

Review

Remediation of Petroleum-Contaminated Soils with Microbial and Microbial Combined Methods: Advances, Mechanisms and Challenges

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Abstract: The petroleum industry's development has been supported by the demand for petroleum and its by-products. During extraction and transportation, however, oil will leak into the soil, destroying the structure and quality of the soil and even harming the health of plants and humans. Scientists are researching and developing remediation techniques to repair and re-control the afflicted environment due to the health risks and social implications of petroleum hydrocarbon contamination. Remediation of soil contamination produced by petroleum hydrocarbons, on the other hand, is a difficult and time-consuming job. Microbial remediation is a focus for soil remediation because of its convenience of use, lack of secondary contamination, and low cost. This review lists the types and capacities of microorganisms that have been investigated to degrade petroleum hydrocarbons. However, investigations have revealed that a single microbial remediation faces difficulties, such as inconsistent remediation effects and substantial environmental consequences. It is necessary to understand the composition and source of pollutants, the metabolic genes and pathways of microbial degradation of petroleum pollutants, and the internal and external aspects that influence remediation in order to select the optimal remediation treatment strategy. This review compares the degradation abilities of microbial–physical, chemical, and other combination remediation methods, and highlights the degradation capabilities and processes of the greatest microbe–biochar, microbe–nutrition, and microbe–plant technologies. This helps in evaluating and forecasting the chemical behavior of contaminants with both short- and long-term consequences. Although there are integrated remediation strategies for the removal of petroleum hydrocarbons, practical remediation remains difficult. The sources and quantities of petroleum pollutants, as well as their impacts on soil, plants, and humans, are discussed in this article. Following that, the focus shifted to the microbiological technique of degrading petroleum pollutants and the mechanism of the combined microbial method. Finally, the limitations of existing integrated microbiological techniques are highlighted.

Keywords: petroleum contaminated soil; composition of petroleum; harm of petroleum; microbial remediation; combined microbial methods; phytoremediation; biochar

1. Introduction

Extraction, processing, and transportation (pipe rupture) all contribute to the entry of petroleum into the soil environment [1,2]. The primary contaminants in petroleum-contaminated soil are toxic and hazardous aliphatic, cycloaliphatic, and aromatic hydrocarbons [3]. They decrease the diversity of plants and microbes in the soil, deplete soil

fertility, disrupt soil ecological balance, and even put human health at risk [4]. Crop germination is delayed, the chlorophyll content is poor, and some crops perish when grown in high petroleum-contaminated soil [5]. Furthermore, pollutants can enter the human body by breathing, skin contact, or eating petroleum-contaminated food, causing contact dermatitis, visual and auditory hallucinations, and gastrointestinal disorders, as well as substantially raising the risk of leukemia in children. Although certain low-molecular-weight hydrocarbon pollutants will weather and decay over time, high-molecular-weight hydrocarbon pollutants will remain in the soil for a long period due to their hydrophobicity, causing secondary contamination in the ecosystem [6,7]. According to statistics, Chevron Texaco's oilfields in Ecuador's Amazon region have harmed human health, the water supply, and the ecosystems in the area. The plaintiff (30,000 individuals of mixed races and indigenous peoples) was awarded USD 9.5 billion by the Cuban Supreme Court in 2013 [8]. This demonstrates that petroleum pollutants have a negative influence on society in addition to destroying the environment. As a result, the issue of restoring petroleum-contaminated soil has become a hot topic.

Incineration, landfill, leaching, chemical oxidation, and microbiological treatment are now used to remediate petroleum-contaminated soil. These technologies can extract, remove, transform, or mineralize petroleum pollutants in a contaminated environment, transforming them into a less damaging, harmless, and stable form [9]. Although incineration and chemical oxidation can remove 99.0% and 92.3% of total petroleum hydrocarbons, respectively, both restoration procedures have disadvantages [10,11]. Toxic substances such as dioxins, furans, polychlorinated biphenyls, and volatile heavy metals will be released into the atmosphere as a result of incomplete petroleum burning [12]. The carbon in the soil is reduced by 49–98% as the incineration temperature rises from 200 °C to 1050 °C, and the organic matter and carbonate in the soil are decomposed into light hydrocarbons (C_2H_2 , C_2H_4 , and CH_4) and carbon dioxide separately [13,14]. The total number of soil microorganisms decrease from 10^4 CFU/g to 10^3 CFU/g to 10^2 CFU/g after chemically oxidizing petroleum pollutants in the soil with 5 percent hydrogen peroxide and persulfate for 10 days. The bacteria will continue to develop slowly over the following 10 days [15]. The incomplete combustion of petroleum increases the hidden dangers of environmental safety, while the loss of carbon and organic matter limits the recovery ability of the soil ecosystem. The addition of oxidants will inhibit the growth of soil microorganisms. Therefore, while reducing the concentration of soil petroleum pollutants, it will not cause secondary pollution to the soil and the surrounding environment, which has become the main consideration for selecting remediation technologies.

Microbial remediation is inexpensive, and it can completely mineralize organic pollutants into carbon dioxide, water, inorganic compounds and cell proteins, or convert complex organic pollutants into other simpler organics [16]. Microorganisms can utilize organic pollutants as their only source of carbon, allowing them to degrade organic pollutants in the soil [17,18]. Microorganisms destroyed 62–75% of petroleum hydrocarbons in the soil in 150 to 270 days [19,20]. Free microorganisms destroyed 2.3–6.8% of petroleum hydrocarbons in 60 days, however when biochar was employed as a carrier, 7.2–30.3% of petroleum hydrocarbons were degraded in 60 days [21]. Petroleum degraded at a rate of 29.8% in the immobilized system (sodium alginate-diatomite beads), whereas free cells degraded at a rate of 21.2% in 20 days [22]. At 4 °C and 10 °C, microbial mineralization of hexadecane generates 45% CO_2 , while at 25 °C, 68% CO_2 is generated in 50 days, indicating that microorganisms can better digest hexadecane [23]. When the soil salinity is higher than 8%, and the pH value is lower than 4 and higher than 9, the activity of *Acinetobacter baylyi* ZJ2 is affected, and a certain amount of lipopeptide surfactant cannot be produced, thereby reducing the degradation of petroleum by microorganisms [24].

In conclusion, extreme environmental conditions (soil temperature below 10 °C, pH below 4 and more than 9) decrease microbial activity, which diminishes the removal impact of petroleum pollutants. Furthermore, a pH 5.5–8.8, temperature 15–45 °C, oxygen content 10%, low clay or silt content soil type, and C/N/P ratio of 100:10:1 are the optimum

conditions for microbial remediation of oily soil, according to current research [25,26]. Long remediation times and low remediation efficacy of free microorganisms are issues with microbial remediation. The microbial combination technique is used to increase the bio-degradation efficiency of microorganisms in order to overcome the challenge of microbial remediation of petroleum in the soil.

The source, categorization, and content of hydrocarbon contamination in soil, as well as its influence on the environment and human health, are discussed in this article. Following that, the different forms of combination microbial repair methods are explained, as well as their benefits. The microbial combination method focuses on the microbial remediation of petroleum pollutants and the interaction of microbial–biochar/nutrients/plants. Finally, the benefits and limitations of contemporary microbial mixed approach repair technologies are discussed.

2. Petroleum-Contaminated Soil

2.1. Sources of Petroleum Pollutants

Figure 1 depicts the major pathways through which petroleum contaminants permeate the soil [27]. Petroleum spills are a major cause of hydrocarbon contamination in the soil. The global leakage of natural petroleum is reported to be 600,000 metric tons per year [28]. Petroleum contamination is estimated to have polluted 3.5 million sites in Europe [29]. In China, about 4.8 million hectares of soil petroleum content may exceed the safe limit [30]. Distinct nations and areas have varied sampling and transportation techniques, as well as different sources and degrees of petroleum contamination. Furthermore, contaminants are leached into the surrounding and deep soil in horizontal and vertical orientations, as well as into the groundwater system, as a result of rainfall washing and leaching.



Figure 1. Major sources of hydrocarbons in the soils.

Low-molecular-weight hydrocarbons are more volatile and more easily penetrate into groundwater than high-molecular-weight hydrocarbons, although volatilization and permeability are influenced by the physical and chemical characteristics of the soil, climate, and vegetation [29]. The natural decay half-life of petroleum hydrocarbons grows

as the concentration of petroleum hydrocarbons increases (when the petroleum concentration is 250 mg/L, the half-life is 217 days) [31]. The natural half-life of alkane and aromatic pollutants rises with increasing molecular weight. Under normal conditions, the half-life of the three-ring molecule phenanthrene is 16 to 126 days, but the half-life of the five-ring molecule benzo[*a*]pyrene is 229 to 1400 days [32]. Though some specific bacteria in polluted soil may biodegrade and bio-transform these hydrocarbons, absorbing them into biomass in the soil [33,34], small quantities of hydrocarbons (such as long chain and high molecular weight hydrocarbons) are still challenging to handle in the environment due to the non-polarity and chemical inertness of pollutants [35].

