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Microbial Trends in Bioremediation of Oil Contaminated Sites: A Comprehensive Review

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Abstract

Hydrocarbons are highly toxic pollutants that are difficult to degrade and can persist in the environment for many years, posing significant threats to the ecological system. Remediating hydrocarbons in contaminated soils has become a challenging task. Currently, bioremediation has gained significant importance compared to other methods, as it is eco-friendly, cost-effective, and can efficiently degrade hydrocarbons in polluted soils. This process relies on microorganisms capable of transforming or mineralizing hydrocarbons. Bacterial degradation processes have proven effective in breaking down various cyclic aromatic and aliphatic hydrocarbons due to the presence of diverse enzymes, which enable bacteria to utilize different hydrocarbons as sole sources of carbon and energy. However, there remains a gap in knowledge regarding microbial capabilities in degrading different hydrocarbons. This review provides comprehensive information on the classification and associated risks of hydrocarbons, focusing on bioremediation methods, mechanisms involved in treating oil-contaminated soils, and factors affecting bioremediation. Additionally, it highlights key findings on bacterial abilities to degrade various types of hydrocarbons, with particular emphasis on aliphatic and aromatic hydrocarbons, due to their abundance in crude oil and its derivatives.

Keywords

Oil contamination, Hydrocarbons, bioremediation, microorganisms.

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1. INTRODUTION

Oil is a primary energy source used extensively in industries, transportation, and other sectors. Although oil plays a significant role, its overuse causes serious issues that can impact our health and the environment due to its mutagenic, toxic, and carcinogenic properties [1]. Both natural and anthropogenic sources contribute to oil pollution. Various anthropogenic sources, including oil extraction, transportation, distribution, and chemical processing [2], leakage from underground storage tanks, and other petrochemical activities, as well as oil release from refinery sites, extraction, and treatment fields, contribute to pollution [3]. Long-term oil pollution causes pollutants like polycyclic aromatic hydrocarbons (PAHs) to accumulate, which can damage soil ecological structure and inhibit plant growth due to their teratogenic, mutagenic, and carcinogenic properties [4]. The soil's physicochemical properties, such as moisture content, porosity, C/N ratio, C/P ratio, and environmental pH, are also altered when contaminated with petroleum hydrocarbons PHS [5].

To address these issues, various methods such as physical, chemical, and biological are employed. The choice of method depends on pollutant characteristics, including physicochemical properties, type, pollution source, and whether the pollution was recent or longstanding [6]. Polluted sites are treated only after analysing pollutant

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type, environmental and human health risks, treatment feasibility, and anticipated effectiveness. Remediation methods can be classified into *ex situ* and *in situ* techniques [7]. *In situ* conventional remediation technologies cannot efficiently volatilize petroleum heavy fractions, and rely on vitrification, which is highly expensive [8], and often require chemicals. Incomplete degradation of petroleum compounds may lead to the formation of even more toxic byproducts. The conventional methods can alter soil structure, subsurface properties, and microbial colonization [9]. Compared to other methods, bioremediation stands out as an eco-friendly and efficient approach for degrading hydrocarbons [10]. Additionally, it can transform highly toxic hydrocarbons into less harmful compounds through enzymatic and metabolic reactions [11]. Microorganisms are widely distributed in the environment due to their ability to thrive in diverse habitats, their intense metabolism, and their versatile nutrition. Certain microorganisms capable of converting, utilizing, or modifying toxic compounds are used in pollutant degradation; this process is termed biodegradation or bioremediation.

Bioremediation is carried out by bacteria, yeast, and fungi. Among these, bacteria play a major role in degrading hydrocarbons [12]. These organisms are also called bioremediators and are used in the cleanup of contaminated sites [13]. The isolation and inoculation of bioremediators enhance the degradation of petroleum hydrocarbons at contaminated sites [14]. Natural attenuation is a type of bioremediation process in which biodegradation efficiency depends on the ability of indigenous microorganisms to degrade pollutants at the contaminated site [15], [9].

