Remediation of Petroleum Contaminated Soils

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Due to the development of the petroleum industrial, numerous petroleum pollutants are discharged into the soil, destroying the structure and properties of the soil, and even endangering the health of plants and humans. Microbial remediation and combined microbial methods remediation of petroleum-contaminated soil are currently recognized remediation technologies, which have the advantages of no secondary pollution, low cost and convenient operation. This entry includes the sources and composition of petroleum pollutants and their harm to soil, plants and humans. Subsequently, the focus is on the mechanism of microbial method and combined microbial methods to degrade petroleum pollutants. Finally, the challenges of the current combined microbial methods are pointed out.

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

1. Introduction

Petroleum enters the soil environment through processes such as extraction com, processing and transportation (pipe rupture) [1][2]. Toxic and harmful aliphatic, cycloaliphatic and aromatic hydrocarbons are the main pollutants of petroleum-contaminated soil [3]. They reduce the diversity of plants and microorganisms in the soil, destroy soil fertility, affect soil ecological balance, and even endanger human health [4]. The germination of crops in high petroleum-contaminated soil is delayed, the chlorophyll content is low, and some crops die [5]. In addition, pollutants can enter the human body through breathing, skin contact, or eating food containing petroleum contaminants, causing contact dermatitis, visual and auditory hallucinations, and gastrointestinal diseases, and even greatly increasing the risk of children suffering from leukemia. Although some low-molecular-weight hydrocarbon pollutants will be weathered and degraded over time, high-molecular-weight hydrocarbon pollutants, due to their hydrophobicity, exist in the soil for a long time and cause secondary pollution to the surrounding environment [6][7]. Therefore, repairing petroleum-contaminated soil has become a topic of widespread concern.

At present, the methods to treat petroleum-contaminated soil include incineration, landfill, leaching, chemical oxidation and microbial treatment. These remediation technologies can extract, remove, transform or mineralize petroleum pollutants in the polluted environment into a less harmful, harmless and stable form [8]. Although 99.0% and 92.3% of total petroleum hydrocarbons(TPH) can be removed by incineration and chemical oxidation, these repair techniques still have drawbacks [9][10]. Toxic substances such as dioxins, furans, polychlorinated biphenyls and volatile heavy metals from incomplete incineration of petroleum will be released into the atmosphere [11]. At the same time, as the incineration temperature rises from 200°C to 1,050°C, the carbon in the soil is lost 49-98%, 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 [12][13]. After chemically oxidizing the petroleum pollutants in the soil with 5% hydrogen peroxide and persulfate for 10 days, the total number of soil microorganisms decreased from $10^4 CFU\,g^{-1}$ to $10^3 CFU\,g^{-1}$ and $10^2 CFU\,g^{-1}$ separately. And the bacteria grow slowly in the next 10 days [14]. 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 [15]. Microorganisms can use organic pollutants as the sole carbon source to grow and metabolize, so as to achieve the purpose of degrading organic pollutants in the soil [16][17]. Within 150-270d, microorganisms degraded 62-75% of petroleum hydrocarbons in the soil [18][19]. Within 60 days, 2.3-6.8% of petroleum hydrocarbons were degraded by free microorganisms, but when biochar was used as a carrier, 7.2-30.3% of petroleum hydrocarbons were degraded [20]. On day 20, the degradation rate of petroleum in the immobilized system (sodium alginate-diatomite beads) was as high as 29.8%, while the degradation rate of free cells was 21.2% [21]. At 4°C and 10°C, the microbial mineralization of hexadecane produced 45% CO_2, but at 25°C, the microbial mineralization of hexadecane produced 68% CO_2 within 50 days [22]. 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 [23].

In summary, extreme environmental conditions (soil environmental temperature less than 10°C, pH less than 4 and greater than 9) reduce microbial activity, which reduces the removal effect of petroleum pollutants. Current research shows that the best conditions for microbial remediation of oily soil are: pH 5.5-8.8, temperature 15-45°C, oxygen content 10%, soil type: low clay or silt content, C/N/P: 100:10:1 [24][25]. Microbial remediation has problems such as long remediation time and poor remediation effect of free microorganisms. In order to overcome the difficulty of microbial remediation of petroleum in the soil, the microbial combination method is used to improve the biodegradation efficiency of microorganisms.

This entry first discusses the source, classification and composition of hydrocarbon pollution in soil, as well as its impact on the environment and human health. Subsequently, the types and advantages of combined microbial repair methods are discussed. The focus is on the microbial remediation mechanism of petroleum pollutants and the microbial-biochar/nutrients/plants interaction in the microbial combined method. Finally, the advantages and challenges of the current microbial combined method repair technology are proposed.

2. Petroleum contaminated soil

2.1. Sources of petroleum pollutants

As shown in Fig. 1, petroleum pollutants leak to the soil through petroleum extraction, petroleum residue and sludge stacking, oily wastewater and accidental petroleum spills, automobile exhaust emissions, and other methods (using pesticides) [26]. Petroleum spills are one of the main sources of hydrocarbon pollution in the soil. The global natural petroleum leakage is estimated to be 600,000 metric tons per year [27]. It is estimated that 3.5 million locations in Europe may be contaminated by petroleum [28]. About 4.8 million hectares of soil petroleum content in China may exceed the safe value [29]. Different countries and regions have different sampling and transportation methods, and the sources and degrees of petroleum pollution are also different. Moreover, through the washing and leaching of rainwater, the pollutants are leached into the surrounding and deep soil in the horizontal and vertical directions, and even into the groundwater system.