2.2. Composition of Petroleum Pollutants

Petroleum-contaminated soil often contains petroleum, water, and solid particles. Petroleum pollutants are often shown as water-in-petroleum (W/O). Petroleum is made up of a variety of hydrocarbons, composed of carbon (83–87%), hydrogen (11–14%), and sulfur (0.06–0.8%), nitrogen (0.02–1.7%), oxygen (0.08–1.82%), and trace metal components (nickel, vanadium, iron, antimony, etc.) [36]. Hydrocarbons formed by the combination of carbon and hydrogen constitute the main component of petroleum, accounting for about 95% to 99%. Various hydrocarbons are classified according to their structure: alkanes, cycloalkanes, and aromatic hydrocarbons.

Alkanes are the main components of gasoline, diesel, and jet fuel [37,38]. The molecular structure is linear, branched, and cyclic. The general formula of linear-alkanes is C_nH_{2n+2} , the general formula of branched alkanes is C_nH_{2n+2} ($n > 2$), and the general formula of cycloalkanes is C_nH_{2n} ($n > 3$). Aromatics are found in gasoline, diesel, lubricants, kerosene, tar, and asphalt [39]. They have a similar molecular structure to cycloalkanes, but they have at least one benzene ring [40]. Aromatics have the general formula C_nH_{2n-6} .

Petroleum is derived from bitumen, and the heaviest and most polar molecules in asphaltene are firmly adsorbed on the source rock, making discharge into the reservoir problematic. As a result, the most frequent are saturated hydrocarbons with the lowest polarity, followed by aromatics [41]. The molecular weight of hydrocarbons influences their degradability. Low-molecular-weight hydrocarbons have better bioavailability than high-molecular-weight hydrocarbons [42,43]. As a result, hydrocarbon susceptibility to microbial breakdown is generally: linear alkanes > branched alkanes > aromatics with low molecular weight > cyclic alkanes [16,44].

2.3. Toxic Effects of Petroleum on the Environment

Saturates, aromatics, and other poisonous and hazardous hydrocarbons are mostly found in petroleum [30]. Highly hazardous petroleum pollutants (PAHs, BTEX) will have an adverse effect on soil, plants, and humans. High levels of polycyclic aromatic hydrocarbons (PAHs) in the soil can induce tumors, reproductive, development, and immunological problems in terrestrial invertebrates [45]. BTEX (benzene, toluene, ethylbenzene, and xylene) can harm a person's personal neurological system, liver, kidneys, and respiratory system [46]. Pollutants obstruct soil pores, alter the content and structure of soil organic matter, diminish the activity and variety of soil microbes and plants, and, as a result, endanger human health via the food chain [47]. Deuterated PAH (dPAH) was utilized by Jose L. Gomez-Eyles et al. to evaluate the bioavailability of PAH in soil [48]. According to research, the dPAH:PAH ratio of benzo(*a*)pyrene in earthworm tissues is greater than the dPAH:PAH ratio obtained by normal chemical methods. The ratio of additional dPAH accumulated by earthworms is increasing as the size of PAH rises. This indicates that the toxicity of petroleum pollution on animals is much worse than previously thought. The petroleum in the soil also pollutes the groundwater environment through diffusion and migration, putting a strain on a variety of elements of human life.

2.3.1. Toxic Effects of Petroleum on Soil

Petroleum degrades the ecological structure and function of soils [6], affecting soil moisture, pH, total organic carbon, total nitrogen, exchangeable potassium, and enzyme activity substantially (urease, catalase and dehydrogenase) [49–52]. As pollutant concentrations rise, the clay content in contaminated soil rises [53], soil porosity declines, and impermeability and hydrophobicity rise [54], inhibiting the growth of plant roots and the number of bacteria in the soil. The root length of *Lepidium sativum*, *Sinapis alba*, and *Sorghum saccharatum* was reduced by 65.1%, 42.3%, and 47.3%, respectively, when the petroleum hydrocarbon concentration in the soil was 7791 mg/kg [55]. Straight-chain alkanes have the greatest influence on the number of bacteria species. The following is the order of influence: 320.5 ± 5.5 (in the control soil) > 289.1 ± 4.7 (in the aromatic hydrocarbon-contaminated soil) > 258.6 ± 2.5 (in the branched-chain-alkane-contaminated soil) > 229.7 ± 2.0 (in straight-chain- and cyclic-alkanes-hydrocarbons-contaminated soil) [56]. According to studies, the major contaminant that causes soil salinization and acidification is benzo[a]pyrene, which is present in petroleum [57].

2.3.2. Toxic Effects of Petroleum on Plants

Petroleum pollutants have the ability to permeate plant surfaces and move via the intracellular space and vascular system. Plant roots may collect petroleum pollutants in the soil, transport them to leaves and fruits, and store them, as well as transmit pollutants from leaves to roots. Corn germination rate, plant height, leaf area, and dry matter yield were all drastically reduced as a result of petroleum contamination [58]. Plant growth is slowed, stem length and diameter are shortened, aboveground tissue length is reduced, and the root length and plant leaf area are altered due to a lack of oxygen and nutrients in the polluted soil (depending on the plant species) [59]. Low concentrations of petroleum hydrocarbon (10 g/kg) have been found to increase plant root vitality, but medium concentrations (30 g/kg) and high concentrations (50 g/kg) have been shown to decrease plant root vitality. Simultaneously, the chlorophyll content of 50 g/kg petroleum-contaminated soil is almost 60% lower than that of non-contaminated soil [60].

2.3.3. Toxic Effects of Petroleum on Human Health

Exposure to petroleum and petroleum products, whether direct (breathing polluted air and direct contact with skin) or indirect (bathing in contaminated water and eating contaminated food), can cause significant health issues in people [61]. Many petroleum pollutants, such as benzene and polycyclic aromatic hydrocarbons, are toxic, mutagenic, and carcinogenic. Some aromatics have a negative impact on human liver and kidney functioning, even causing cancer [62]. Furthermore, because PAHs are extremely lipophilic, they are easily absorbed by animals through the digestive tract [45]. Long-term exposure to polluted areas can cause tiredness, respiratory problems, eye irritation, and headaches, and women are more likely to have spontaneous abortions [8]. Oil extraction in residential areas, particularly in low- and middle-income nations, has been shown to affect the health of a huge number of non-occupational contacts, according to studies. It is estimated that 638 million people in low- and middle-income countries live in rural areas close to conventional oil reservoirs [8]. Individuals who are more exposed to oil-related pollution and are not typically exposed to occupational areas, such as infants, children, pregnant women, the elderly, or people with prior health conditions, will use daily activities (such as bathing, agricultural activities, and so on) that will be affected. Simultaneously, natural gas burning in oil wells can produce volatile organic compounds (VOCs), nitrogen dioxide (NO₂), sulfur dioxide (SO₂), polycyclic aromatic hydrocarbons, and benzo[a]pyrene, all of which are harmful to non-occupationally exposed individuals.

3. Advances in the Utilization of Microorganisms in Petroleum Remediation

Articles were searched for in “web of science” databases. Databases contain the Core Collection (WOS), the Derwent Innovations Index (DII), the Korean Journal Database (KJD), MEDLINE, the Russian Science Citation Index (RSCI), and Scientific Electronic Library Online (SCIELO) six databases. The items that were retrieved were only published between 1950 and 2020. “Microbial degradation petroleum” is the result of a specific search phrase. The deadline for the search is 17 September 2020, and the findings will be analyzed statistically.

Figure 2 depicts the unprecedented number of research findings on microbial petroleum pollution cleanup from 1950 to 2020. The number of published study findings has risen year after year, suggesting that petroleum microbial remediation technology has attracted the interest of academics both at home and abroad in recent years. Figure 3 shows the statistics on different sorts of research outputs and the percentage of countries/regions that were re-searched. The data shows that the article is the most common kind of research output. The majority of research on microbial remediation of petroleum pollution takes place in Asia (34%) and Europe (34%).