Two methods of biodegradation are used: bioaugmentation and biostimulation [16]. Bioaugmentation involves the addition of highly competent oil-degrading bacteria to enhance degradation. Biostimulation, on the other hand, modifies environmental conditions to stimulate bacterial activity. In this process, indigenous microorganisms are activated by adding nutrients, including large amounts of carbon sources (i.e., contaminants), which can result in the rapid depletion of essential inorganic nutrients, such as phosphorus (P) and nitrogen (N) [17]. Many studies report positive effects of biostimulation in oil decontamination, especially in cold ecosystems, mainly using N-P-K oleophilic or N-P-K fertilizers [8]; [18].

Bioremediation of hydrocarbon-contaminated soils is versatile and can be applied both in situ (on-site) and offsite [19]. The importance of oil bioremediation spans various ecosystems, including marine, cold terrestrial ecosystems, alpine, arctic and Antarctic soils, and sea ice [20]. Alaskan groundwater is also considered in such efforts. The nature and extent of hydrocarbon metabolism are significantly influenced by field temperatures, which affect the physical and chemical characteristics of hydrocarbons as well as the rate of biodegradation [21]. For low-risk, oil-contaminated sites, intrinsic bioremediation is widely accepted as a cost-effective alternative. While biodegradation is the primary mechanism for contaminant breakdown, other processes- such as chemical and physical processes like dilution, dispersion, abiotic transformation, volatilization, and absorption—also play important roles [6].

The aim of this review is to provide an overview of hydrocarbon pollution, the diversity of hydrocarbons, the variety of hydrocarbon clastic bacteria, and their degradation potential, as well as an update on the different bioremediation strategies adopted for treating polluted environments. The mechanisms by which hydrocarbon clastic bacteria degrade hydrocarbons will also be discussed.

2. HYDROCARBON POLLUTION IN CONTAMINATED SITES

Soil pollution is defined as the accumulation in soils of persistent toxic compounds, chemicals, salts, radioactive materials, or disease-causing agents, all of which adversely affect plant growth and animal health. Soil pollution can occur through several pathways, including (1) seepage from landfills, (2) discharge of industrial waste into the soil, (3) percolation of contaminated water into the soil, (4) rupture of underground storage tanks, (5) excessive application of pesticides, herbicides, or fertilizers, (6) solid waste seepage, and (7) improper installation, detonation, or dismantling of munitions.

Petroleum and its combustion by-products are the primary hydrocarbon sources contributing to soil pollution. Based on their origin, hydrocarbon sources are categorized as phytogenic, petrogenic, or pyrogenic. Phytogenic hydrocarbons naturally enter the ecosystem through processes like evaporation, dispersion, adsorption, and dissolution of petroleum and petroleum combustion products. Petrogenic sources, such as fuels from leaking tanks and lubricants, can significantly pollute groundwater systems [22]. Pyrogenic hydrocarbons, released during fuel combustion at high temperatures and in low-oxygen conditions, are another major source, with incomplete combustion and fat pyrolysis near power plants adding further hydrocarbon pollutants. Globally, over 50% of crude oil is supplied by the Arabian Gulf region (both onshore and offshore oil reservoirs), making oil spills frequent in this area. Oil production wells located near coastlines pose an additional threat to marine and water systems.

3. DIFFERENT CLASSES OF HYDROCARBONS

Hydrocarbons are organic compounds composed exclusively of hydrogen and carbon atoms. They are the primary components of fossil fuels, natural gas, and petroleum. Crude oil contains tens of thousands of hydrocarbons, ranging from simple C1 (methane) molecules to more complex structures exceeding C30. According to Saturate Aromatic Resin and Asphaltene (SARA) analysis, alkanes (including open-chain, linear, branched, and cycloalkanes) are a major part of aliphatic hydrocarbons. Aromatic hydrocarbons, present in high quantities, contribute monomers to resin formation. The composition of crude oil depends on both hydrocarbons and non-hydrocarbons, as well as their origins. Persistent organic pollutants (POPs), including polycyclic aromatic hydrocarbons (PAHs), are lipophilic and resistant to degradation, leading to their accumulation in aquatic systems.

3.1. Aliphatic Hydrocarbons

Aliphatic hydrocarbons consist solely of hydrogen and carbon atoms and may be linear, branched, cyclic, saturated, or unsaturated. This group includes alkanes, alkenes, and alkynes. Among these, alkanes are the most abundant in crude oil and are typically the first compounds to degrade.