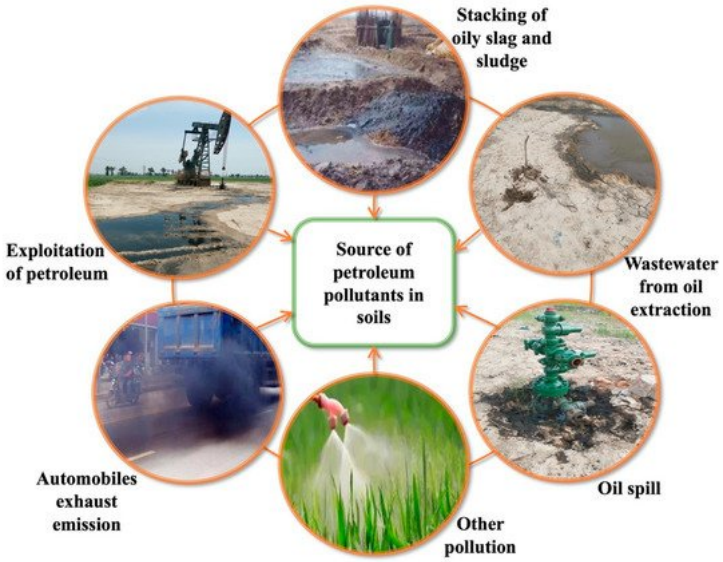

Figure 1. Major sources of hydrocarbons in the soils.

Compared with high-molecular-weight hydrocarbons, low-molecular-weight hydrocarbons are more volatile and easier to penetrate into groundwater, but volatilization and permeability are affected by the physical and chemical properties of soil, climate, and vegetation [28]. Although low-molecular-weight hydrocarbons will weather and degrade over time, high-molecular-weight hydrocarbons can remain in the soil for a long time due to their hydrophobicity [6][7]. The natural decay half-life of petroleum hydrocarbons increases as the concentration of petroleum hydrocarbons increases (when the petroleum concentration is 250mg/L, the half-life is 217d) [30] . As the molecular weight increases, the natural half-life of alkane and aromatic contaminants increases. For example, the half-life of three-ring molecule phenanthrene under natural conditions is 16 to 126 days, while the half-life of five-ring molecule benzo[α]pyrene is 229 to 1,400 days [31]. Although the contaminated soil itself has some special microorganisms that can biodegrade and bio-transform these hydrocarbons, assimilating them into biomass in the soil [32][33]. However, due to the non-polarity and chemical inertness of pollutants, small amounts of hydrocarbons (such as long chain and high molecular weight hydrocarbons) are still difficult to handle in the environment [34].

2.2. Composition of petroleum pollutants

Petroleum-contaminated soil usually consists of petroleum, water and solid particles. Petroleum contaminants are usually shown in the form of water-in-petroleum (W/O). Petroleum is composed of a mixture of different hydrocarbons. The chemical elements that make up petroleum are mainly carbon (83% ~ 87%), hydrogen (11% ~ 14%), and the rest are

sulfur (0.06% ~ 0.8%) and nitrogen (0.02% ~ 1.7%), oxygen (0.08% ~ 1.82%) and trace metal elements (nickel, vanadium, iron, antimony, etc.) [35]. 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 [36][37]. The molecular structure is linear, branched and cyclic. The general formula of linear-alkanes is CnH2n+2, the general formula of branched alkanes is CnH2n+2(n > 2), and the general formula of cycloalkanes is CnH2n(n > 3). Aromatics are found in gasoline, diesel, lubricants, kerosene, tar and asphalt [38]. The molecular structure is similar to cycloalkanes, but they contain at least one benzene ring [39]. The general formula of aromatics is C_nH_{2n-6}.

Petroleum comes from the source rock bitumen, and the heaviest and most polar molecules in the asphaltene are strongly adsorbed on the source rock and are difficult to discharge into the reservoir. Therefore, saturated hydrocarbons with the lowest polarity are the most common, followed by aromatics [40]. The degradability of hydrocarbons is affected by their molecular weight. The bioavailability of low-molecular-weight hydrocarbons is higher than that of high-molecular-weight hydrocarbons [41][42]. Therefore, the sensitivity of hydrocarbons to microbial degradation is generally: linear alkanes> branched alkanes> low-molecular-weight aromatics> cyclic alkanes [15][43].

2.3. Toxic effects of petroleum on the environment

Petroleum mainly contains saturates, aromatics and other toxic and harmful hydrocarbons [29]. Specific petroleum pollutants (PAHs, BTEX) that are highly toxic will have a negative impact on soil, plants and humans. High concentrations of polycyclic aromatic hydrocarbons (PAHs) in the soil can cause toxic effects on terrestrial invertebrates such as tumors, reproduction, development and immunity [44]. Low concentration (10 mg/kg) PAHs can promote tomato growth. However, when the concentration of PAHs was greater than 20 mg/kg, tomato growth was inhibited [45]. Benzene, toluene, ethylbenzene, and xylene (BTEX) can create problems for nervous system, liver kidney, and respiratory system of human being [46]. Pollutants block soil pores, change the composition and structure of soil organic matter, reduce the activity and diversity of soil microorganisms and plants, and ultimately threaten human health through the food chain [47]. The petroleum in the soil also pollutes the groundwater environment through diffusion and migration, which poses unfavorable pressure on many aspects of the human living environment.

2.3.1. Toxic effects of petroleum on soil

Petroleum destroys the stability of soil ecological structure and function [6], and significantly affects the content of soil moisture, pH, total organic carbon, total nitrogen, exchangeable potassium, and enzyme activity (urease, catalase and dehydrogenase)[48][49][50][51]. As the concentration of pollutants increases, the clay content in the contaminated soil increases [52], the soil porosity decreases, and the impermeability and hydrophobicity increase [53], which inhibits the growth of plant roots and soil the number of bacteria. When the petroleum hydrocarbon content in the soil was 7791 mg/kg, the root length of *Lepidium sativum, Sinapis alba*, and *Sorghum saccharatum* was suppressed by 65.1%, 42.3% and 47.3% [54]. Straight-chain alkanes have the greatest influence on the number of bacteria species. The order of influence is as follows: 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 contaminate soil) [55]. Studies have found that benzo[a]pyrene in petroleum is the main pollutant that causes soil salinization and acidification [56].

