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Progress and Prospect of Bioremediation Technology for Petroleum and Petrochemical Contaminated Sites

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Abstract: While petroleum resources provide a steady stream of power for economic and social development, they also result in a large area of oil and petrochemical contaminated sites, causing increasingly serious pollution to the environment of the sites. Bioremediation technology has attracted extensive attention due to its advantages of being green, environment-friendly, and secondary-pollution-free. This review mainly summarizes the current status and progress of research on bioremediation technology based on microbes and phytoremediation of petroleum and petrochemical contaminated sites, and analyzes limitations of bioremediation technology and prospects for future research focus, aiming to improve the development of bioremediation for petroleum and petrochemical contaminated sites, and provide a scientific basis for the development of bioremediation technology and its all-round applications.

Keywords: Petroleum and petrochemical contaminated site; Soil pollution; Bioremediation; Microbial remediation; Phytoremediation

In the past decades, due to the acceleration of industrialization and urbanization, china and other countries with rapid economic development are facing increasingly serious soil pollution problems [1]. Organic pollutants such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), phthalate esters (PAEs) and polycyclic aromatic hydrocarbons (PAHs) are characterized by high toxicity, persistence and bioaccumulation in the environment [2]. Their pollution in soil has attracted more and more public attention. As an important reservoir of organic pollutants, soil is also the emission source of air and water pollution [3-4]. Under normal ecological conditions, most organic pollutants in the soil are difficult to biodegrade. Their residues in the soil can enter the food chain through animals, plants and microorganisms, and eventually pose potential risks to human health through nutrition transfer [5]. Oil is known as the "blood of industry" and an important power fuel for the development of industrial society. Oil and its products are not only necessities for people's livelihood, but also important materials for modern industry, agriculture and national

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defense. As early as the early 20th century, countries all over the world began the competition of oil exploitation and resource plundering. During the exploitation of oil, due to poor management, equipment defects, high pressure, blowout, pipeline rupture, tank oil leakage and other accidents, as well as the landing crude oil, oil sludge and waste mud in various stages, large areas of oil contaminated sites will inevitably be generated [6]. The petroleum pollutants in the soil of oil contaminated sites mainly include organic pollutants such as c15~c36 alkanes, pAHs, olefins, benzene homologues and phenols [7]. Petroleum pollutants cause significant changes in soil physical and chemical properties, affect soil pH, permeability, bulk density, oxygen, minerals and nutrient content, reduce soil permeability, hinder the transportation of water, oxygen, minerals and nutrients, reduce soil fertility and hinder plant growth [8]. At present, the remediation methods for oil contaminated sites are mainly divided into biological remediation, chemical remediation, physical remediation and physical-chemical remediation, mainly including phytoremediation [9], microbial remediation [10], chemical oxidation [11], photocatalytic degradation [12], electrokinetic remediation (ER) [13], incineration [14], thermal desorption [15], solvent extraction [16], steam extraction [17] and bioelectrochemical remediation [18]. Among them, bioremediation has been widely studied because of its green, practical, low cost, easy in-situ repair, no secondary pollution and other advantages. It is an environmentally friendly remediation method [19]. Because a single bioremediation has certain limitations in the actual repair process, the Combined Bioremediation Technology Based on microorganism and phytoremediation has gradually become the focus of bioremediation technology research.

1 Microbial based combined remediation technology

Microbial remediation of oil began to be used in the 1940s, and became popular after the Exxon Valdez oil spill in the 1980s [20]. Petroleum pollutant degrading microorganisms use carbon compounds as energy sources to grow and reproduce. Microbial remediation of petroleum hydrocarbon degradation by selected microorganisms has attracted extensive research. Some of the most common petroleum hydrocarbon degrading bacteria, such as Pseudomonas, rhodococcus, alcaligenes, ralstonia solanacearum, acinetobacter, nocardia, vibrio and Achromobacter, can effectively degrade petroleum hydrocarbons into simpler compounds. In addition, fungi such as Penicillium, fusarium and Rhizopus have been isolated and used for bioremediation of petroleum hydrocarbon contaminated soils and sediments. Current research shows that more than 200 microorganisms belonging to 100 genera of bacteria, fungi, actinomycetes, yeasts and molds can effectively degrade petroleum hydrocarbon pollutants [23]. After years of research, combined with the actual situation of contaminated sites, microbial based remediation technology has gradually developed and become more and more perfect.

