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# Current Status of Bioremediation Technology for Petroleum-Contaminated Soil

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**Abstract:** Leakage accidents occur frequently in the process of oil exploitation, storage and transportation. Effective treatment of contaminated soil has become the focus of social attention. In this paper, the problem of petroleum pollution and bioremediation methods are introduced. The key factors that restrict the bioremediation effect, such as screening and planting of degrading microorganisms and improving the bioavailability of petroleum hydrocarbons, are discussed.

**Keywords:** Petroleum pollution, soil, remediation technology, microorganism, bioremediation.

## 1. Introduction

As an important industrial raw material, petroleum plays a vital role in energy, materials, chemical industry and other fields. However, the abnormal leakage of petroleum in the process of exploitation, processing, storage and transportation will cause soil and water pollution, which will endanger human health through plants in the soil or drinking water. In April 2010, the Deepwater Horizon drilling platform in the Gulf of Mexico exploded and triggered a fire. Oil leaks at the bottom of the drilling platform continued. Half a month later, no effective remedial measures were available. The average daily oil leak was about 5,000 barrels, which caused huge economic and environmental losses to the United States [1].

After the soil is polluted by petroleum, the content of organic matter and carbon increases greatly, the nutrients such as nitrogen and phosphorus are seriously lacking, the change of soil pH value destroys the growth of indigenous microorganisms, and then affects the uptake of nutrients by surface crops, and destroys the local ecological environment [2]. At the same time, petroleum hydrocarbons with strong migration will migrate with soil moisture, through the soil aeration zone to the underground aquifer and pollute the groundwater, and then endanger human health with the transportation and use of groundwater. In addition, some refractory petroleum hydrocarbons can be accumulated in the soil for a long time, enter the human body through the food chain, affect the liver, kidney and cardiovascular system, and cause harm to human beings [3].

## 2. Research status of bioremediation of petroleum-contaminated soil

Soil bioremediation technology originated in the 1980s.Bioremediation technology has the advantages of simple operation, complete degradation of organic matter, no secondary pollution, in situ treatment and so on. It has attracted great attention from academia, industry and government departments in various countries. It is the frontier of the current research field of soil remediation technology, and also one of the preferred soil bioremediation technologies recommended by the United States Environment Agency. Phytoremediation means that plants can degrade organic pollutants in soil by directly absorbing and degrading organic pollutants and releasing various secretions to stimulate microbial activity, so as to enhance their biological transformation and strengthen the mineralization of organic pollutants in the root region of plants. This method has the advantages of low energy consumption, low cost, simple operation and conforming to the sustainable development strategy. It is suitable for remediation of large-scale contaminated soil. However, most plant roots concentrate on the surface of the soil, unable to remediate deep soil pollutants, and are greatly affected by season and environmental temperature, so phytoremediation technology has some limitations. Zhang[4] studied the degradation of petroleum hydrocarbons in petroleum-contaminated soils by alfalfa plus optimized microbial group inhibitors and regulating soil temperature and nutrients. After 99 days of restoration, the degradation rate of petroleum hydrocarbons in soils reached 95%.

Soil fauna play an important role in soil ecosystem and maintain the stability of soil ecological structure. Soil animals can break up, digest, absorb and transform some organic matter, and convert the pollutants in soil into manure with good performance. However, the organic species and content of soil animals are limited, which is suitable for slightly polluted soil. The activities of some animals in soil, such as earthworms, can promote the growth and transfer of microorganisms and make the effect of microbial remediation more obvious. Therefore, the combination of animal remediation and microbial remediation is more beneficial to the process of soil remediation.

Microbial remediation is widely used in bioremediation of petroleum contaminated soil. The method utilizes or enhances the ability of environmental microorganisms to metabolize and decompose toxic substances, removes toxic and harmful pollutants from soil, and decomposes organic pollutants into other harmless substances. Cao[5] enriched and isolated the dominant strain B-3 which can degrade diesel oil from soil samples polluted by diesel oil. It belongs to Tetra thio bacter kashmirensis. It was cultured in diesel oil medium with a mass concentration of 3.78 g/L, and the degradation rate was as high as 60.98%.The results provide a theoretical basis for bioremediation of diesel contaminated soil.