2.3.2. Toxic effects of petroleum on plants

Petroleum contaminants can penetrate the surface of plants and migrate in the intercellular space and vascular system. Plant roots can absorb petroleum pollutants in the soil, move to leaves and fruits and accumulate, and can also transfer pollutants from the leaves to the roots. Petroleum pollution significantly reduced the germination rate, plant height, leaf area and dry matter yield of corn [56]. Due to the lack of oxygen and nutrients in the contaminated soil, plant growth is retarded, stem length and diameter are shortened, aboveground tissue length is reduced, and root length and plant leaf area changes(depending on the plant species) [57]. Studies have shown that low concentration(10 g/kg) petroleum hydrocarbon can promote plant root vitality, while medium concentration(30 g/kg) and high concentration(50 g/kg) petroleum hydrocarbon can inhibit plant root vitality. At the same time, the chlorophyll content of 50 g/kg petroleum-contaminated soil is nearly 60% lower than that of non-petroleum-contaminated soil [58].

2.3.3. Toxic effects of petroleum on human health

Direct (breathing polluted air and direct contact with skin) or indirect (bathing in contaminated water and eating contaminated food) exposure to petroleum and petroleum products can cause serious health problems to humans [59]. Many petroleum contaminants are toxic, mutagenic and carcinogenic, such as benzene and polycyclic aromatic hydrocarbons. Some aromatics affect the human normal functions of liver and kidney and even causing cancer [60]. And PAHs are highly lipophilic, so they are easily absorbed by mammals through the gastrointestinal tract [44]. Workers who have been exposed to contaminated sites for a long time have symptoms such as fatigue, breathing, eye irritation and headaches, and women have an increased risk of spontaneous abortion [61].

3. Advances in the utilization of microorganisms in petroleum remediation

Search for articles in "web of science" databases , databases contain the Core Collection (WOS), Derwent innovations index(DII), Korean Journal Database (KJD), MEDLINE (MEDLINE), Russian Science Citation Index (RSCI) and Scientific Electronic Library Online (SCIELO) six databases. The searched articles were published limited to 1950-2020. The specific search terms result is “Microbial degradation petroleum”. The search time is September 17, 2020, and the results are statistically analyzed.

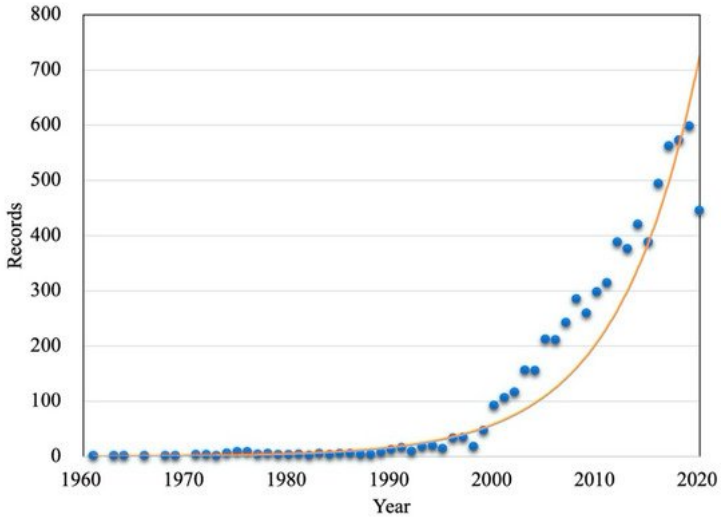

Figure 2. The record number of research results of microbial remediation of petroleum pollution.

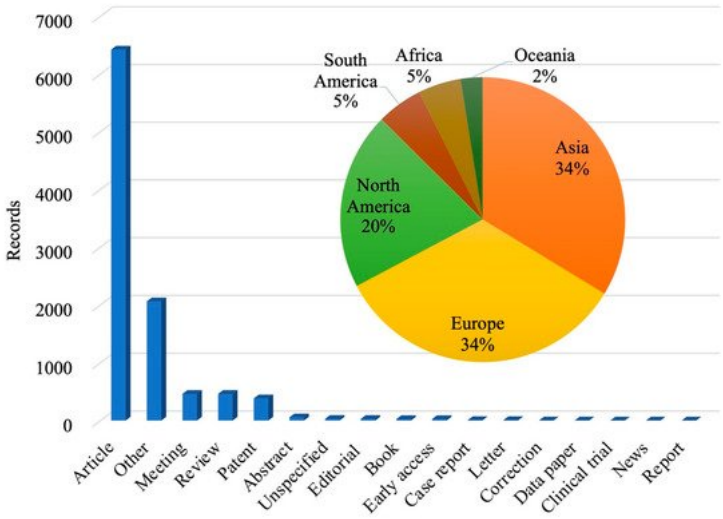

Figure 3. Statistics of research output types and the percentage of countries/regions researched.

Fig. 2 summarizes the record number of research results of microbial remediation of petroleum pollution from 1950 to 2020. The number of recorded research results has increased year by year, indicating that the microbial remediation technology of petroleum has attracted the attention of scholars at home and abroad in recent years. Fig. 3 Statistics of research output types and the percentage of countries/regions researched. The aggregated data show that the article is the main research output

4. Microbial remediation

Compared with physical and chemical remediation methods, bioaugmentation shows high feasibility and economic applicability [62][63]. Bioaugmentation can be accomplished by introducing lipophilic bacteria [64]. Oleophilic bacteria widely exist in petroleum contaminated environment[6], such as seawater, coastline, sludge and soil. They can use various hydrocarbons as the sole carbon source for growth, while decomposing or mineralizing toxic and harmful petroleum pollutants [65][66].