3.2. Aromatic Hydrocarbons

Aromatic hydrocarbons are closed-chain hydrocarbons containing a benzene ring or exhibiting the basic characteristics of aromatic compounds. They can be divided into monocyclic aromatic hydrocarbons, polycyclic aromatic hydrocarbons, and non-benzene aromatic hydrocarbons.

***3.2.1. Monocyclic Aromatic Hydrocarbons*:** These compounds contain only one benzene ring and include substances like toluene, xylene, ethylbenzene, styrene, benzene, and acetylene. Naphthalene, anthracene, and phenanthrene are also significant pollutants often found at oil-contaminated sites. Due to their complex structure, these compounds are highly persistent and challenging to degrade.

***3.2.2. Polycyclic Aromatic Hydrocarbons (PAHs)*:** Polycyclic aromatic hydrocarbons (PAHs) are a significant class of chemicals consisting of 2 to 7 fused aromatic rings. Approximately 10,000 PAH compounds have been identified as pollutants in the atmosphere, water, and soil. Based on the fusion of benzene rings, PAHs can be classified into three categories:

1. Polybenzo-aliphatic hydrocarbons - Examples include diphenyl methane, stilbene, styrene, and triphenylmethane.
2. Biphenyl and polybenzene hydrocarbons - Examples include biphenyl, terphenyl, and tetraphenyl.
3. Polycyclic aromatic hydrocarbons - Examples include phenanthrene, indene, naphthalene, anthracene, fluorene, acenaphthene, pyrene, and coronene.

PAHs are highly resistant to degradation and can persist in the environment for extended periods. They are considered mutagenic, carcinogenic, and toxic, posing significant environmental and health risks [23]. PAHs in soil contribute to increased levels of environmental contamination [24]. Major sources of PAHs include road surfaces, domestic waste oil spills, leaks from aging storage tanks, tanker spillages, incomplete fossil fuel combustion, and seepage from natural oil reservoirs.

3.3. Heterocyclic Compounds

Heterocyclic compounds are organic molecules containing at least one heterocyclic ring, where nitrogen, oxygen, or sulphur atoms replace carbon atoms within the ring structure. Examples include quinolines (containing nitrogen), dibenzothiophenes (containing sulphur), and xanthenes (containing oxygen). These compounds are particularly challenging to degrade.

4. RISK HAZARDS OF HYDROCARBON POLLUTION

4.1. Impact on Human Health

Epidemiological and toxicological research indicates that the constituents of crude oil and oil slicks pose numerous health risks to humans. The release of hydrocarbons into the environment causes various health hazards, including encephalopathy, acidosis, arrhythmias, and dermatitis, commonly reported due to acute hydrocarbon exposure [25]. Another significant consequence of hydrocarbon aspiration is pneumonitis. Hydrocarbons, due to their low viscosity and surface tension, can penetrate deeply into the lungs, resulting in severe necrotizing pneumonia. Ingestion of hydrocarbons can irritate the gastrointestinal tract, leading to symptoms such as abdominal pain, nausea, vomiting, and hematemesis.

Toluene, a common component in hydrocarbons, can cause renal tubular acidosis, glomerulonephritis, hypokalaemia, and hyperchloremia. The central nervous system may also be affected, with effects that may be either short-term or long-term. Symptoms include headache, ataxia, nausea, hallucination, dizziness, disorientation, slurred speech, and depression. Several studies have shown that hydrocarbons impact neurotransmitter pathways and receptors, such as serotonin, glutamate, nicotinic acid receptors, GABA, and dopamine pathways, as well as voltage-gated channels in the brain. Prolonged exposure can lead to brain atrophy and cardiovascular effects.

The U.S. Environmental Protection Agency and the European Union have identified seven polycyclic aromatic hydrocarbons (PAHs) as carcinogenic and sixteen PAHs as major pollutants [26]. According to the American Association of Poison Control Centres' Toxic Exposure Surveillance System, there were 318,939 exposures due to substances like lamp oil, benzene, toluene, halogenated hydrocarbons, and kerosene [27].