1.1 Synergetic combination technology of degrading microorganisms

Because a single microorganism does not have enough metabolic capacity to efficiently degrade all oil components, people tend to pay attention to the combined degradation technology of various types of bacteria, fungi and other microorganisms for the remediation of oil contaminated soil. Utilizing the synergy of multiple microorganisms, that is, multiple microorganisms promote, strengthen and work together to improve the remediation efficiency of oil contaminated sites. Varjani et al. [24] conducted a field test on the in-situ synergistic degradation of petroleum hydrocarbon pollutants by the hydrocarbon utilization flora composed of six bacteria at the oil development pollution site in India, and achieved a removal efficiency of 83.7% within 75 days. COVINO et al. [25] degraded the clay soil polluted by petroleum hydrocarbons by using the local fungi *in* the soil polluted by petroleum hydrocarbons and adding Pseudomonas sp for enhancement. After 60 days, the removal efficiency reached 79.7%. Ramadass et al. [26] introduced Pseudomonas putida tphk-1 or Pseudomonas aeruginosa tphk-4, a bacterial strain that degrades petroleum hydrocarbon pollutants, to treat the soil of oil contaminated sites in South Australia. The results show that the two bacteria have obvious biological synergistic effect. Qu Yandun et al. [27] screened efficient degrading bacteria Pseudomonas aeruginosa and colourless bacilli from the oil sludge respectively, and studied the degradation law of crude oil by their independent use and combined use. The results showed that the degradation rate of soil oil pollution by microbial synergy was higher than that by single use. It can be seen that the synergistic combination of multiple degrading microorganisms has greatly improved the bioremediation efficiency of oil contaminated soil, and made up for the shortcomings of poor applicability, low degradation efficiency and poor pollutant selectivity of a single degrading microorganism.

1.2 Microbial physical combination technology

Physical remediation is a widely used soil remediation technology, which uses single or mixed solvents to extract and remove pollutants from soil. The effectiveness of extraction depends on the close contact between soil and solvent mixture. Research shows that the solvents for removing petroleum from soil include a variety of organic solvents, surfactant assisted aqueous solutions, supercritical and subcritical fluids [28]. The main disadvantages of physical extraction include high operating cost and secondary pollution caused by the solvent used.The combination of microorganism and physical remediation technology can not only improve the remediation efficiency, but also effectively reduce the risk of secondary pollution. Wu et al. [29] evaluated the efficiency and sustainability of the combined technology of solvent extraction and microbial degradation for the remediation of oil contaminated soil, indicating that the solvent extraction pretreatment reduced the concentration and toxicity of TPH in the soil, thus providing a more favorable environment for the application of microbial remediation technology. After solvent extraction for 15 min, about 90% of the pollutants were removed. After 132 days of follow-up bioremediation, the oil pollutants were further reduced to 97%. Therefore, this combined method has high efficiency and sustainability, and shows good performance in the remediation of high concentration weathered hydrocarbons. Yan et al. [30] studied the removal efficiency of four soil washing methods (water washing, surfactant washing (Tween80), bioremediation + water washing and bioremediation + surfactant washing) on oil pollutants in oil contaminated sites, and found that surfactants promoted the transportation and delivery of microorganisms, improved the bioavailability of pollutants, and thus improved the bioremediation efficiency. In general, surfactant flushing combined with microbial remediation is a promising remediation strategy for oil contaminated sites. In addition, the development of environmentally friendly solvents and the use of biosurfactants or non-ionic surfactants are the development trend of microbial physical combination technology. Biosurfactant is a kind of green solvent that can cause biosafety. Because of its low toxicity, it is easy to extract from renewable resources and may be reused through regeneration.

1.3 Degrading microorganisms - chemical enhancement technology

Microbial remediation has the advantages of green, safe, sustainable and no secondary pollution, but it has some limitations in practical application due to its low treatment efficiency and long treatment cycle. Microbial remediation combined with chemical technology can not only reduce secondary pollution but also improve remediation efficiency. It is a common joint remediation technology in the remediation of oil contaminated sites. Lu et al. [31] studied the method of removing oil from soil by microbial degradation after Fenton like pretreatment. The results show that when the optimal volume ratio of H_2O_2: Fe^{3+} is 300: 1, the removal rate of Fenton like wastewater can reach 67.3%. After 10 W of microbial treatment, 50.6% of the pollutants were removed. The total oil removal efficiency of the combined remediation technology was about 83.5%. Pretreatment with chemical oxidation method not only destroys the target compounds, but also reduces the overall toxicity of pollutants to microorganisms in the later biodegradation process. Kim et al. [32] have shown that adding H_2O_2 will not damage the biodegradation process, and this combined technology can effectively remove pollutants from contaminated sites. Gong et al. [33] first carried out microbial degradation on the oil contaminated site to repair the weathered crude oil contaminated soil, and used additional nutrients and peanut shells as fillers to carry out microbial remediation for up to 8 W, and then carried out chemical enhancement with improved Fenton oxidation technology, adding H_2O_2 directly to the soil for oxidation. The removal of pollutants in the bioremediation phase showed a removal efficiency of 38.6%, and then increased to 88.9% by adding H_2O_2.