Currently, studies have shown that a variety of microorganisms can degrade petroleum pollutants, such as *Rhodococcus* sp., *Pseudomonas* sp. and *Scedosporium boydii* [67][68][69]. Strains mainly degrade hydrocarbons through aerobic pathways [70]. Generally, the catabolism of hydrocarbons is faster when oxygen acts as an electron acceptor [71]. The reactions that mediate degradation are oxidation, reduction, hydroxylation and dehydrogenation that occur in aerobic mode. Enzymes such as monooxygenase, dioxygenase, cytochrome P450, peroxidase, hydroxylase and dehydrogenase play an important role in the biodegradation of hydrocarbons [70][72][73][74][75].

At present, microorganisms that degrade alkanes and PAHs in inorganic salt liquid medium have been successfully separated(as shown in Table 1). That microorganism metabolism alkanes and PAHs main through terminal oxidation, subterminal oxidation, ω-oxidation and β-oxidation. Alkanes are main degraded by terminal oxidation pathway. Molecular oxygen is introduced into hydrocarbons by alkane hydroxylase to oxidize terminal methyl to form alcohols, which are further oxidized to aldehydes and fatty acids, and finally, carbon dioxide and water are generated through β-oxidation pathway to form tricarboxylic acid cycle (TCA)[76][77][78]. On the contrary, PAHs show strong recalcitrance to biodegradation due to the structural stability. PAHs are main metabolized by cytochrome P450 enzyme mediated mixed functional oxidase system with oxidation or hydroxylation as the first step, and the formation of diols as intermediate products. These intermediates undergo ortho-cleavage or meta-cleavage pathways to form catechol intermediates, which are finally incorporated into the TCA cycle [70][77].

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

Substrates	**Microorganisms**	**Source of strain**	**Main findings**		
			Substrate concentration	Incubation conditions	Degradation rat

PAHs	bacteria	*Achromobacter* sp.HZ01	Crude oil-contaminated seawater, China.	100mg/kg anthracene, phenanthrene and pyrene.	109 cells mL^{-1}bacterial suspension /28 °C/150 rpm/30 days.	Strain remove anthracene, phenanthrene a pyrene about 29.8%, 50.6% a 38.4% respecti
		Acinetobacter sp. WSD	Crude oil-contaminated groundwater, Shanxi province of northern China.	1 mg/kg phenanthrene, 2 mg/kg fluorine and 0.14 mg/kg pyrene.	5 % actively growing cells /33 °C/150 rpm/6 days.	Approximately 9 of fluorine, 90 % phenanthrene a 50 % of pyrene were degraded.
		Bacillus subtilis BMT4i (MTCC 9447)	Automobile contaminated soil, Uttarakhand, India.	50 g /ml Benzo [a] Pyrene.	1x108 cells ml^{-1} /37 °C/120 rpm/28 days.	Strain started degrading Benz Pyrene achievi maximum degradation of approximately 8 %.
		Caulobacter sp. (T2A12002)	From King Fahd University of Petroleum and Minerals Department of Life Sciences laboratory.	100 ppm pyrene.	2 ml inoculum suspension(in 100 ml mineral salts) /37 °C and 25 °C /120 rpm/18 days/ pH 5.0 and pH 9.0.	Strain degraded 35% and 36% d pyrene at 25 °C 37 °C.
		Enterobacter sp. (MM087)	Engine oil contaminated soil, Puchong and Seri Kembangan, Selangor Malaysia.	500 mg/l phenanthrene and 250 mg/l pyrene.	5% v/v bacterial cells $1x10^6$cells ml^{-1}/37±0.5^{0}C/200 rpm/24 hours.	Strain with 80.2 degradations fo phenanthrene a 59.7% degrada for pyrene.
		*Klebsiella pneumoniae*AWD5	Automobile contaminated soil, Silchar, Assam.	0.005% PAH (Pyrene, Chrysene, Benzo(a)pyrene).	OD600=0.4/30°C/140 rpm/9 days.	Strain degraded pyrene (56.9%) chrysene (36.5 and benzo(a)py (50.5%), respectively.
		Mycobacterium vanbaalenii PYR-1	Oil contaminated sediment and water, the watershed of Redfish Bay near Port Aransas, Tex.	0.5 ug/ml pyrene.	1.5x106 cells ml^{-1}/ 24°C /150 rpm/48 to 96 h.	After incubation 47.3 to 52.4% d pyrene was mineralized to C

Raoultella planticola	Near a car repair station, Hangzhou, China.	20 mg L−1 pyrene and 10 mg L−1 benzo[a]pyrene.	2.0 × 108 cells mL^{-1}/30°C /180 rpm/10 days.	Strain degrade 52.0% of pyren and 50.8% of benzo[a]pyrene
Rhodococcus sp. P14	Oil contaminated sediments, Xiamen, China.	50 mg/L phenanthrene, pyrene and benzo[a]pyrene.	200 µl cell suspension(in 20ml Inorganic salt medium)/ 30°C /150 rpm/30 days.	Strain degrade 34% of the pyre about 43% of th phenanthrene a 30% of the Benzo[a]pyrene
Pseudomonas sp. MPDS	PAHs and petrochemicals contaminated soil and mud, Tianjin[89].	1mg/ml naphthalene, 0.1mg/ml dibenzofuran, 0.1mg/ml dibenzothiophene, 0.1mg/ml fluorene.	OD600 = 5.0(in 50ml)/25°C /200 rpm/84 h, 96 h and 72 h.	Strain could completely deg naphthalene in A total of 65.7% dibenzofuran ar 32.1% dibenzothiophe could be degra in 96 h and 40.3 fluorene could b degraded in 72
Pseudoxanthomonas sp. DMVP2	Hydrocarbon contaminated sediment, Gujarat, India.	300 ppm phenanthrene	cell suspension (4%, v/v)/ 37 °C/ 150 rpm/72 h.	Strain was able degrade 86% phenanthrene.
Sphinogmonas sp.	Typical mangrove swamp(surface sediment (0–2 cm)), Ho Chung, Hong Kong.	5000 mg L-1 phenanthrene.	180rpm/ 7 days.	Strain was obta to degrade 99.4 phenanthrene at the end of 7 days.
Stenotrophomonas sp. IITR87	—[1]	phenanthrene(10ppm), pyrene(10ppm), and benzo-α-pyrene(10ppm).	200 µl of cells suspension(in 25 ml minimal medium)/ 30 °C/ 175rpm/15 days.	Strain showed 9 98 and <50% degradation of phenanthrene, pyrene, and be α-pyrene respectively.
Streptomyces sp. (ERI-CPDA-1)	Oil contaminated soil, Chennai, India.	naphthalene(0.1%), phenanthrene(0.1%).	3%, v/v cells suspension/30 °C/ 200rpm/7 days.	Strain could rer 99.14% naphthalene an 17.5% phenanthrene.