Recent studies have shown that in order to improve the degradation rate of pollutants in soil, a combination of various remediation techniques can be adopted. For example, the optimal combination of electro-remediation technology and bioremediation technology has become an efficient and environmentally friendly composite remediation technology. Li[6] used electro-microbial combined remediation technology to remove petroleum pollutants. After applying electric field, the number of oil-degrading bacteria increased, and the oil-degrading rate was 2.4 times that of the control group.

## 3. Classification and application of microbial remediation technology

Microbial remediation refers to the degradation of organic pollutants into carbon dioxide and water by microorganisms through their life metabolism, so as to achieve the purpose of remediation of contaminated soil. Microbial remediation technology can be divided into natural attenuation method, biological stimulation method, biological strengthening method [7] and enzymatic reaction method according to the types of preparations added to soil and the sources of microorganisms in the process of remediation. The classification, advantages and disadvantages of microbial remediation techniques are listed in Table 1.

Table 1 Classification and characteristics of microbial remediation techniques

| Method | Strain | Advantage | Shortcoming |
|---|---|---|---|
| Natural attenuation [8-9] | Protozoa or microorganisms in the environment | In the natural environment, there is no need to consume manpower, material resources and financial | The degradation ability is weak, and it takes a long time to ensure the repair efficiency. |

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|---|---|---|---|
| | | resources. | |
| Bio stimulation [10-12] | Indigenous microorganisms | It has no effect on soil physical and chemical properties and is easy to operate. | Refractory petroleum hydrocarbons still exist, which can not guarantee the repair efficiency and safety in the repair process. |
| Bioaugmentation [13] | A highly efficient degrading bacteria or microbial flora screened | Pollutants are degraded rapidly and thoroughly without secondary pollution to the environment. | Input of microorganisms is limited by the physical, chemical and biological characteristics of the soil environment. |
| Enzyme reaction | Extraction from microorganisms | Widening reaction conditions, accelerating degradation speed and efficiency | The operation is complex, and the current experimental research needs to be improved. |

### 3.1 Natural attenuation method

Natural attenuation relies on natural processes in soil or groundwater systems to remove or weaken pollution. When the environment is polluted by chemical substances, the natural attenuation of pollutants occurs in most polluted areas. However, due to the weak degradation ability of pollutants in the natural attenuation process, it usually takes 5 to 25 years to complete the purification of the environment [14].

### 3.2 Biostimulus

Biostimulus remediation technology strengthens the degradation of pollutants by degradation bacteria in indigenous microorganisms by regularly adding nutrients such as nitrogen and phosphorus to soil. Wang[15] studied the degradation of 13 kinds of PAHs in soil by adding $(NH_4)HPO_4$ to the soil polluted by polycyclic aromatic hydrocarbons (PAHs). After 13 weeks of aerobic culture, the content of each kind of PAHs decreased in varying degrees, and the degradation rates of 2 and 4 rings were the highest, 24% and 38% respectively. This is due to the high content of organic carbon in contaminated soil and the relative deficiency of N and P nutrient sources. After adding $(NH_4)HPO_4$, the growth conditions of microorganisms were optimized, and the activity of indigenous microorganisms was activated, thus promoting the degradation of PAHs. Recent studies have shown that the addition of organic and inorganic fertilizers can improve the degradation rate of petroleum hydrocarbons to varying degrees. Ayotamuno[16] applied fertilizer in situ addition method to remediate oil-contaminated agricultural soil. The degradation rate of petroleum hydrocarbons in treated samples reached 50%-65%, which was an effective method for petroleum hydrocarbon degradation. Margesin[17] studied the degradation of petroleum hydrocarbons by applying lipophilic and inorganic fertilizers. It was found that fertilizers could significantly improve the degradation rate of petroleum hydrocarbons and the activity of lipase. The higher the initial concentration of pollutants, the more significant the effect of fertilizers.