4.2. Effect of Hydrocarbon Toxicity on Microorganisms

Microbial metabolism is influenced by organic components, nutrients, temperature, and the soil ecosystem. Hydrocarbons alter the soil's physico-chemical properties, such as moisture content, C/N ratio, porosity, and pH. Dong *et al.* [5] reported changes in bacterial populations following pH contamination in samples from the permafrost region of the Qinghai-Tibet Plateau. Oil pollution affects microbial distribution both vertically and horizontally, leading to a homogeneous microbial population and the emergence of rare populations at the site [28]. Several hydrocarbons, particularly cyclic ones, are toxic to microbes due to their high partition coefficient values (log P) with water and octanol. Lipophilic compounds accumulate in cell membrane bilayers, resulting in hydrocarbon buildup that compromises membrane integrity and disrupts proton and ion permeability [29], which, in turn, disturbs intracellular pH homeostasis. To counteract this, microbial cells employ tolerance mechanisms, such as modifying fatty acid structures by altering the cis-to-trans configuration of fatty acyl side chains. Another adaptive mechanism involves increasing cell wall cross-linking and altering cell wall hydrophobicity.

5. HYDROCARBON BIOREMEDIATION

Bioremediation is the process by which microorganisms, or their enzymes convert pollutants or contaminants back to non-toxic states, returning them to the environment. It is particularly effective for pollutants such as chlorinated pesticides. For degradation, bacterial strains may be added as biosurfactants in oil spills to facilitate decomposition [30]. Biodegradation can be categorized as either 1) aerobic or 2) anaerobic [31]. For complex pollutants, a sequential aerobic–anaerobic process is often employed to enhance remediation effectiveness. Fungal strains are also used for efficient biodegradation [9], as seen in Mediterranean Sea oil-polluted sites [32].

Bioremediation techniques can be classified as in situ or ex situ, depending on the location of waste treatment. In ex situ methods, contaminated soil and water are removed from the environment for treatment. Conversely, in situ methods involve treating pollutants directly at the site [33]. Bioremediation efficacy depends on microbial composition, the specific contaminated site, and environmental conditions. Key strategies to enhance bioremediation efficiency include [13]:

A. Introduction of microorganisms into the polluted environment: This can involve two approaches such as Bioaugmentation and Use of engineered microbes.
B. Engineered bioremediation: This strategy encompasses two main methods including Biostimulation and Surfactant-assisted bioremediation.

5.1. Bioaugmentation

Bioaugmentation is defined as the addition of specific microbial strains (natural, exotic, or engineered) with particular catabolic abilities to polluted sites [34]; [35]. This method is used to enhance biodegradation efficiency. To stimulate the growth of native microorganisms that primarily feed on pollutants at the site, microbes are isolated from the polluted environment, cultured separately, and potentially genetically modified before reintroduction for remediation [36]. This technique can be applied using either allochthonous or autochthonous microbial strains.

In autochthonous bioaugmentation (ABA), indigenous microorganisms were added to contaminated sites, and these isolated microbes are already adapted to the polluted environment [37], [38]. In contrast, allochthonous strains are non-native to the site and may struggle to adapt or integrate effectively with the indigenous microbial community. The biodegradation efficiency of autochthonous strains depends on suitable conditions for their growth and metabolism [39].

According to Fodelianakis *et al.* [40], allochthonous bioaugmentation did not significantly enhance hydrocarbon degradation in contaminated sediments, making autochthonous bioaugmentation generally more effective [41]. Bioaugmentation is commonly conducted using mixed bacterial consortia. This approach has shown success in remediating sites contaminated with different PAHs, such as pyrene, anthracene, naphthalene, and dibenzoanthracene [42]. For example, adding *Absidia cylindrospora* increased polycyclic aromatic hydrocarbon degradation, removing more than 90% of fluorene within 288 hours [43].

In another study, Sarkar *et al.* [44] used a bacterial consortium isolated from drill cuttings, reintroduced via the autochthonous bioaugmentation method, achieving up to 66% alkane degradation within 12 weeks. Similarly, Koolivand *et al.* [45] reported successful biodegradation of over 91% within 12 weeks by reintroducing autochthonous isolates into the population. However, some studies indicate a decrease in biodegradation efficiency over time.

5.2. Use of Engineered Microbes

Natural microbial species are often insufficient for the effective breakdown of certain pollutants, necessitating modifications through DNA manipulation. Genetically engineered microorganisms have demonstrated enhanced remediation efficiency for a broad range of pollutants, leveraging their diverse metabolic pathways to convert complex contaminants into simpler, harmless products [46]; [34]. Genetically modified organisms can degrade contaminants more rapidly than natural species and are competitive with indigenous microbial species, as well as resilient to various abiotic factors like temperature and pH. These GM organisms show significant potential for the biodegradation of a wide range of chemical and physical pollutants [47] and are used in the remediation of soil, groundwater, and activated sludge.