		Aspergillus 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% v/v cells suspension/30 °C/ 120rpm/7 days.	Biodegradation efficiencies of c oil, naphthalene phenanthrene, pyrene were 84 50.3%, and 55. respectively.
		Nocardia sp. H17-1	Oil-contaminated soil	aliphatic and aromatic(1%, w/v).	30 °C/6 days.	The aliphatic ar aromatic fractio were degraded ± 0.1% and 23. 0.8%, respectiv
	fungus	*Penicillium* sp. CHY-2	Soil, Antarctic.	100 mg L−1 butylbenzene, naphthalene, acenaphthene, ethylbenzene, and benzo[a]pyrene.	20 °C/110rpm/28days.	Strain showed t level of degrada for butylbenzen (42.0%), naphthalene (15.0%), acenaphthene (10.0%), ethylbenzene (4.0%), and benzo[a]pyrene (2.0%).
		Trichoderma sp.	—[1]	100 mg kg-1 pyrene and benzo(a)pyrene.	240 h	Strain degraded 63% of pyrene(mg kg-1) and 3 of benzo(a)pyrene mg kg-1) after 2 of incubation.
		Fusarium sp.	—[1]	100 mg kg-1 pyrene and benzo(a)pyrene.	240 h	Strain degraded 69% of pyrene(mg kg-1) and 3 of benzo(a)pyrene mg kg-1) after 2 of incubation.

alkanes		*Achromobacter* sp.HZ01	Crude oil-contaminated seawater, China.	2% (w/v) diesel oil	28 °C/150 rpm/10 days.	Strain degrade total n-alkanes reached up to 96.6%.
		Acinetobacter sp. *(KC211013)*	Coal chemical industry wastewater treatment plant, northeast China.	700mg/L alkanes.	35˚C	The degradatio rate reached 5
	bacteria	*Bacillus subtilis*	Petroleum-polluted soil, Shengli Oilfield, China.	0.3% (w/v) crude oil.	6%(v/v) cells suspension/30 °C/ 150rpm/5 days.	The results indicated that 3 80% of the n-alkanes (C13–C were degraded strain.
		Pseudomonas sp. WJ6	Xinjiang oilfield, China.	0.5% (w/v) n-alkanes.	1010 CFU ml-1/ 37 °C/ 180 rpm/20 days.	N-dodecane (C was degraded b 46.65%. 42.62% 31.69% and 23.62% of C22, and C40 were degraded, respectively.
		Rhodococcus sp.	Bay of Quinte, Ontario, Canada.	0.1% (v/v) diesel fuel.	OD600=0.025/ 0°C/ 150 rpm/ 102 days.	After 102 days incubation at 0° strain mineraliz C12 (8%), C16 (6.1%), C28 (1. and C32 (4.3%)
	fungus	*Cladosporium resinae*	Soil, Australian.	12.5%(v/v) n-alkanes.	0.3-0.5ml cells suspension(in 40ml minimal medium)/ 35°C/ 35 days.	All higher n-alka from n-nonane octadecane we assimilated by t fungus.
		Penicillium sp. CHY-2	Soil, Antarctic.	100 mg L^{-1} decane, dodecane and octane.	20 °C/110 rpm/28 days.	Strain was degraded decan (49.0%), dodec (33.0%) and oc (8.0%).

actinomycetes	*Gordonia* sp.	Hydrocarbon-contaminated mediterranean shoreline, west coast of Sicily, Italy.	1 g L^{-1} eicosane and octacosane.	30 °C /28 days.	Eicosane and octacosane wer degraded from to 99% in 28 da
	Tsukamurella sp. MH1	Petroleum-contaminated soil, Pitești, Romania.	0.5% (v/v) liquid alkanes.	30 °C	Strain capable t use a wide rang n-alkanes as th only carbon sou for growth.

[1] There is no clear description in the entry.

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 [104]. The order 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 [105]. After 45 days of degradation of asphaltenes by *Bacillus subtilis* and *Pseudomonas aeruginosa*, the methylene content in asphaltenes decreased by 14% and 8%, respectively [105]. *Pseudomonas aeruginosa* can degrade 63.8% of n-hexadecane within 60 days [106]. Most of the microbial degradation of petroleum pollutants experiments remain in the laboratory tests in the mineral basal medium (liquid)(as shown in Table 1), and lack of application in actual petroleum contaminated soil. At the same time, a single bioremediation technology faces challenges such as long repair time, unstable microbial activity and poor degradation of free microorganisms. Therefore, the combined microbial methods (synergistic repair involving microorganisms in the degradation process) is used to improve degradation effect and practical applicability.