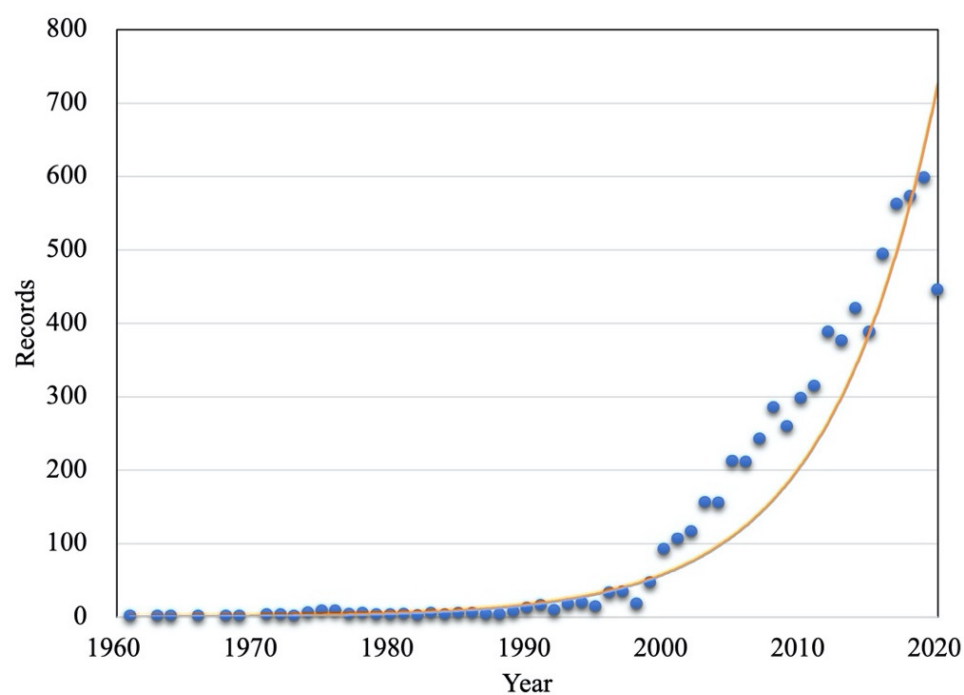


Figure 2. The record number of research results of microbial remediation of petroleum pollution.

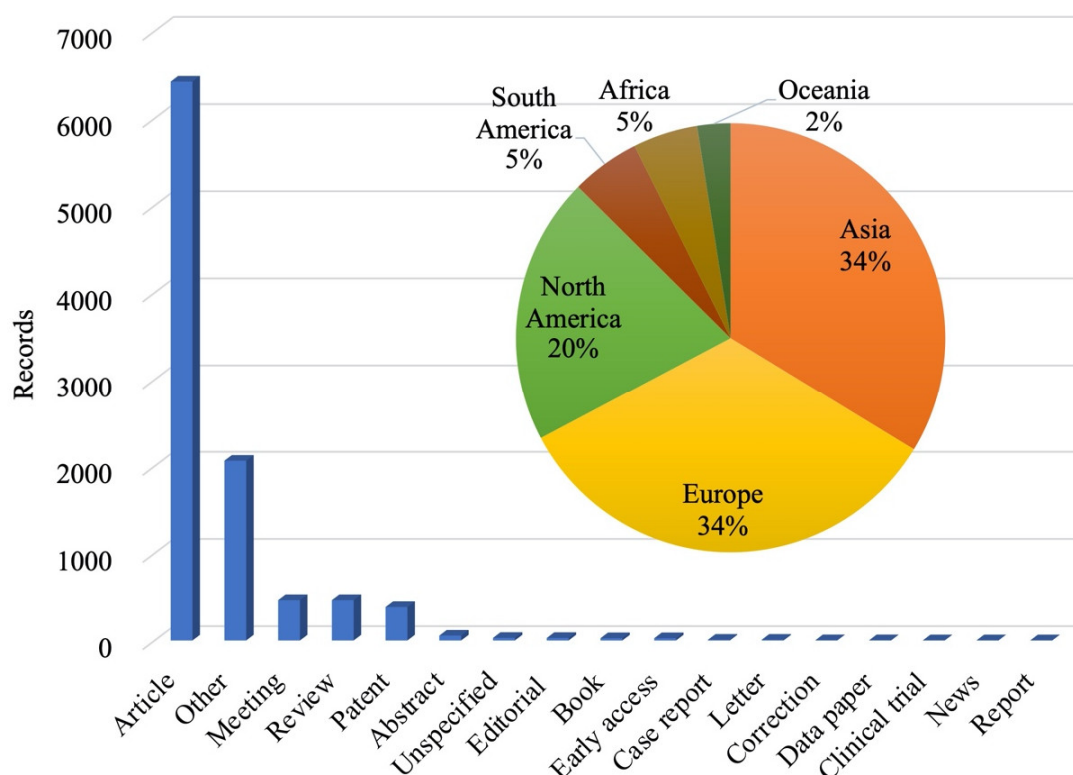


Figure 3. Statistics of research output types and the percentage of countries/regions researched.

4. Microbial Remediation

Bioaugmentation has a high practicality and economic application when compared to physical and chemical remediation techniques [63,64]. By adding lipophilic bacteria, bioaugmentation can be accomplished [65]. Oleophilic bacteria may be found in a wide range of petroleum-contaminated environments, including saltwater, coastlines, sludge, and soil [6]. They may thrive only on hydrocarbons while decomposing or mineralizing harmful and hazardous petroleum contaminants [66,67]. Different types of degrading bacteria can be found in different sorts of polluted environments. To determine the kinds and activities of soil organisms, DNA-based stable isotope probing (DNA-SIP) technology is used [68]. In soil contaminated by polycyclic aromatic hydrocarbons, actinomycetes are a common phylum. *Acidovorax*, *Rhodoferrax*, *Hydrogenophaga* and *Polaromonas* were found in the soil contaminated in the Philippines. *Acidobacteria* exists in the soil contaminated with petroleum, phenanthrene, pyrene, and fluoranthene.

Studies have demonstrated that a number of bacteria, including *Rhodococcus* sp., *Pseudomonas* sp., and *Scedosporium boydii*, can degrade petroleum contaminants [69–71]. Hydrocarbons are mostly degraded by bacteria via aerobic pathways [72]. When oxygen serves as an electron acceptor, hydrocarbon catabolism is often accelerated [73]. In aerobic mode, the processes of oxidation, reduction, hydroxylation, and dehydrogenation mediate degradation. The biodegradation of hydrocarbons is assisted by enzymes such as monooxygenase, dioxygenase, cytochrome P450, peroxidase, hydroxylase, and dehydrogenase [72,74–77].

Microorganisms that degrade alkanes and PAHs in an inorganic salt liquid media have been effectively isolated for the time term (as shown in Table 1). *Pseudomonas* sp., *Acinetobacter* sp., and *Rhodococcus* sp. are currently the bacteria that have the most effect on the degradation of petroleum pollutants. Short-chain and medium-chain alkanes (C5–C16) can be oxidized by the integral membrane non-heme iron oxygenase (AlkB) or cytochrome P450 enzyme (CYP153) in the strain, according to studies. Putative flavin-binding

monooxygenase (AlmA) and long-chain alkane monooxygenase (LadA) is involved in the oxidation of long-chain alkanes [78]. Several degradation genes can coexist in a single bacterium. There are at least two AlkB-type genes (AlkMa and AlkMb) and one AlmA-type gene (AlmA) in *Acinetobacter strain* DSM17874 that are responsible for degrading alkanes of varying chain lengths [79]. *Pseudomonas* sp. also contained nahAc, catechol dioxygenase (C12O and C23O), AlkB, and cytochrome P450, which are important for the degradation of alkanes and polycyclic aromatic hydrocarbons [80–84].

The major pathways for alkane and PAH metabolism in microorganisms include terminal oxidation, subterminal oxidation, ω -oxidation, and β -oxidation. The terminal oxidation pathway is the most common mechanism for alkanes to be destroyed. Alkane hydroxylase introduces molecular oxygen into hydrocarbons to oxidize terminal methyl to form alcohols, which are next oxidized to aldehydes and fatty acids, and eventually, carbon dioxide and water are produced by the β -oxidation pathway [85–87]. PAHs, on the other hand, are resistant to biodegradation due to their structural stability. PAHs are metabolized primarily through a mixed functional oxidase system mediated by the cytochrome P450 enzyme, with oxidation or hydroxylation as the initial step and the production of diols as intermediate products. These intermediates are converted to catechol intermediates via ortho- or meta-cleavage pathways, which are then integrated into the tricarboxylic acid cycle (TCA) [73,86].

Table 1. Common microorganisms that degrade alkanes and polycyclic aromatic hydrocarbons.