1.4 Degradation microorganism electrochemical enhancement technology

Microbial remediation is the most commonly used remediation technology for contaminated soil because of its low cost and convenience. However, microbial remediation has some limitations, including ecological conditions, electron receptors and nutrients, the nature of pollutants, and the growth of microorganisms and metabolism [34]; electrokinetic technology can solve these limitations and improve treatment efficiency, i.e. transfer of pollutants, nutrients and microorganisms through electroosmosis, electrophoresis and electromigration processes [35]. In recent years, electrokinetic soil flushing – bioremediation (EKSF–Bio), which is the combination of electrokinetic flushing and bioremediation, has attracted the attention of many researchers. This technology mainly uses electric technology to increase the biodegradation rate of microorganisms in soil pores, and the use of solubilizers such as surfactants can improve the biodegradation process. For example, prakash et al. [36] showed that the electrokinetic biodegradation efficiency of crude oil by Bacillus subtilis AS2, bacillus licheniformis AS3 and Bacillus AS4 was 88%, 92% and 97% respectively. Adding biosurfactants to crude oil dissolves them, making it easier for bacterial strains to break them down. The combined remediation of eksf bio and surfactant can be used to repair the soil contaminated by crude oil in an eco-friendly way.

In recent years, the research group has also taken the lead in developing a new energy-saving bioremediation process, called bioelectrochemical system (BES), also known as microbial electrochemical system, to enhance and accelerate the remediation of oil contaminated soil [18]. BES provides two redox reactions (oxidation and reduction) to form an integrated microbial electrochemical removal mechanism, which has high removal efficiency even for complex pollutants [37-38]. Lu et al. [39] showed that the application of BES in a 50 L pilot scale reactor can repair diesel contaminated soil, with a maximum removal efficiency of 89.7%. At present, the remediation of oil contaminated sites by bioelectrochemical system is still in the exploration stage. Due to the complexity and diversity of this technology, more process development is needed to apply it to the in-situ remediation of oil contaminated soil [40].

2 Combined remediation technology based on green plants

Phytoremediation of oil contaminated sites is a promising bioremediation technology. This technology uses plants and their root related microorganisms to fix and degrade petroleum pollutants in soil, so as to achieve the role of soil remediation [1, 40]. Phytoremediation for oil contaminated sites has many advantages, mainly including green and safe, no secondary pollution, easy on-site repair, low maintenance costs.At present, many plants are used for the restoration of oil contaminated sites, such as arthrodendron, barley, tall sheep spear, lilac tree, salt grass, wild Iris and iris [40-45]. The traditional phytoremediation technology has the disadvantages of low efficiency and poor environmental applicability, so the combination technology of green plants and other remediation technologies has gradually become the research direction and focus.

2.1 Green restoration plant combination technology

Green phytoremediation is a potential green technology solution, but due to the diversity and complexity of contaminated soil, a single green phytoremediation may be challenging. By using the unique repair functions of different plants, a variety of repair plant combinations have the possibility to repair all kinds of polluted soil and can improve the efficiency of soil repair. Brereton et al. [45] have shown that there are more species of rhizosphere related bacteria in CO cultivation of two plants than in single plant cultivation. These bacteria jointly promote the growth and development of degrading plants, thus improving the repair efficiency of CO cultivation plants. Dacunha et al. *[47]* studied *the* remediation of oil contaminated soil by CO planting Salix Rubens and Salix triandra. In the contaminated soil planted by the two plants, the total hydrocarbon concentration decreased by nearly 98% and the PAHs content also decreased significantly. Desjardins et al. [48] also proved that the co cultivation of multiple plants makes their biological characteristics complementary, thus bringing higher phytoremediation efficiency.