5. Combined microbial methods remediation

The microbial combination method is mainly summarized into three categories: microorganism-physical, microorganism-chemical, and microorganism-biology. Many materials and methods have been used in microbial combined method to degrade petroleum-contaminated soil (Table 2). Due to the hydrophobicity and fluidity of petroleum, most remediation combined methods are designed to improve microbial activity and aeration of contaminated soil. Therefore, electric field, nutrients, biocarrier, biochar, biosurfactants and plants were added to the petroleum-contaminated soil to improve the degradation rate of the system [107][108][109]

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

Methods	Materials	Main findings			Reference
		Substrate concentration	Incubation conditions	Degradation rate	

	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 biochar/ 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 10000 mg/kg(the US EPA clean up standard).	[107]
	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).	1,000 g soil/ 2% (w/w) biochar/ 60% water holding capacity/ C:N:P ratio 100:10:5/ 80 days.	TPH removal rate was 84.8%.	[110]
	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%.	[108]
microorganism-physical	β-cyclodextrin	1,000mg/kg PAHs	1.5, 3.0, 5.0 mmol/kgβ-cyclodextrin/ 25 °C.	Compared with the co-metabolism of glucose, the addition of β-cyclodextrin more strongly enhanced oil remediation in soil.	[111]
	bulking agents (chopped bermudagrass-hay/sawdust/vermiculite) aeration (tillage/forced aeration).	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%.	[112]
	biocarrier(activated carbon/zeolite)	49.81 mg g−1 TPH	800g 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.	[113]

	biostimulation	19.8 ± 0.38 g kg−1 TPH	0.8 kg soil/ 108 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.	[114]
microorganism-chemical	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% (w/w) 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.	[109]
	permanganate/activated persulfate/modified-Fenton/Fenton	263.6 ± 73.3 and 385.2 ± 39.6 mg·kg−1Σ16 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 (81.5%–86.54%) > modified Fenton (81.5%–85.4%) > Fenton (54.1%–60.0%).	[10]
	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.	[115]

microorganism-biology					
	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%.	[116]
	Medicaga sativa	30%(40% TPH oily sludge)+70% non-pollution soil.	1kg soil/ N:P 10:1/ 75-80% moisture content/ 60 days.	Consortium degraded more than 63% TPH.	[117]
	Medicaga sativa/vicia faba/Lolium perenne	1.13% TPH.	2kg 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.	[118]
	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%.	[119]
	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%.	[120]
	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.	[121]

earthworms (Eisenia fetidaAllolobophora chlorotica/Lumbricus terrestris)	10,000 mg/kg TPH	1000 g soil/ ten adult worms per container/ 28 days.	The TPH concentration decreased by 30–42% in samples with L. terrestris, by 31–37% in samples with E. fetida, and by 17–18% in samples with A. chlorotica.	[122]

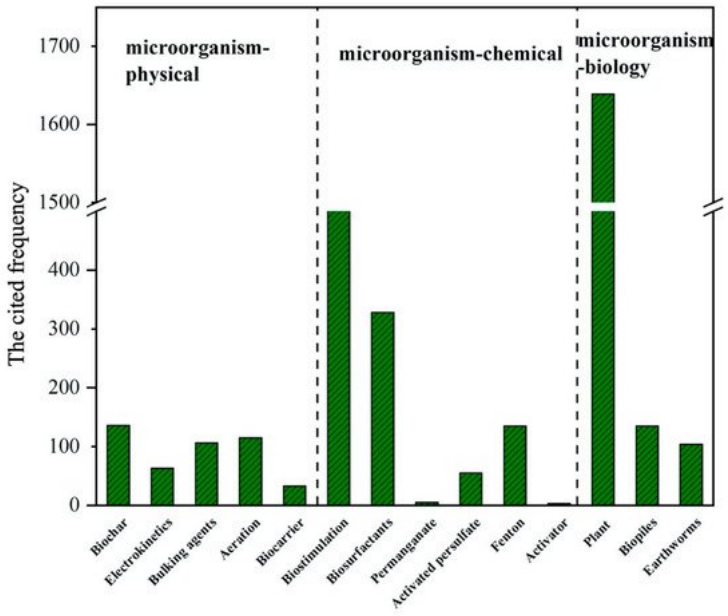

Figure 4. The frequency of citations of the article with three microbial combined methods for remediation of oil contaminated soil.

Through six databases in “web of science”, the time limit is limited to 2016-2020. The specific search terms results are “Microbial biochar(electrokinetics/ bulking agents/ aeration/ biocarrier/ biostimulation/ biosurfactants/ permanganate/ activated persulfate/ fenton/ activator/ plant/ biopiles/ earthworms) remediation of petroleum contaminated soil”. The search time is September 17, 2020, and the results are statistically analyzed. Fig. 4 shows the highest citation frequency of articles using the three combined microbial methods to remediate petroleum contaminated soil from 2016 to 2020. The compiled data indicate that microorganism-biochar, microorganism-nutrients and microorganism-plant combined microbial methods have been widely adopted for hydrocarbon degradation in current research.

5.1. Microorganism-biochar interactions in remediation of hydrocarbons

Biochar has a high carbon content, strong adsorption capacity, good stability, and the best immobilization capacity for bacteria and nutrients. The porous structure of biochar can provide attachment sites and suitable habitats for the survival of microorganisms. The addition of biochar of different properties to the soil is conducive to the enrichment of specific functional groups of microorganisms and the enhancement of biological activity [123][124][124][124][124][124][124][124][124][124][124][124][124][124][124][125]. The functional groups on the surface of biochar, easily decomposable carbon source and nitrogen source help to improve the activity of microorganisms and affect the growth, development and metabolism of microorganisms. Using biochar to immobilize microorganisms with different functional characteristics can strengthen the release of some nutrients in the soil and improve the degradation efficiency of pollutants. Studies have shown that biochar can absorb pollutants in petroleum, thereby reducing soil toxicity, and has no obvious negative impact on soil microorganisms [112]. In addition, the joint input of biochar and petroleum-degrading bacteria improves the diversity of microbial populations and the bioavailability of hydrocarbons [126].