Substrates	Microorganisms	Source of Strain	Main Findings			Reference	
			Substrate Concentration	Incubation Conditions	Degradation Rate		
PAHs	bacteria	<i>Achromobacter</i> sp. HZ01	Petroleum-contaminated seawater, China.	100 mg/kg anthracene, phenanthrene and pyrene.	10 ⁹ cells mL ⁻¹ /28 °C/150 rpm/30 days.	Strain remove anthracene, phenanthrene and pyrene about 29.8%, 50.6%, and 38.4%, respectively.	[88]
		<i>Acinetobacter</i> sp. WSD	Petroleum-contaminated groundwater, Shanxi province of northern China.	1 mg/kg phenanthrene, 2 mg/kg fluorine, and 0.14 mg/kg pyrene.	5% cells suspension/33 °C/150 rpm/6 days.	Approximately 90% of fluorine, 90% of phenanthrene, and 50% of pyrene were degraded.	[89]
		<i>Bacillus subtilis</i> BMT4i (MTCC 9447)	Automobile contaminated soil, Uttarakhand, India.	50 g/mL Benzo[a]Pyrene.	1 × 10 ⁸ cells mL ⁻¹ /37 °C/120 rpm/28 days.	Strain started degrading Benzo[a]Pyrene achieving maximum degradation of approximately 84.66%.	[90]
		<i>Caulobacter</i> sp. (T2A12002)	From King Fahd University of Petroleum and Minerals Department of Life Sciences laboratory.	100 ppm pyrene.	2% cells suspension/37 °C and 25 °C/120 rpm/18 days/pH 5.0 and pH 9.0.	Strain degraded 35% and 36% of pyrene at 25 °C and 37 °C, respectively.	[91]
		<i>Enterobacter</i> sp. (MM087)	Engine-oil-contaminated soil, Puchong and Seri Kembangan, Selangor Malaysia.	500 mg/L phenanthrene and 250 mg/L pyrene.	5% cells suspension and 1 × 10 ⁶ cells mL ⁻¹ /37 ± 0.5 °C/200 rpm/24 h.	Strain with 80.2% degradations for phenanthrene and 59.7% degradations for pyrene.	[92]
		<i>Klebsiella pneumoniae</i> AWD5	Automobile-contaminated soil, Silchar, Assam.	0.005% PAH (Pyrene, Chrysene, Benzo(a)pyrene).	Cells(OD600 = 0.4)/30 °C/140 rpm/9 days.	Strain degraded pyrene (56.9%), chrysene (36.5%) and benzo(a)pyrene (50.5%), respectively.	[93]
		<i>Mycobacterium vanbaaleenii</i> PYR-1	Petroleum-contaminated sediment and water, the watershed of Redfish Bay near Port Aransas, Tex.	0.5 ug/mL pyrene.	1.5 × 10 ⁶ cells mL ⁻¹ /24 °C /150 rpm/48 to 96 h.	After incubation, 47.3 to 52.4% of pyrene was mineralized to CO ₂ .	[94]
		<i>Raoultella planticola</i>	Near a car repair station, Hangzhou, China.	20 mg L ⁻¹ pyrene and 10 mg L ⁻¹ benzo[a]pyrene.	2.0 × 10 ⁸ cells mL ⁻¹ /30 °C /180 rpm/10 days.	Strain degraded 52.0% of pyrene and 50.8% of benzo[a]pyrene.	[95]
		<i>Rhodococcus</i> sp. P14	Petroleum-contaminated sediments, Xiamen, China.	50 mg/L phenanthrene, pyrene and benzo[a]pyrene.	1% cells suspension/30°C/150 rpm/30 days.	Strain degraded 34% of the pyrene, about 43% of the phenanthrene and 30% of the Benzo[a]pyrene.	[96]
	<i>Pseudomonas</i> sp. MPDS	PAH- and petrochemical-contaminated soil and mud, Tianjin.	1 mg/mL naphthalene, 0.1 mg/mL dibenzofuran, 0.1 mg/mL dibenzothiophene, 0.1mg/mL fluorene.	Cells(OD600 = 5.0)/25°C/200 rpm/84 h, 96 h, and 72 h.	Strain could completely degrade naphthalene in 84 h. A total of 65.7% dibenzofuran and 32.1% dibenzothiophene could be degraded in 96 h	[97]	

					and 40.3% fluorene could be degraded in 72 h.	
		<i>Pseudoxanthomonas</i> sp. DMVP2	Petroleum-contaminated sediment, Gujarat, India.	300 ppm phenanthrene	4% cells suspension/37 °C/150 rpm/72 h.	Strain was able to degrade 86% phenanthrene. [98]
		<i>Sphinogmonas</i> sp.	Typical mangrove swamp(surface sediment (0–2 cm)), Ho Chung, Hong Kong.	5000 mg L ⁻¹ phenanthrene.	180 rpm/7 days.	Strain was obtained to degrade 99.4% phenanthrene at the end of 7 days. [99]
		<i>Stenotrophomonas</i> sp. IITR87	— ¹	Phenanthrene(10 ppm), pyrene(10 ppm), and benzo- α -pyrene(10 ppm).	0.8% cells suspension/30 °C/175 rpm/15 days.	Strain showed >99, 98, and <50% degradation of phenanthrene, pyrene, and benzo- α -pyrene respectively. [100]
		<i>Streptomyces</i> sp. (ERI-CPDA-1)	Petroleum-contaminated soil, Chennai, India.	Naphthalene(0.1%), phenanthrene(0.1%).	3% cells suspension/30 °C/200 rpm/7 days.	Strain could remove 99.14% naphthalene and 17.5% phenanthrene. [101]
		<i>Aspergillus</i> sp. RFC-1	Rumaila oilfield(surface polluted sludge (1–10 cm)), Basra, Iraq.	50 mg/L naphthalene, 20 mg/L phenanthrene, 20 mg/L pyrene.	10% cells suspension/30 °C/120 rpm/7 days.	Biodegradation efficiencies of crude oil, naphthalene, phenanthrene, and pyrene were 84.6%, 50.3%, and 55.1%, respectively. [102]
		<i>Nocardia</i> sp. H17-1	Petroleum-contaminated soil	Aliphatic and aromatic (1%, w/v).	30 °C/6 days.	The aliphatic and aromatic fractions were degraded 99.0 \pm 0.1% and 23.8 \pm 0.8%, respectively. [103]
	fungus	<i>Penicillium</i> sp. CHY-2	Soil, Antarctic.	100 mg L ⁻¹ butylbenzene, naphthalene, acenaphthene, ethylbenzene, and benzo[a]pyrene.	20 °C/110 rpm/28 days.	Strain showed the level of degradation for butylbenzene (42.0%), naphthalene (15.0%), acenaphthene (10.0%), ethylbenzene (4.0%), and benzo[a]pyrene (2.0%). [104]
		<i>Trichoderma</i> sp.	— ¹	100 mg kg ⁻¹ pyrene and benzo(a)pyrene.	240 h	Strain degraded 63% of pyrene (100 mg kg ⁻¹) and 34% of benzo(a)pyrene (100 mg kg ⁻¹) after 240 h of incubation. [105]
		<i>Fusarium</i> sp.	— ¹	100 mg kg ⁻¹ pyrene and benzo(a)pyrene.	240 h	Strain degraded 69% of pyrene (100 mg kg ⁻¹) and 37% of benzo(a)pyrene (100 mg kg ⁻¹) after 240 h of incubation. [105]
alkanes	bacteria	<i>Achromobacter</i> sp. HZ01	Petroleum-contaminated seawater, China.	2% (w/v) diesel oil	28 °C/150 rpm/10 days.	Strain degraded the total n-alkanes reached up to 96.6%. [88]

	<i>Acinetobacter</i> sp. (KC211013)	Coal chemical industry wastewater treatment plant, northeast China.	700 mg/L alkanes.	35 °C	The degradation rate reached 58.7%.	[106]
	<i>Bacillus subtilis</i>	Petroleum-polluted soil, Shengli Oilfield, China.	0.3% (<i>w/v</i>) crude oil.	6% cells suspension/30 °C/150 rpm /5 days.	The results indicated that 30–80% of the n-alkanes (C13–C30) were degraded by strain.	[107]
	<i>Pseudomonas</i> sp. WJ6	Xinjiang oilfield, China.	0.5% (<i>w/v</i>) n-alkanes.	1010 CFU mL ⁻¹ /37 °C/180 rpm/20 days.	N-dodecane (C12) was degraded by 46.65%, 42.62%, 31.69%, and 23.62% of C22, C32, and C40 were degraded, respectively.	[108]
	<i>Rhodococcus</i> sp.	Bay of Quinte, Ontario, Canada.	0.1% (<i>v/v</i>) diesel fuel.	Cells(OD600 = 0.025) /0 °C/150 rpm/102 days.	After 102 days of incubation at 0 °C, strain mineralized C12 (8%), C16 (6.1%), C28 (1.6%), and C32 (4.3%).	[109]
fungus	<i>Cladosporium Resinae</i>	Soil, Australian.	12.5%(<i>v/v</i>) n-alkanes.	0.75–1.25% cells suspension/35 °C/35 days.	All higher n-alkanes from n-nonane to n-octadecane were assimilated by the fungus.	[110]
	<i>Penicillium</i> sp. CHY-2	Soil, Antarctic.	100 mg L ⁻¹ decane, dodecane and octane.	20 °C/110 rpm/28 days.	Strain was degraded decane (49.0%), dodecane (33.0%), and octane (8.0%).	[104]
actinomycetes	<i>Gordonia</i> sp.	Hydrocarbon-contaminated Mediterranean shoreline, west coast of Sicily, Italy.	1 g L ⁻¹ eicosane and octacosane.	30 °C /28 days.	Eicosane and octacosane were degraded from 53% to 99% in 28 days.	[111]
	<i>Tsukamurella</i> sp. MH1	Petroleum-contaminated soil, Pitești, Romania.	0.5% (<i>v/v</i>) liquid alkanes.	30 °C	Strain capable to use a wide range of n-alkanes as the only carbon source for growth.	[112]

¹ There is no clear description in the article.

