metabolism for hydrocarbons by microorganisms[6.] Anaerobic bacteria exhibit a gradual degradation of these molecules, generating essential energy and carbon sources for the growth of other microorganisms engaged in catabolism. Additionally, methane produced during anaerobic degradation serves as a resource for various aerobic organisms, including some animals that rely on methane-oxidizing microorganisms. A diverse range of facultative and obligatory anaerobic microorganisms (both bacteria and archaea) have been identified, demonstrating the ability to anaerobically degrade hydrocarbon molecules.

These microorganisms employ various mechanisms, including anaerobic respiration (using nitrate, nitrite, nitrous oxide, sulfate, thiosulfate, carbonate, and metal ions), fermentation, or anoxygenic phototrophic reactions, to donate electrons and hydrogen for substrate catabolism activities. In the case of anaerobic microorganisms, both sulfate- and nitrate-reducing bacteria find shorter-chain alkanes more resistant compared to mid- to long-chain length alkanes . The lack of evaporation for short-length hydrocarbons (up to n-C17) in anaerobic conditions results in their accumulation, exerting a toxic effect on microorganisms' cell membranes and hindering their degradation. Additionally, for sulfate-reducing bacteria, the degradation of branched alkanes, such as pristane and phytane, proves to be more efficient than that of normal alkanes [19].

Denitrifying Bacteria

Bacteria, which possess the capability to degrade hydrocarbons, are members of diverse microbial phyla, primarily within the *Betaproteobacteria* (order Rhodocyclales and genera *Azoarcus*, *Georgfuchsia*, *Thauera* and *Aromatoleum*), *Castellaniella defragrans* (a species belonging to the family *Alcaligenaceae* and the order Burkholderiales) and *Magnetospirillum* (belonging to the Alphaproteobacteria), and degrade different aliphatic and aromatic hydrocarbons at the expense of nitrate reduction [18].

Sulphate-Reducing Microorganisms

Numerous bacteria, including Deltaproteobacteria such as *Desulfobacula toluolica*, *Desulfococcus oleovorans, Desulfatibacillum alkenivorans, Desulfosarcina cetonicum, Desulfoglaeba alkenexedens,* and *Desulfatibacillum aliphaticivorans*, as well as *Desulfotomaculum* and the archaeon *Archaeoglobus fulgidus*, demonstrate the ability to degrade various hydrocarbons [6,] including long/short-chain saturated/unsaturated aliphatic hydrocarbons, non-substituted aromatics (e.g., naphthalene and benzene), and alkyl-benzenes (such as xylenes, toluene, and ethylbenzene). Strains like *Desulfatiferula* BE2801 and LM2801T and *D. aliphaticivorans* CV2803T exhibit this capability [18]. Sulphate-reducing bacteria oxidize the double bond of 1-alkenes, transforming them into corresponding 1-alkanols and further converting them to fatty acids and eventually to CO2.

Metal-Oxidizing Microorganisms

In addition to nitrogen and sulfur-containing inorganic compounds, certain bacteria can use other inorganic compounds as electron acceptors while metabolizing hydrocarbons. For example, Geobacter and Georgfuchsia reduce metal ions (Fe (III), Mn(IV), or U(VI)) through anaerobic degradation of hydrocarbons. Apart from these genera, a toluene-degrading bacterium (strain G5G6) belonging to Betaproteobacteria can completely mineralize toluene to CO2 while utilizing Fe3+, Mn4+, and NO3− as terminal electron acceptors. Additionally, Dechloromonas aromatica RCB and strain Y5 oxidize toluene in reactions coupled with (per)chlorate and arsenate, respectively (Callaghan *et al.,* 2012). Given that oil-contaminated sites may contain arsenic along with aromatic compounds, the ability of microorganisms to degrade aromatics in association with As(V) reduction proves beneficial for environmental remediation.

Conclusion

Petroleum hydrocarbons pose a significant threat to human and environmental health due to their high toxicity. Bioremediation employing bacteria capable of degrading petroleum hydrocarbons is widely acknowledged as an environmentally friendly and effective approach. While numerous bacterial species with this ability have been harnessed for bioremediation, practical applications have revealed challenges that hinder optimal biodegradation. Based on the current state of knowledge, several crucial investigations are required before successfully applying bioremediation to restore petroleum oil-contaminated

environments. The following key recommendations emerge: (1) Advance the theoretical understanding of the interfacial interaction mechanism between bacteria and petroleum hydrocarbons to overcome barriers hindering microbial uptake, (2) innovate the development of biocompatible surfactants to enhance bacterial interaction with petroleum hydrocarbons, (3) employ new biotechnological methods, such as high-throughput screening, to discover untapped resources of petroleum hydrocarbon-degrading bacteria, (4) optimize artificial microbial consortia strategies, including metagenome enrichment approaches, to cultivate and enhance effective consortia, (5) investigate novel functional genes that control hydrocarbon degradation pathways to gain new insights into molecular mechanisms and microbial remediation, and (6) utilize synthetic biology technology to genetically engineer bacteria, enhancing their capability for petroleum hydrocarbon degradation.

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