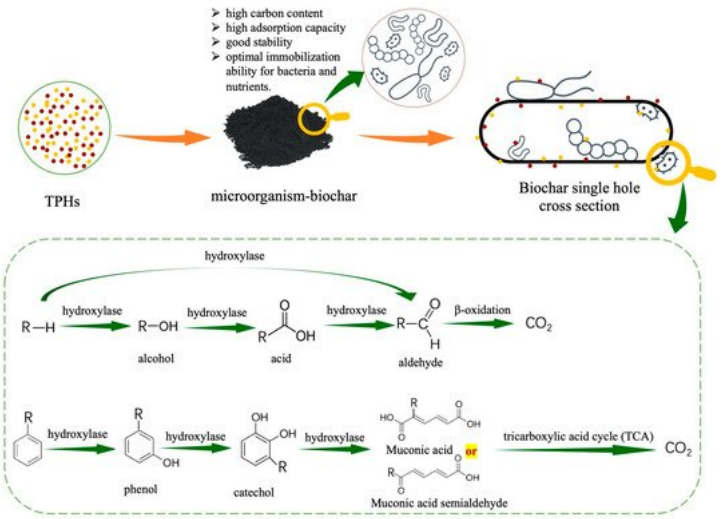

Figure 5. Proposed mechanism for the microbial metabolization of alkanes and aromatic hydrocarbons.

Fig. 5 outlines the general interactions that occur in the microorganism-biochar remediation of contaminants. Microorganism-biochar remediation mechanism can be divided into the adsorption, biodegradation and mineralization or a combination of these three methods. Due to the large specific surface area and rough surface structure of biochar, it is beneficial to the attached microorganisms to secrete biofilm, which increases the adsorption and degradation rate of hydrocarbons, and also increases the abundance of soil and active microorganisms. At the same time, studies have shown that fixed bacteria can use carbon chains more widely than free bacteria, and the removal rate of hydrocarbons has increased by about 21%-49% [126].

5.2. Microorganism-nutrients interactions in remediation of hydrocarbons

The input of a large amount of carbon sources (petroleum pollutants) often leads to the rapid depletion of the available pools of the main inorganic nutrients (such as nitrogen(N) and phosphorus(P)) in the soil, while the essential nutrients (such as N, P and terminal electron acceptors (TEA), etc.) is a key factor in reducing the rate of microbial metabolism [88]. Although the microorganisms in the soil show obvious pollution remediation potential, the lack of essential nutrients or the lack of stimulation of the degradation metabolic pathways leads to the inhibition or delay of microbial remediation. Therefore, it is necessary to add nutrients from external sources to stimulate the biodegradation of inorganic pollutants [127].

If the soil environment is anaerobic for a long time and the carbon content of the pollutant is high, the metabolism of denitrifying bacteria in the soil will reduce the total nitrogen content of the soil, thereby limiting this nutrient [128]. Studies have shown that the content of ammonium nitrogen (NH4+-N) and phosphorus (PO43−-P) in the soil drops rapidly after 15 days of restoration [129]. Nitrate has a significant advantage in increasing the potential of soil biodegradation of organic pollutants. Adding N to nutrient-deficient samples with rich hydrocarbons can increase the rate of cells growth and hydrocarbon degradation. Because nitrate has thermodynamic advantages as TEA, it participates in the assimilation and/or dissimilatory reduction process under oxygen limitation and anaerobic conditions, which promotes the heterotrophic or autotrophic denitrification process and simultaneously oxidizes organic matter (especially alkanes) [130]. At the same time, in the terrestrial underground environment, the phosphorus content is very low. Although some areas contain a lot of apatite, it cannot be used by biology. Several inorganic and organic forms of phosphate have been successfully used to stimulate environmental pollution [131]. Therefore, the addition of nutrients nitrogen and phosphorus contributes to the effective oxidation of carbon substrates and accelerates bacterial growth and hydrocarbon catabolism[88]. At present, the best C:N:P for efficient biodegradation of petroleum hydrocarbons has been reported as 100:10:1 [132].

5.3. Microorganism-plant interactions in remediation of hydrocarbons

The microorganism-plant combined method is the most common method for in-situ remediation. Studies have shown that in phytoremediation, organic pollutants are mainly mineralized by plant-related microorganisms. It has also been suggested that the remediation potential of plants depends to some extent on the number of bacteria in their surrounding environment [133].Therefore, in the process of remediation of pollutants, the synergy between plants and microorganisms accelerates the degradation and mineralization of pollutants. Plants and microorganisms have special enzymes and other substances, which can convert many toxic and complex chemical substances into simple and less toxic compounds. This process is conducive to their growth under polluted conditions. Plant rhizosphere can provide nutrients for microorganisms, oxygen and provide space for their attachment and growth [134][135]. These microorganisms increase the surface area of plant roots, allowing the roots to contact the soil and obtain more nutrients necessary for plant growth. Therefore, the inoculated bacteria are more concentrated in the soil around the roots of the vegetation [136]. At the same time, plant root exudates can stimulate the degradation process of microorganisms by changing the composition of the microbial community and improving microbial activity[137].

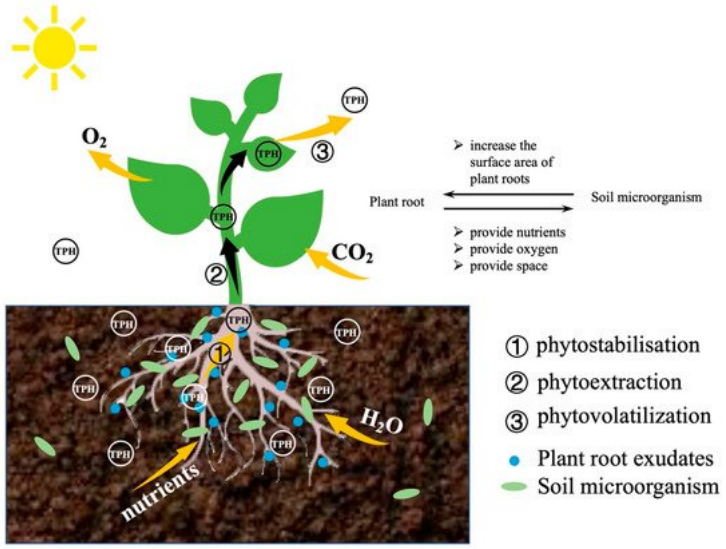

Figure 6. The general interactions that occur in the microorganism-plant remediation of contaminants.