Low-molecular-weight saturated hydrocarbons and aromatic hydrocarbons are easily degraded by microorganisms, while petroleum hydrocarbons with higher-molecular-weight have strong resistance to microbial degradation [113]. The sequence of microbial degradation is as follows: N-alkanes > branched-chain alkanes > branched alkenes > low-molecular-weight n-alkyl aromatics > monoaromatics > cyclic alkanes > polynuclear aromatics > asphaltenes [114]. The methylene concentration in asphaltenes dropped by 14% and 8%, respectively, after 45 days of degradation by *Bacillus subtilis* and *Pseudomonas aeruginosa* [115]. *Pseudomonas aeruginosa* can degrade 63.8% of n-hexadecane within 60 days [116].

The most critical issue affecting the globe is the elimination of persistent environmental contaminants. PAHs have emerged as one of the most significant environmental contaminants, due to their hydrophobicity [117]. The following is a ranking of PAHs based on the order of the mineralization rate and the estimated half-life (in weeks): naphthalene (2.4–4.4), hexadecane (2.2–4.2), phenanthrene (4–18), 2-methyl Base naphthalene (14–20), pyrene (34→90), 3-methylcholanthrene (87→200), and benzo[a] pyrene (200→300) [118]. Long-term exposure to low-level petroleum hydrocarbons lasts two to four times longer than PAHs surviving in the original environment. Despite the discovery of microbes able to degrade naphthalene, phenanthrene, and pyrene, the biodegradation of polycyclic aromatic hydrocarbons with large molecular weight remains a challenge.

The majority of microbial degradation of petroleum pollutants research are conducted in the laboratory using a mineral basal medium (liquid) (as indicated in Table 1) and have not been applied to actual petroleum-contaminated soil. Although some studies have shown that a single strain may degrade petroleum-contaminated soil, there are still issues with a single bioremediation technique, such as lengthy repair times, unstable microbial activity, and inadequate destruction of free microorganisms. Within 30 days, *S. changbaiensis* and *P. stutzeri* may decompose $39.2 \pm 1.9\%$ and $47.2 \pm 1.2\%$ of TPH in soil, respectively (the initial oil concentration is 1026 ± 50 mg/kg) [119]. *T. versicolor* can degrade 50% of TPH within 280 days (the initial oil content of the soil is 1727 mg/kg) [120]. Therefore, to increase degradation impact and practical application, combined microbial methods (synergistic repair incorporating microorganisms in the degradation process) are utilized.

5. Combined Microbial Methods Remediation

Microorganism–physical, microorganism–chemical, and microorganism–biology are the three primary types of microbial combination methods. In the microbial combined method of decomposing petroleum-contaminated soil, a variety of materials and procedures have been employed (Table 2). Most remediation combination methods are designed to enhance the microbial activity and aeration of polluted soil because of the hydrophobicity and fluidity of petroleum. To increase the system's degradation rate, an electric field, fertilizers, biocarrier, biochar, biosurfactants, and plants were applied to the petroleum-contaminated soil [121–123]. As shown in Table 2, the combination of microbes and physical or chemical technologies can improve the efficiency of microbial degradation of petroleum pollutants. In high-concentration petroleum-contaminated soil ($\geq 10,000$ mg/kg), the addition of biochar, electric fields, nutrients, and biosurfactants can all make the removal rate of petroleum pollutants reach more than 60%. The combination of ryegrass and mixed microbial strains had the best degradation effect within 162 days, with a degradation rate of 58% (the initial oil content was 6.19%). The combination of alfalfa and microorganisms can degrade 63% of petroleum hydrocarbons within 60 days (the initial oil content is 12%).

Table 2. The microbial combined materials and methods were used for the degradation of petroleum-contaminated soil.

Methods	Materials	Main Findings			Reference
		Substrate Concentration	Incubation Conditions	Degradation Rate	
Microorganism–physical	Biochar (walnut shell biochar (900 °C)/pinewood biochar (900 °C))	24,000, 16,000 and 21,000 mg/kg total petroleum hydrocarbons (TPH).	50 g soil/5% pinewood bio-char/C:N:P at 800:13.3:1/25 °C/60 days.	The combined remediation of biochar and fertilizer reduces the TPH in the soil to 10,000 mg/kg (the US EPA clean up standard).	[121]
	Biochar (rice straw (500 °C))	16,300 mg kg ⁻¹ TPH (saturated hydrocarbons, 8260 mg kg ⁻¹ ; aromatic hydrocarbons, 5130 mg kg ⁻¹ ; polar components, 2910 mg kg ⁻¹).	1000 g soil/2% (<i>w/w</i>) biochar/60% water holding capacity/C:N:P ratio 100:10:5/80 days.	TPH removal rate was 84.8%.	[124]
	electrokinetics	12,500 mg/kg TPH.	600 g soil/C:N:P 100:10:1/30 days.	The degradation rate of TPH was 88.3%.	[122]
	β-cyclodextrin	1000 mg/kg PAHs	1.5, 3.0, 5.0 mmol kg ⁻¹ β-cyclodextrin/25 °C.	Compared with the co-metabolism of glucose, the addition of β-cyclodextrin more strongly enhanced oil remediation in soil.	[125]
	bulking agents (chopped bermudagrass-hay/sawdust/vermiculite)	10% TPH	C:N:P 1000:10:1/15–35 °C/12 weeks.	Tillage and adding bulking agents enhanced remediation of oil-contaminated soil. The most rapid rate of remediation occurred during the first 12 weeks, where the TPH decreased 82% and the initial concentration of TPH was 10%.	[126]
	aeration (tillage/forced aeration).				
	Biocarrier (activated carbon/zeolite)	49.81 mg g ⁻¹ TPH	800 g soil/50 g biocarrier + 150 mL planktonic bacterial culture/C:N:P 100:10:1/30 °C/33 days.	Biocarrier enhanced the biodegradation of TPH, with 48.89% removal, compared to natural attenuation with 13.0% removal.	[127]
	biostimulation	19.8 ± 0.38 g kg ⁻¹ TPH	0.8 kg soil/10 ⁸ cfu g ⁻¹ petroleum degrading flora/15% soil moisture/C:N:P 100:10:1/24 °C/12 weeks.	Biostimulation achieved 60% oil hydrocarbon degradation.	[26]
	biosurfactants(rhamnolipids)	47.5 g kg ⁻¹ TPH	500 g soil/7 g of rhamnolipids (dissolved in 1 L deionized water)/500 mL bacterial consortium (in sterile 0.9% NaCl solution)/20% (<i>w/w</i>) moisture content/C:N:P 100:10:1/30 days.	TPH degradation of 77.6% was observed in the soil inoculated with hydrocarbon-degrading bacteria supplemented with rhamnolipids and nutrients.	[123]
	permanganate/activated persulfate/modified-Fenton/Fenton	263.6 ± 73.3 and 385.2 ± 39.6 mg·kg ⁻¹ Σ16 PAHs.	50 g soil/the final volume of the Milli-Q water and oxidant was 100 mL/150 rpm/15 days.	The removal efficiency of PAHs was ordered: permanganate (90.0–92.4%) > activated persulfate	[11]