2.2 Collaborative combination technology of green remediation plants and degrading microorganisms

The collaborative remediation of contaminated soil by green plants, bacteria, fungi and other microorganisms promotes the absorption of pollutants by plants to a certain extent, which is also one of the most important research directions in the field of phytoremediation [1, 40, 49]. The addition of microorganisms can not only degrade petroleum pollutants directly, but also promote the growth of green plants, improve soil structure, reduce soil pH, and form environmental conditions conducive to the degradation of pollutants through their own metabolism. It has a good application prospect for the joint treatment of stone oil contaminated sites with green plants. In order to study the synergistic degradation and repair effect of green plants and Bacillus megaterium Bb-1, zhouqixing et al. [46, 50] added the strain to the benzo[a].pyrene (b[a].p) contaminated soil planted with purple jasmine through comparative study. In the contaminated soil not added with Bb-1, the degradation rates of b[a].p by purple jasmine at flowering stage and mature stage were (27.42 ± 1.99)% and (51.31 ± 3.06)% respectively; in the polluted soil added with Bb-1, the degradation rate of b[a].p increased to (68.22 ± 1.21)% and (77.16 ± 0.62)% respectively. It can be seen that adding Bb-1 can significantly improve the degradation efficiency of b[a].p in soil. Shangqiongqiong et al. [51] have shown that the remediation method of straw and microbial immobilization has a good synergistic remediation effect on oil contaminated soil, which not only accelerates the decomposition of straw, but also improves the activity of microorganisms, thus improving the degradation efficiency. Tangjingchun et al. [52] invented a ryegrass high-efficiency microorganism technology to repair oil contaminated saline alkali soil. The combined treatment of microorganism and ryegrass can play a good synergistic effect and improve the degradation effect of petroleum hydrocarbons. After 5 months of growth, the degradation rate of petroleum hydrocarbons can reach 57%. In recent years, the addition of plant growth promoting rhizobacteria (PGPR) to soil has aroused great interest. PGPR can help promote plant growth, reduce environmental pressure and enhance the degradation capacity of existing PGPR. In addition, pGPR can also improve the ability of plants to resist the toxicity of petroleum pollutants [53].

2.3 Physical / chemical enhancement technology of green restoration plants

The physical/chemical enhancement technology of green plants mainly includes adding nutrients, plant growth regulators, surfactants and other chemical solvents to improve the efficiency of phytoremediation. Contaminated soils usually have low nutrient levels and may hinder the growth of microorganisms and plants, thereby reducing the dissipation of pollutants [1, 40]. Therefore, adding fertilizer to balance nutrition can reduce the competition between plants and microorganisms, so as to improve the efficiency of rhizosphere remediation. The addition of nutrients can also improve the survival rate of plants in the soil polluted by petroleum pollutants and promote plant growth, so as to produce enough root biomass to promote the development of rhizosphere microorganisms and improve the efficiency of plant rhizosphere degradation of pollutants. Wang et al. [54] reported that applying compost could *increase* the degradation rate of *oil* pollutants in soil by 46% for L. perenne and M. sativa. In recent years, studies have shown the feasibility of using plant growth regulators to improve the effect of phytoremediation [55-56]. The effective plant growth regulators to improve phytoremediation mainly include auxin, gibberellin, cytokinin and salicylic acid. The activity of these substances depends on their concentration, the environmental factors that affect their absorption and the physiological state of plants. The use of these regulators can increase the biomass of plants and reduce the negative effects caused by the presence of pollutants in plants [1, 40]. The molecular structure of surfactants consists of both hydrophilic and hydrophobic parts. This unique structure can enhance the water solubility of soil pollutants, especially for some hydrophobic organic compounds, such as petroleum hydrocarbons and PAHs, so as to promote their absorption and utilization by plants [57]. Studies have shown that a variety of chemically synthesized or natural surfactants (such as anionic, cationic, zwitterionic and non-ionic surfactants) have been used to enhance the phytoremediation of petroleum pollutants [58]. Fadhile et al. [59] compared the phytoremediation performance of biosurfactants with hydrocarbon degrading bacteria, sodium dodecyl sulfate and bacterial culture supernatant on 2 g/kg gasoline contaminated soil. The results showed that the removal rate of biosurfactant was 93.5%, while the removal rates of bacteria, culture supernatant and sodium dodecyl sulfate were 85.4%, 70.3% and 86.3% respectively. The physical / chemical enhancement of green plants can also enhance the oxidation-reduction potential in soil by applying chelators and acidifiers to promote plant growth and improve phytoremediation efficiency [60]; nano materials can not only directly remove pollutants [40, 61], but also promote plant growth and increase the plant utilization of pollutants, playing an important role in phytoremediation systems [62]. Nanoscale zero valent iron is the most used nano material for phytoremediation [63-65]. Fullerene nanoparticles can also increase the plant utilization of pollutants. Generally, the use of nano materials to promote phytoremediation of contaminated soil is an effective strategy, but it is still in the exploration and trial stage [66]. As a soil conditioner, biochar has been paid more and more attention in the field of Phytoremediation in recent years. Biochar has the potential of long-term carbon fixation, reducing soil N2O emissions, increasing soil water and nutrient retention, and regulating soil pH [67], and can stimulate plant growth by promoting the reproduction of beneficial microorganisms [68].