It has been identified four microbial consortia capable of degrading petroleum hydrocarbons up to 92% within two months. The degradation-related genes identified were *alkB*, *ndoB*, *xylA*, *cat23*, and *nidA1* [37]. In another study, mutant strains of *Pseudomonas putida* were utilized for crude oil degradation, achieving a 46.3% improvement compared to the parent strain [48]. Additionally, the *gyrB* gene responsible for crude oil and diesel oil degradation was isolated from *Gordonia* species, which were obtained from oil refinery-polluted soil.

Now a days, isotope probing techniques, such as active ^13C labeling, are used to identify phenanthrene degraders. This method has facilitated the identification of various fungal and bacterial species capable of degrading polycyclic aromatic hydrocarbons (PAHs) [49].

5.3. Biostimulation

Biostimulation involves the addition of specific nutrients to contaminated sites to enhance bioremediation by indigenous microbes. This method typically includes adding limited nutrients to boost the population of native microbial communities. The marine environment, for example, is often low in oxygen and essential nutrients, such as phosphorus and nitrogen, which are critical for microbial activity. Additionally, pollutants in these environments have low bioavailability, which is a limiting factor. To address this, biostimulation is achieved by adding specific substrates that act as promoters to stimulate pollutant degradation [50]. In a study, adding organic nutrients to polluted soil containing 38,000 mg/kg of TPH resulted in a 100% removal of polycyclic PAHs within three months [51]. Compared to bioaugmentation, biostimulation has been found more effective, as bioaugmentation can lead to decreased biodiversity among soil microorganisms and a community enriched in PAH-degrading species [52].

5.4. Biosurfactants

Biosurfactants decrease surface tension and enhance the contact between pollutants and microorganisms, thereby increasing the bioavailability of pollutants and facilitating biodegradation. Several studies have demonstrated the effect of biosurfactants on the bioremediation process [53], [54]. Biosurfactants are used in various fields, including cosmetics, industrial applications, textiles, crude oil recovery, detergents, pharmaceuticals, biomedicine, and food processing industries. These compounds are non-toxic, biodegradable, and can function under extreme conditions of temperature, salinity, and pH [55], [56]. This makes biosurfactants more advantageous than chemical surfactants. Recently, biosurfactants have become a promising alternative for biodegradation. Numerous studies have highlighted their biotechnological applications.

Significant biosurfactant production and emulsification activities have been identified in bacteria such as *Stenotrophomonas*, *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Kocuria*, and *Bacillus* strains isolated from hydrocarbon-polluted environments [57]. Various bacterial genera, including *Corynebacterium*, *Rhodococcus*, *Pseudomonas*, *Bacillus*, *Achromobacter*, and *Ochrobactrum*, are also effective biosurfactant producers [58], [59] and are frequently used in biotechnological applications.

Biosurfactants produced by *Pseudomonas* species, known for their glycolipid structure, were characterized as rhamnolipids, which enhanced both biodegradation processes and oil recovery [54], [58]. *Bacillus subtilis* produces a lipopeptide biosurfactant called surfactin, widely used in commercial applications [60]. Additionally, a study reported the use of rhamnolipids from *Burkholderia plantarii* in pharmaceutical and industrial applications [61].

Bacillus subtilis produces surfactin, a lipopeptide biosurfactant used in commercial applications [60]. *Brevibacillus* species have been found to produce biosurfactants that facilitate phenanthrene degradation. Similarly, phenanthrene biodegradation is stimulated by trehalose lipids produced by *Rhodococcus erythropolis*.

5.5. Landfarming

Landfarming is a bioremediation method, also known as land application or land treatment. In this method, sediments or contaminated soils are spread in a thin layer on a prepared, suitable surface. Microbial activity is stimulated by the addition of suitable nutrients and minerals or through aeration. The contaminated soil is periodically tilled or mixed to aerate the mixture. This method has been successful in degrading various hydrocarbons at polluted sites [62]. Landfarming has several advantages over other methods, including low cost, low energy consumption, and ease of implementation. Consequently, it is widely practiced for hydrocarbon degradation at polluted sites [63], [64].