Microorganism–biology	(81.5–86.54%) > modified Fenton (81.5–85.4%) > Fenton (54.1–60.0%).				
	activator (low ammonia and acetic acid)	29,500 mg kg ⁻¹ TPH.	18–20% moisture content/12 weeks.	Macro-alkanes in soils were efficiently degraded.	[128]
	Lolium perenne	6.19% TPH	750 g soil/20–30% moisture content/162 days.	The results show that the combination of ryegrass with mixed microbial strains gave the best result with a degradation rate of 58%.	[129]
	Medicago sativa	30% (40% TPH oily sludge)+70% non-pollution soil.	1 kg soil/N:P 10:1/75–80% moisture content/60 days.	Consortium degraded more than 63% TPH.	[130]
	Medicago sativa/vicia faba/Lolium perenne	1.13% TPH.	2 kg soil/18 months.	The TPH degradation in the soil cultivated with broad beans and alfalfa was 36.6% and 35.8%, respectively, compared with 24% degradation in case of ryegrass.	[131]
	biopiles (bark chips)	700 mg kg ⁻¹ TPH	soil to bulking agent was approximately 1:3/15–20 °C/5 months.	The TPH content in the pile with oil-contaminated soil decreased with 71%.	[132]
	biopiles (peanut hull powder)	29,500 mg kg ⁻¹ TPH	5 kg of soil/15% w/w peanut hull powder/18–20% moisture content/C:N:P 100:10:1/25–30 °C/12 weeks.	Biodegradation was enhanced with free-living bacterial culture and biocarrier with a TPH removal ranging from 26% to 61%.	[133]
	biopiles (food waste)	2% diesel oil	soil [77% (w/w)] and food waste [23% (w/w)]/C:N 11:1/13 days.	84% of the TPH was degraded, compared with 48% of removal ratio in control reactor without inoculum.	[134]
	earthworms (<i>Eisenia fetida</i> / <i>Allolobophora chlorotica</i> / <i>Lumbricus terrestris</i>)	10,000 mg kg ⁻¹ TPH	1000 g soil/ten adult worms per container/28 days.	The TPH concentration decreased by 30–42% in samples with <i>L. terrestris</i> , by 31–37% in samples with <i>E. fetida</i> , and by 17–18% in samples with <i>A. chlorotica</i> .	[135]

The time period is confined to 2016–2020 according to six databases in the “web of research.” “Microbial biochar (electrokinetics/bulking agents/aeration/biocarrier/biostimulation/biosurfactants/permanaganate/activated persulfate/fenton/activator/plant/biopiles/earthworms) remediation of petroleum polluted soil,” according to the results of the particular search keywords. The limit for the search is 17 September 2020, and the findings will be examined statistically. From 2016 to 2020, the papers employing the three combined microbial approaches to treat petroleum-polluted soil had the highest citation frequency (Figure 4). The microorganism–biochar, microorganism–nutrients, and microorganism–plant combined microbiological techniques have been extensively used for hydrocarbon degradation in current study, according to the gathered data.

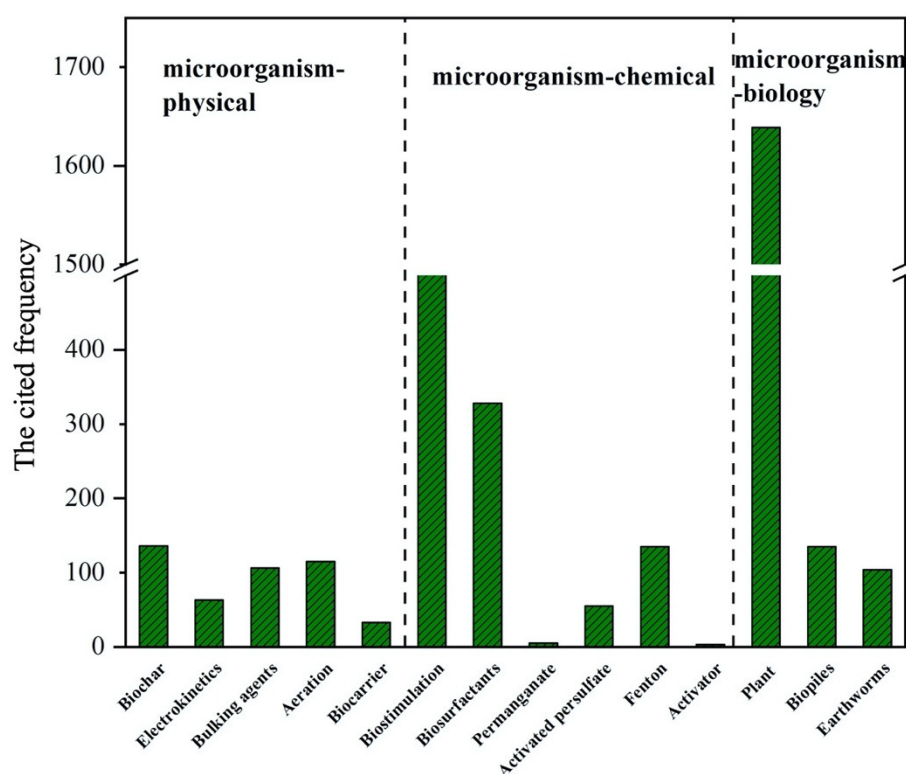


Figure 4. The frequency of citations of the article with three microbial combined methods for remediation of petroleum contaminated soil.

5.1. Microorganism–Biochar Interactions in Remediation of Hydrocarbons

Biochar has a high carbon content, excellent adsorption capacity, good stability, and the best bacteria and nutrient immobilization capacity. Biochar’s porous structure can provide attachment sites and appropriate habitats for microorganisms to survive. The addition of various types of biochar to the soil promotes the enrichment of particular functional groups of microorganisms as well as an increase in biological activity [48,136]. The functional groups on the surface of biochar, as well as the easily decomposable carbon source and nitrogen source, assist to increase microbial activity and influence their growth, development, and metabolism. The use of biochar to immobilize microorganisms with various functional properties can enhance the release of particular nutrients in the soil and the efficiency with which pollutants are degraded. Biochar has been found in studies to absorb contaminants in petroleum, decreasing soil toxicity while having no discernible detrimental influence on soil microbes [124]. Furthermore, combining biochar with petroleum-degrading bacteria enhances the variety of microbial populations as well as the hydrocarbon bioavailability [137].

The basic interactions that occur in the microorganism–biochar remediation of pollutants are illustrated in Figure 5. The three modes of microorganism–biochar remediation include adsorption, biodegradation, and mineralization, or a combination of these three. Because of the huge specific surface area and rough surface structure of biochar, associated microorganisms produce biofilm, which improves the adsorption and degradation rate of hydrocarbons while also increasing the quantity of soil and active microorganisms. Simultaneously, studies have demonstrated that fixed bacteria may employ carbon chains more broadly than free bacteria, and the clearance rate of hydrocarbons has risen by around 21% to 49% [137].