Studies have shown that plants such as *Merr.,Setaria viridis Beauv.*, *Plantago asiatica L.*, *Phragmites communis*, *Medicago sativa*, *Festuca elata Keng ex E.Alexeev* and *Lolium perenne L.* are suitable for the climate and environment in China, and these are candidates for phytoremediation of petroleum-contaminated soil in China [138]. After 90 days of repairing petroleum-contaminated soil by *Festuca elata Keng ex E.Alexeev*, the petroleum removal rate is about 64% [139]. *Festuca elata Keng ex E.Alexeev* can not only effectively remove benzopyrene from the soil [140], but its growth also promotes soil biological activity in saline-alkali areas with petroleum pollution [52]. Fig. 6 outlines the general interactions that occur in the microorganism-plant remediation of contaminants.

Microorganism-plant remediation mechanism can be divided into the degradation, extraction, inhibition and a combination of three methods. Roots not only provide oxygen to rhizosphere bacteria through respiration, but also promote the secretion of root exudates and the degradation of rhizosphere pollutants [141]. Subsequently, through the expression of special enzymes(such as nitroreductase, dehalogenase, laccase and peroxidase etc.), plants and strains degrade hydrocarbons into simpler organic compounds [142]. Some pollutants are adsorbed on the root surface and accumulate in the root through the hemicellulose of the plant cell wall and the lipid bilayer of the plasma membrane [143]. Part of the pollutants are absorbed by phytoextraction/plant transfer to the upper part (stems and leaves) of plants [144]. Finally, some pollutants are released into the atmosphere through phytovolatilization [145]. Due to the self-protection mechanism of plants, some plants restrict the transportation of hydrocarbons from the roots to the ground, so that more hydrocarbons are retained in the root tissues. This restriction protects the chlorophyll and other nutrient synthesis systems of plants and ensures the normal operation of photosynthesis [144]. This is to ensure the normal photosynthesis activities of plants to generate more energy for survival and repair process.

6. Advantages and challenges in combined microbial methods application for hydrocarbon removal

With the exploitation of petroleum, the release of toxic pollutants in the soil environment has increased dramatically. Bioremediation has the characteristics of convenient operation, economic feasibility and no secondary pollution etc., which is currently a research hotspot for remediation of oily soil[64]. The combination of biochar, nutrients and plant with microorganisms not only increases the biological stability and activity of microorganisms, but also improves the ability of microorganisms to degrade petroleum pollutants. Three combined microbial methods have the following advantages. These methods will not damage the soil environment, physical, chemical and biological properties, and even better than the original properties after restoration. In addition, they can degrade organic pollutants into completely harmless inorganic substances (carbon dioxide and water) without secondary pollution problems.

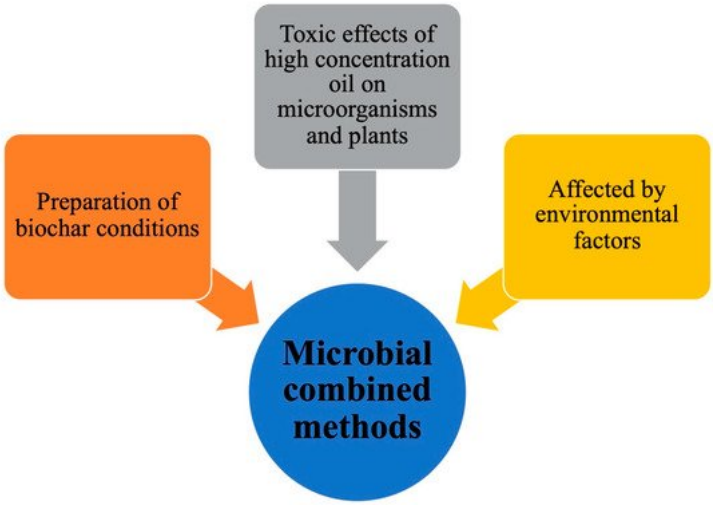

Figure 7. Some challenges of three microbial combined methods.

At present, the three repair methods only stay in the laboratory stage, and there are few strains used in engineering repair. Many influencing factors and degradation mechanisms are not yet clear, so further research is needed. Some challenges of three combined microbial methods are summarized in Fig. 7. The long-term stability and tolerance of biochar is one of the challenges faced by microorganism-biochar combined restoration. The high temperature pyrolysis biochar has a lower H/C ratio, which leads to enhanced electron donor-acceptor interaction, which makes the adsorption efficiency of biochar higher [146]. However, changes in environmental conditions (such as pH) and the abiotic or biodegradation of biochar will promote the desorption of PAH from biochar to sediment.

The ability of strains and plants to metabolize petroleum pollutants is the main challenge for microorganism-nutrients and microorganism -plants combined remediation. Excessive petroleum pollution in contaminated soil will negatively affect the growth and metabolism of microorganisms and plants, and soil environmental factors(temperature, humidity and pH) also affect the ability of microorganisms and plants to metabolize pollutants.

7. Conclusions

This entry outlines the method of remediation of petroleum-contaminated soil by the combined microbial methods. Although a combination of microorganisms-biochar/nutrients/plants can be used to remediate petroleum-contaminated soil to remedy the problems of a single remediation, there is no single method that is most suitable for all types of pollutants and various specific location conditions that occur in the affected environment. Therefore, it is necessary to construct an effective joint remediation technology based on the physical and chemical properties of soil at different contaminated sites and the types of pollutants. And study the migration, distribution, degradation mechanism of pollutants in the combined system, and the interaction and relationship with microorganisms. Select specific remedial measures by clarifying the internal and external factors that affect the restoration. Therefore, constructing a microbial joint remediation technology with a wide range of degradable pollutants, high degradation efficiency, strong stability and eco-friendly is the best choice for the current petroleum-contaminated soil remediation technology.

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