2.4 Electrochemical enhancement technology for green restoration plants

It is found that applying an electric field near the green repair plants can promote plant growth and development, and enhance the repair ability of plants by moving nutrients and pollutants, making these nutrients and pollutants easier for plants to absorb [69-70]. Therefore, green remediation plants can improve the remediation efficiency of oil contaminated sites through electrochemical enhancement, which is a promising joint remediation technology [46]. Rocha et al. [70] studied the use of graphite electrode to repair oil contaminated soil and enhance the efficiency of phytoremediation through ER. The results showed that electric treatment can improve the bioavailability of nutrients and reduce evaporation flux to improve the efficiency of phytoremediation. Acosta santoyo et al. [69] found that applying 0.2 dcv/cm to the clean soil near ryegrass seeds can increase the germination rate by 75%, thus improving the remediation of contaminated sites by ryegrass. The existence of pollutants in soil hindered the germination and growth of ryegrass. The application of DC electric field was conducive to the germination and growth of plants, thus compensating for the negative effects of pollutants. In the electrochemical enhancement technology of green remediation plants, the removal or degradation of pollutants is completed by plants, while the electric field enhances the activity of plants by increasing the mobility and bioavailability of pollutants, so as to make up for the negative impact of pollutants on phytoremediation. Since the electric field effectively pushes more and more soluble heavy metals to the plant roots, resulting in the stress state of plants, the high accumulation plants with rapid growth period are considered to be the best choice for the combination of electrochemical technology [40, 70].

3 Outlook

As oil is still the main energy for economic and social development, a large number of oil exploration, exploitation and production are bound to produce a large area of oil and petrochemical contaminated sites. The remediation of contaminated sites has become a difficult problem in the field of soil remediation in China and even the world. Therefore, it is urgent to develop low-cost, efficient, sustainable and eco-friendly remediation technologies to deal with more oil contaminated sites.Due to the shortcomings of traditional remediation technology and poor environmental benefits, bioremediation technology has more advantages than physical and chemical methods. It is an environmentally safe and sustainable method, which has aroused widespread interest. A series of remarkable achievements have been made in the research on bioremediation of oil contaminated sites at home and abroad. These achievements provide theoretical basis, data support and technical support for promoting the development of bioremediation technology.However, bioremediation technology has some limitations in the actual remediation process of petroleum hydrocarbon contaminated sites. For example, different microorganisms and plant species can only degrade one or several specific pollutants, and the general applicability is not strong; the degradation of pollutants is not complete, the mineralization rate is not high, and the toxicity of some intermediates is even higher than that of the parent; site conditions and environmental factors have a great impact on the survival of microorganisms and plants and the efficiency of bioremediation.Therefore, the research and application of bioremediation technology need more in-depth discussion to overcome the limitations of biotechnology and achieve better remediation effect. Based on the current progress of bioremediation technology [1, 40, 70-71], the following suggestions are put forward in combination with the repair background and needs of practical application:

(1) Improving the level of joint restoration will become the focus of future research. On the one hand, improve the level of microbial plant joint remediation of microbial remediation and phytoremediation, and constantly improve the bioremediation technology to make its system more systematic and mature; on the other hand, how to effectively combine bioremediation technology with traditional physical and chemical remediation technology to improve the remediation efficiency of oil contaminated sites is still the focus of research. Bioremediation technology and traditional technology learn from each other, maintain the green and environment-friendly characteristics of bioremediation technology, and introduce the advantages of high efficiency and universal applicability of traditional remediation technology to pollutants, so as to achieve an organic combination of the two, give play to their respective advantages and jointly improve the remediation efficiency of oil contaminated

soil.

(2) The key of bioremediation technology is to cultivate and screen plants or microorganisms that can degrade petroleum pollutants. In the process of practical application, local plants and microorganisms in the polluted site should be taken as the research focus, and their bioremediation potential should be developed through bioremediation means and mechanisms; when it is necessary to strengthen the bioremediation effect by exogenous plants and microorganisms, the impact on other local biological communities and soil health should be considered to avoid systemic damage to the local ecosystem.

(3) Applying genomics technology to better understand the interaction between plants and their rhizosphere microbial communities can help us understand how complex organic compounds (such as petroleum hydrocarbons, benzene homologues, etc.) are metabolized and degraded in the soil system, and help to establish new and efficient bioremediation systems.

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