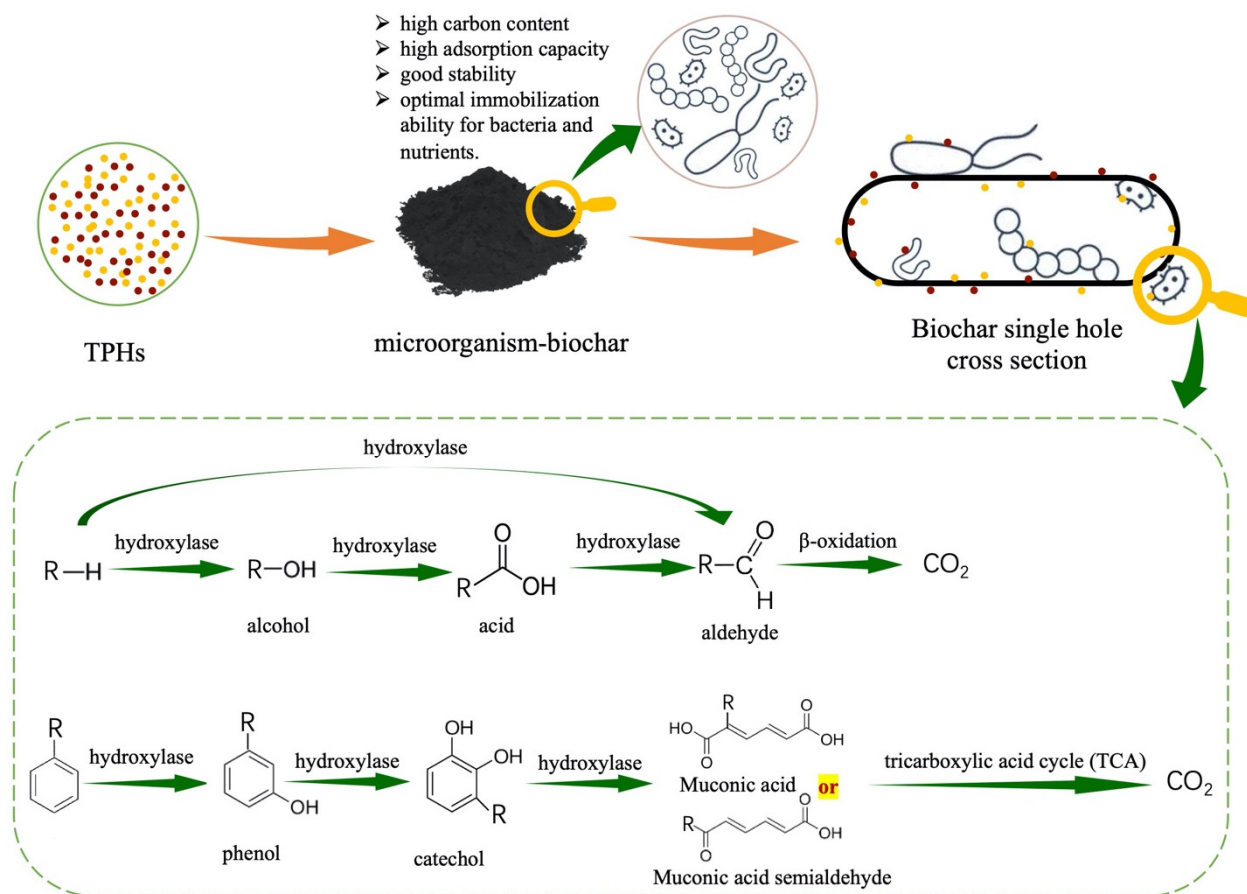


Figure 5. Proposed mechanism for the microbial metabolization of alkanes and aromatic hydrocarbons.

5.2. Microorganism–Nutrients Interactions in Remediation of Hydrocarbons

The input of a large amount of carbon sources (petroleum pollutants) frequently results in the rapid depletion of the available pools of the main inorganic nutrients (such as nitrogen (N) and phosphorus (P)) in the soil, whereas the essential nutrients (such as N, P, and terminal electron acceptors (TEA), etc.) are key factors in reducing the rate of microbial metabolism [138]. Although soil microorganisms have apparent pollution remediation potential, a shortage of necessary nutrients or activation of the degradation metabolic pathways inhibits or delays microbial repair. As a result, additional nutrients must be added to stimulate the biodegradation of inorganic pollutants [139].

If the soil environment is anaerobic for an extended period of time and the pollutant has a high carbon content, the metabolism of denitrifying bacteria in the soil will lower the total nitrogen level of the soil, therefore restricting this nutrient [140]. According to research, the amount of ammonium nitrogen (NH_4^+-N) and phosphorus ($PO_4^{3-}-P$) in soil decreases quickly 15 days after restoration [141]. Nitrate has a major benefit in enhancing

the capacity for organic pollutant biodegradation in soil. Adding N to nutrient-deficient samples rich in hydrocarbons can accelerate cell growth and hydrocarbon degradation. Because nitrate has thermodynamic benefits over TEA, it participates in the absorption and/or dissimilatory reduction process under oxygen limitation and anaerobic circumstances, promoting heterotrophic or autotrophic denitrification while oxidizing organic matter (especially alkanes) [142]. At the same time, the phosphorous concentration of the terrestrial subsurface environment is quite low. Although apatite is common in some locations, it cannot be utilized by life. Several inorganic and organic forms of phosphate have been effectively utilized to stimulate pollution in the environment [143]. As a result, the addition of nutrients nitrogen and phosphorus promotes the efficient oxidation of carbon substrates while also accelerating bacterial growth and hydrocarbon catabolism [138]. Currently, the optimum C:N:P ratio for effective biodegradation of petroleum hydrocarbons has been observed to be 100:10:1 [144].

5.3. Microorganism–Plant Interactions in Remediation of Hydrocarbons

The most popular technique for in situ remediation is the microorganism–plant combination method. Organic contaminants are mostly metabolized by plant-related microorganisms in phytoremediation, according research. It has also been reported that the remediation capacity of plants is influenced by the quantity of bacteria in their surroundings [145]. As a result, in the process of pollution remediation, the synergy between plants and microbes increases pollutant degradation and mineralization. Special enzymes and other chemicals found in plants and microbes can transform many hazardous and complicated chemical molecules into simpler and less poisonous ones. Under polluted environments, this mechanism promotes their development. Plant rhizospheres can offer microorganisms with nutrition, oxygen, and area for growth and development [146,147]. These bacteria expand the surface area of plant roots, allowing them to make contact with the soil and acquire more nutrients required for plant growth. As a result, the inoculation bacteria are more concentrated in the soil near the vegetation's roots [148]. Simultaneously, plant root exudates can promote the destruction of microorganisms by altering the composition of the microbial community and increasing microbial activity [149].

Plants such as *Merr.*, *Setaria viridis* Beauv., *Plantago asiatica* L., *Phragmites communis*, *Medicago sativa*, *Festuca elata* Keng ex *E.Alexeev*, and *Lolium perenne* L. have been shown in studies to be suitable for the climate and environment in China and are candidates for the phytoremediation of petroleum-contaminated soil in China [150]. The petroleum removal rate after 90 days of restoring petroleum-contaminated soil by *Festuca elata* Keng ex *E.Alexeev* is around 64% [151]. *Festuca elata* Keng ex *E.Alexeev* not only successfully removes benzopyrene from soil [152], but its development also improves soil biological activity in saline-alkali regions contaminated with petroleum [54]. The basic interactions that occur in the microorganism–plant remediation of pollutants are depicted in Figure 6.

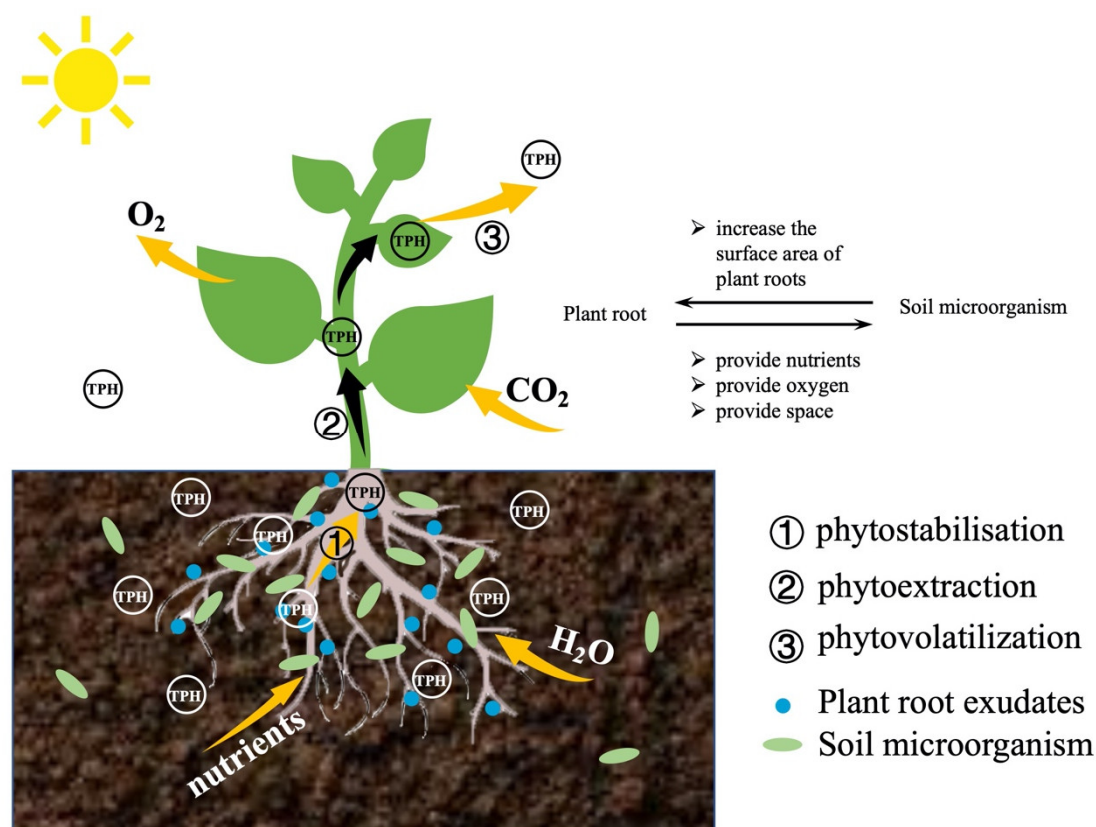


Figure 6. The common interactions that occur during pollutant remediation by microorganisms and plants.