6.FACTORS AFFECTING BIOREMEDIATION TREATMENT

Various physical and chemical factors influence the rate of hydrocarbon degradation. Key factors include the amount, nature, and type of hydrocarbons; the type of matrix (water, soil, sediment, or effluents); the activity of microbial communities; and environmental conditions such as humidity, pH, temperature, oxygen levels, and pollutant bioavailability [65].

6.1. Nutrient Availability

Microbial activity, growth, and metabolism depend on the availability of inorganic elements like phosphorus, nitrogen, hydrogen, oxygen, and potassium [66]. Micronutrients such as manganese, nickel, iron, cobalt, zinc, chloride, and copper are also needed in trace amounts. The rate of biodegradation is influenced by carbon/nitrogen or carbon/phosphorus ratios, which tend to be high in hydrocarbon-polluted sites, thereby decreasing the rate of remediation.

In aquatic environments, biodegradation was slow due to the low availability of nutrients [67]; [68], [69]. In open sea and intertidal environments, nutrients are often washed away. To address this, nutrient delivery systems have been developed for these environments. Fertilizers or oleophilic nutrients, which act as inorganic nutrient sources, are released gradually in these sites. Nitrogen and phosphorus sources are coated with hydrophobic compounds like vegetable oil or paraffin, providing a solution to nutrient washout and rapid dilution of water-soluble nutrients. Oleophilic components are located at the oil-water interface, promoting bacterial growth and metabolism. This method was considered to be highly effective [70]. Adding essential nutrients (N, P) balances microbial growth and reproduction, thereby enhancing biodegradation. In cold environments, carefully added nutrients can stimulate microbial metabolic activity, increasing the rate of biodegradation [20], [71].

6.2. Temperature

Temperature is a critical factor for microorganisms involved in hydrocarbon degradation [72], [73], [74], [75]. Higher temperatures increase the bioavailability, solubility, distribution, and degradation rates of hydrocarbons, which enhances the ability of microorganisms to degrade them and raises the overall rate of biodegradation. However, at elevated temperatures, oxygen solubility decreases, potentially halting the degradation activity of aerobic microorganisms.

Biodegradation is most efficient at optimal temperatures, rather than at excessively high or low temperatures. Lofthus *et al.* [76] reported that microorganisms can degrade PAHs in seawater at extreme temperatures, from as low as 0°C to as high as 50°C. Along the Mediterranean coast, Elsaeed *et al.* [77] studied the genes of microorganisms involved in biodegradation and found a correlation between temperature and the presence of genes related to biodegradation. At lower temperatures, the percentage of gene sequences associated with biodegradation ranged from 0.56% to 1.30%, but this increased by 5.6 times in medium-temperature sites (13-24°C) [57], [67].

Some researchers have confirmed that low temperatures reduce bacterial populations and catabolic diversity. In the Arctic, natural degradation is extremely slow due to very low temperatures. Oleophilic microorganisms became inactive because transport channels in the cell wall closed, and the cytoplasm frozen entirely [78], [70]. Enzymes involved in the degradation pathways are most active at optimal temperatures, with specific pollutants requiring specific temperature ranges for effective degradation. Microbial activity increases with a rise in temperature, reaching a maximum before sharply decreasing if temperatures continue to rise or fall beyond a certain point, ultimately halting at extreme temperatures.

6.3. Oxygen Limitations

Degradation of petroleum hydrocarbons (PHs) can occur under either aerobic or anaerobic conditions. During hydrocarbon metabolism, fungi and bacteria utilize molecular oxygen to oxidize substrates through oxygenases, making oxygen essential for most organisms involved in biodegradation. Although anaerobic degradation occurred in various environments, it was generally considered less significant [79], [13], as oxygen enhanced the catabolism of PHs [80]. Oxygen limitation is commonly encountered in aquatic soils and sediments, where dissolved oxygen levels can drop to zero, leading to anaerobic degradation. Various technologies are available to deliver oxygen to hydrocarbon-polluted sites, though it remains challenging in marine environments. For aerating the upper layers of polluted areas, tilling was used during low tide [20], [81], [8].