The mechanisms of microorganism–plant remediation can be classified as degradation, extraction, inhibition, or a combination of the three. Roots not only give oxygen to microorganisms in the rhizosphere through respiration, but they also stimulate the release of root exudates and the degradation of rhizosphere contaminants [153]. Plants and microorganisms then degrade hydrocarbons into simpler organic molecules by expressing specific enzymes such as nitroreductase, dehalogenase, laccase, and peroxidase, among others [154]. Some pollutants are adsorbed on the root surface and accumulate in the root via the hemicellulose of the plant cell wall and the lipid bilayer of the plasma membrane [155]. A part of the pollutants are absorbed via phytoextraction/plant transfer to the upper section of plants (stems and leaves) [156]. Finally, phytovolatilization releases certain contaminants into the atmosphere [157]. Some plants, as a self-protection strategy, limit the transfer of hydrocarbons from the roots to the ground, retaining more hydrocarbons in the root tissues. This limitation preserves the chlorophyll and other nutrient synthesis mechanisms of plants and ensures that photosynthesis continues to function normally [156]. This is to guarantee that photosynthetic processes of the plants are regular, allowing them to produce more energy for survival and repair.

6. Advantages and Challenges in Combined Microbial Methods Application for Hydrocarbon Removal

The discharge of hazardous contaminants into the soil environment has increased substantially as a result of petroleum extraction. Bioremediation offers the advantages of ease of use, economic feasibility, and no secondary contamination, among other things, and is currently a research hotspot for oily soil remediation [64]. The addition of biochar, nutrients, and plants to microorganisms not only enhances their biological stability and activity, but it also improves their capacity to degrade petroleum pollutants. The benefits of three combined microbial methods are as follows. These methods will not harm the soil ecosystem, physical, chemical, or biological characteristics, and will actually improve

them following restoration. They may also degrade organic pollutants into entirely non-polluting inorganic molecules (carbon dioxide and water) without causing secondary contamination. The study found that after integrating oily soil remediation with microbial biomass and the number of PAH-degrading bacteria, soil enzyme activity, microbial biomass, and the number of polycyclic-aromatic-hydrocarbon-degrading bacteria were significantly higher than in other treatments [158,159]. The diversity, richness, and homogeneity of soil microbial communities have altered following restoration, according to Biolog analysis [160]. Joint restoration has enhanced the genetic variety of soil microbial communities, according to a DNA sequencing study of soil microbial diversity [161].

The three repair approaches are currently only at the laboratory stage, and few strains are utilized in engineering repair. Many contributing variables and degradation processes are yet unknown, necessitating more investigation. Figure 7 summarizes some of the problems of the three integrated microbiological techniques. The long-term stability and tolerance of biochar is one of the challenges with microbial–biochar composite repair. The most essential feature influencing the thermal decomposition of biomass and the characteristics of biochar is the pyrolysis temperature. The physicochemical characteristics and structure of biochar, such as element composition, pore structure, surface area, and functional groups, are affected by the pyrolysis temperature [162]. Biochar is rich in oxygen-containing functional groups when the pyrolysis temperature is 300 to 500 °C. There are less oxygen-containing functional groups, a higher mineral concentration, and more micropores when the pyrolysis temperature is 500 to 700 °C [163]. Their environmental activities are determined by these features. Furthermore, the pyrolysis temperature affects carbon retention throughout the pyrolysis process as well as biochar carbon stability [164]. According to research, the higher the temperature, the lower the H/C ratio, the greater the electron donor–acceptor interaction, the higher the quantity of non-decomposable carbon, and the higher the adsorption effectiveness of biochar [165]. However, investigations have indicated that at a moderate temperature of around 500 °C, the residual carbon in biochar is only around 50% on average [164]. Soil microorganisms will mineralize a portion of the biochar after joint remediation. As a result, certain techniques for adjusting the pyrolysis process should be presented in order to maximize biochar’s overall carbon sequestration capability while taking carbon retention and carbon persistence into account.

Most microorganisms and plants are more suitable to soil remediation with a petroleum pollution concentration of less than 5%, according to previous studies [26,151,166]. The remediation potential of microorganisms and plants is rapidly negatively affected when the concentration of petroleum pollutants in the soil increases (5%). The original oil content was 1.21%, and the removal rate of *Testuca arundinacea* for TPH was $64.0 \pm 1.6\%$ after 90 days of repair, and the removal rate of biological flora was $54.6 \pm 1.3\%$ [151]. After 90 days of repair, the stem and root biomass of ryegrass is lower than the control group when the soil oil concentration is 3% [167]. Tall fescue can remove 48.4% of oil pollution after 70 days of restoration when the soil oil concentration is 5% [168]. Microorganisms could remove 15% of petroleum pollutants after 70 days of remediation when the soil oil content is 5.6% [169]. When soil oil levels are too high, it is hazardous to plants and microorganisms, reducing their capacity to degrade petroleum contaminants and potentially causing deaths in microorganisms and plants. Extreme climatic circumstances (soil temperature below 10 °C, pH value below than 4 and higher than 9), on the other hand, will limit the activity of microorganisms and plants, lowering the removal of petroleum pollutants. Changes in soil pH and abiotic or biodegradation of biochar, on either hand, will increase the desorption of PAH from biochar into sediments. In conclusion, despite the benefits of minimal secondary contamination and low cost, microbial remediation still confronts significant challenges.

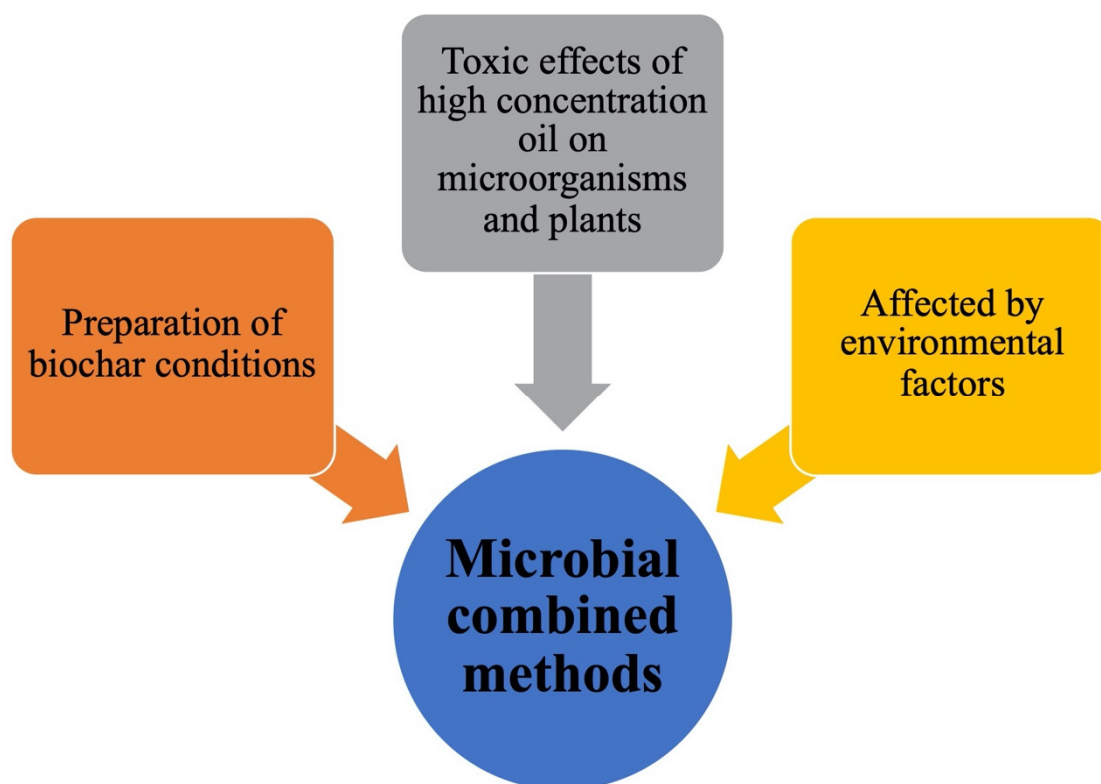


Figure 7. Some challenges of three microbial combined methods.

7. Conclusions

This article explains the use of a combination of microbiological methods to remediate petroleum-contaminated soil. Although a combination of microorganisms–biochar/nutrients/plants can be utilized to remediate petroleum-contaminated soil to solve the issues of a unique remediation, no one method is best for all types of pollutants or all unique site circumstances that occur in the impacted area. As a result, an efficient combined remediation method based on the physical and chemical characteristics of soil at various polluted sites as well as the kinds of contaminants is required. In addition, scientists are working at the movement, distribution, and degradation mechanisms of contaminants in the combined system, as well as their interactions and relationships with microorganisms. Clarify the internal and external elements that impact the restoration before selecting particular therapeutic treatments. Therefore, to find out the key factors and mechanisms that increase the degradation rate of microbial joint remediation, and to design a microbial joint remediation technology with high degradation efficiency, sustainability, and environmental friendliness is a problem that needs to be solved urgently.

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