6.4. pH

pH is another crucial factor affecting microbial activity and the biodegradation process. Soil pH levels indicate the potential for microbial growth, and even slight changes in pH can significantly impact metabolic activity. Extreme pH ranges reduced microbial activity and biodegradation efficiency [82], [83], [84]. Most fungi and bacteria degrade PHs best at neutral pH. For instance, phenanthrene was degraded by *Burkholderia cocovenenans* at pH 5.5 up to

40%, while at neutral pH, the degradation rate reached 80% [85], [86], [87]. Leahy and Colwell [88] reported that naphthalene degradation was reduced at pH 5.0, with the highest degradation occurring at pH 7. Some studies have shown that *Pseudomonas* species degraded PHs at alkaline pH levels [46]. (Liu et al., 2019), while other studies have reported hydrocarbon degradation at pH 2 in acidic environments [89]. The appropriate pH for bioremediation depends on the specific microorganisms used.

6.5. Bioavailability of Hydrocarbons

Bioavailability is a key factor in hydrocarbon degradation, as it determines the rate at which a substrate is transferred into the microbial cell. Polycyclic aromatic hydrocarbons (PAHs), being less soluble in aqueous environments, have low bioavailability, which made them resistant to degradation and persistent in nature [90]. Studies have indicated that the bioavailability of PHs decreased over time. When PAHs dissolve or evaporate, they become more bioavailable [91]. Photo-oxidation can also enhance the bioavailability of petroleum hydrocarbons, thus increasing microbial activity and biodegradation [92]. The use of surfactants, applied or sprayed in polluted areas, can increase the bioavailability of contaminants at these sites, promoting more effective bioremediation.

7. DISTRIBUTION OF HYDROCARBON-DEGRADING MICROORGANISMS

Many studies have shown that a wide range of microorganisms isolated from polluted areas can degrade petroleum hydrocarbons (PHs). A recent study reported 103 fungal, 79 bacterial, 9 cyanobacterial, and 14 algal species capable of utilizing hydrocarbons as carbon and energy sources [70]. Numerous studies have indicated that microorganisms from polluted environments found to be highly efficient in hydrocarbon degradation [93]. Approximately 25 bacterial genera capable of degrading PHs have been found in marine ecosystems [38]. Although oil spills in the sea were toxic to many microbes, some were resistant and able to degrade pollutants; nearly 25 hydrocarbon-degrading bacterial genera have been isolated in such environments [66], [12].

Bacteria that use hydrocarbons as their sole carbon source were referred to as obligate hydrocarbon clastic bacteria (OHCB) [94], [95]. Before hydrocarbon contamination, OHCBs represented about 0.1% of the total microbial population [96], [97], but after contamination, their population increased significantly, with OHCBs becoming 1-2% of the most dominant species. Only a limited number of these organisms could be cultivated. Another study reported three bacterial strains isolated from the oil-polluted environment of the Khurais oil field, Dhahran, Saudi Arabia, identified as *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Bacillus cereus*, based on their ability to grow in the presence of hydrocarbons [98].

In another study, bacterial communities were investigated in four soil samples collected from the top layers of diesel oil-contaminated and engine oil-contaminated soils, as well as top and middle layers of uncontaminated soils. This study identified 25 phyla, 53 classes, 108 orders, 199 families, and 292 genera in various soil samples, with biodiversity differing significantly in oil-polluted samples. In engine oil-contaminated soil, seven bacterial genera (*Sulfuritalea*, *Sphingomonas*, *Alkanindiges*, *Rhodococcus*, *Nocardioides*, *Rhodoplanes*, and *Actinobacteria norank*) were found to be effective biodegraders, with *Rhodococcus* and *Nocardioides* known to produce biosurfactants [5]. *Pseudomonas* NCIM 5514, a petroleum-degrading organism, was identified in the crude oil-contaminated area of Ankleshwar, Gujarat, India [99].

Several polycyclic aromatic hydrocarbon (PAH)-degrading microbial species, such as *Microbacterium* sp. BPw, *Novosphingobium* sp. PCY, *Alcaligenes* sp. SSK1B, *Ralstonia* sp. BPH, and *Achromobacter* sp. SSK4, have also been identified in mangrove sediments [100], [101].

8. MECHANISM OF HYDROCARBON DEGRADATION

The biodegradation rate of petroleum hydrocarbons generally follows this trend: *n*-alkanes > branched alkanes > low molecular weight aromatics > cyclic alkanes. Resins and asphaltenes are the most recalcitrant compounds among petroleum hydrocarbons [102].

8.1. Alkane-Degrading Bacteria

Alkanes are saturated hydrocarbons primarily composed of carbon and hydrogen atoms. Alkanes constitute the major fraction of crude oil, accounting for more than 50%, depending on the oil type and source. Various aerobic and anaerobic microorganisms, including bacteria, fungi, and yeasts, have the metabolic capacity to utilize alkanes as their sole carbon and energy source. Bacterial degradation of alkanes, primarily through aerobic processes, has been well-studied [103]. Degradation of *n*-alkanes is particularly well-understood compared to other types of petroleum hydrocarbons. Alkane biodegradation typically requires oxygen, with alkane hydroxylase as a key enzyme in the process of degradation [104]. Methane monooxygenase was involved in the degradation of short-chain alkanes (C2–C4) [105].

Medium-chain alkanes (C5–C17) are degraded by multiple hydroxylases, including cytochrome P450 and monooxygenases such as *AlkB* [105]. Long-chain *n*-alkanes (C18 and above) required alkane hydroxylases for degradation [106], [107]. For the breakdown of these long chains, multiple alkane hydroxylases were often involved

[107]. *Alcanivorax* spp. was commonly associated with alkane degradation in polluted sites [94]. The *alkB* gene, responsible for encoding alkane hydroxylase, plays a crucial role in degrading medium to long-chain alkanes. This gene was first identified in *Pseudomonas putida* and has since been found in numerous hydrocarbon-degrading organisms, known as hydrocarbon clastic bacteria [104]. Approximately 250 *alkB* gene homologs have been identified in hydrocarbon-degrading *Alcanivorax* spp.

8.2. Polycyclic Aromatic Hydrocarbon (PAH) Degradation

The first step in PAH degradation in hydroxylation, with monooxygenases and dioxygenases serving as the primary enzymes involved in this process. Dioxygenases are enzyme complexes composed of ferredoxin, reductases, and terminal oxygenase subunits. Many bacteria metabolize PAHs through cytochrome P450, converting them to carbon dioxide and water in the presence of oxygen [101].

Anaerobic biodegradation of PAHs was found to be a very slow process. While few PAHs could be degraded under anaerobic conditions, some reported examples include anthracene, fluorene, phenanthrene, fluoranthene, and acenaphthene [104].

9. CONCLUSION AND FUTURE PROSPECTS

Hydrocarbons in crude oil mixtures pose hazardous risks to humans and animals. Conventional techniques for reclaiming oil-contaminated soils are often costly. Bioremediation techniques, such as biostimulation and bioaugmentation, involve the use of microorganisms to transform hazardous PAHs and other hydrocarbons into less or non-hazardous compounds, making this approach cost-effective and time efficient. Biodegradation is influenced by various factors, including environmental conditions (temperature, pH), types of microorganisms, pollutant types, and hydrocarbon bioavailability. Numerous studies have shown that microorganisms isolated from oil-contaminated sites can effectively degrade PAHs. Genetically modified organisms (GMOs) can degrade pollutants faster than natural species, competing well with indigenous microbial species, predators, and various abiotic factors such as temperature and pH. Alkane hydroxylase is a key enzyme in the degradation of alkane hydrocarbons, with *alkB* genes playing a role in the breakdown of medium to long-chain alkanes by encoding alkane hydroxylase. Monooxygenases and dioxygenases are involved in PAH degradation.

In our view, several strategies can be implemented to make microbial bioremediation processes more efficient, as outlined below:

i. Screening microorganisms capable of effectively degrading PAHs in oil-contaminated soils.
ii. Constructing novel bacterial consortia for enhanced hydrocarbon degradation.
iii. Investigating the biochemical pathways and molecular mechanisms involved in PAH biodegradation.
iv. Using genetic engineering to create engineered bacteria capable of growing in extreme reservoir conditions and producing adequate metabolites with desired properties.
v. Applying biosurfactants to enhance oil recovery, as heavy oil resources represent a substantial potentially recoverable petroleum energy resource.
vi. Optimizing environmental conditions on-site to promote biodegradation.

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