### 3.3 Bioaugmentation

Bio-enhanced remediation technology refers to the technology of adding efficient exogenous bacteria or flora to the pollution control system to improve the ability of biological remediation of pollutants, so as to achieve efficient removal of target pollutants. It has become a hot research topic in the field of bioremediation in recent years. Ma[18] used diesel oil as the sole carbon source, screened and isolated the bacteria q41c, q41e, z41b and z41h degrading diesel oil from crude oil contaminated soil of Qingyang and Zhongyuan oilfields in Gansu Province by enrichment culture and plate marking method, and found the most suitable pH value and temperature for their growth, which provided the bacterial reserve for in situ remediation of diesel-contaminated soil. A diesel degrading yeast KML was isolated from soil samples not contaminated by diesel oil in Cameroon by researchers of Zhejiang University of Technology[19]. It was identified as Yarrowia lipolytica. The degradation rate was 78% at suitable temperature, pH and nutrient concentration. GC-MS analysis showed that the bacteria mainly degraded C9-C25 long-chain hydrocarbons and a small amount of branched-chain hydrocarbons. Zhao[20] took the soil near the storage tank of Zhongcheng Satellite Linkage Gas Station soaked by diesel oil for a long time as the bacterial source and diesel oil as the sole carbon source for domestication. After domestication, the dominant strains for diesel oil degradation were isolated. Ammonium chloride, potassium dihydrogen phosphate and potassium hydrogen phosphate were used as nutrient sources, and the degradation rate was 47.8%. Zhao[21] inoculated thermophilic hydrocarbon-degrading bacteria screened in laboratory into soil samples contaminated by petroleum at stations 8-8 in Karamay Oilfield, Xinjiang, for indoor bioremediation experiments. The soil microecology and physical and chemical properties changed greatly at different stages of restoration. The activities of catalase and urease and dehydrogenase increased while the activities of urease and dehydrogenase decreased during the restoration process. After 75 days of

repairing, the degradation rate of petroleum hydrocarbons reached 56.31%.
The results showed that the strains with long stable growth period and high tolerance could be obtained by mutagenesis of the strains which were screened and separated to degrade petroleum hydrocarbons efficiently, so as to improve the degradation rate. Zheng[22] isolated two strains of highly efficient degrading petroleum bacteria W1 and W2 from contaminated soil of Qingshan Petrochemical Plant in Wuhan. They are fixed rod bacteria and Bacillus bacteria respectively. The suspension was placed in a sterilized culture dish and the mutagenic bacteria were obtained by irradiating 40 S with 30 W ultraviolet lamp at a distance of 30 cm. The experimental results show that the mutagenic bacteria are more and more efficient with the increase of petroleum concentration. In addition, by comparing the growth curves, we can see that the mutant strains have better genetic stability. Lu[23] has enriched and isolated bacteria that can efficiently degrade diesel oil from oil contaminated soil. It is identified as Rhodococcus sp. by morphological characteristics and physiological and biochemical characteristics. By using ultraviolet irradiation and adding lithium chloride mutagen, the strain was induced to degrade diesel oil efficiently, and its degradability was 16.9% higher than that before mutagenesis.

### 3.4 Enzyme reaction method

For some biodegradable petroleum hydrocarbon microorganisms, the degradation efficiency is not high because of their slow growth. Therefore, it is a feasible way to extract petroleum hydrocarbon degradable enzymes from microorganisms and construct enzymatic reactions. Lai[24] used diesel oil and petroleum as carbon source. After enriching and culturing deep seawater in the Indian Ocean, a strain of bacteria P40 with strong diesel degradability was isolated. It was identified that it had the highest similarity with Alcanivorax dieselolei B25T and A. dieselolei NO1A, and the similarity was 99.8%.Two fragments of alkane hydroxylase gene were cloned from the strain. It was preliminarily determined that the strain might be a deep-sea phenotype strain of diesel alkanobacteria. Catechol dioxygenase is an important enzyme catalyzing the cracking of benzene rings during the degradation of aromatic hydrocarbons in petroleum. The catechol 2,3-dioxygenase gene was amplified from Sphingomona ssp. B2-7 by primer design[25]. Wu[26] extracted catechol 2,3-dioxygenase gene from soil samples near the refinery and cloned it. Seven different gene sequences were obtained. The results showed that these genes might originate from Pseudomonas spp. in soil, and the abundance of these genes was related to the number of aromatic hydrocarbon-degrading bacteria. Therefore, the diversity of Degrading Genes in soil provided abundant resources for bioremediation. Hua[27] used phthalic anhydride to modify laccase which degraded polycyclic aromatic hydrocarbons in white rot fungi. Anthracene was used as model substrate to study its degradation effect. The results showed that compared with natural laccase, modified laccase had higher affinity to substrate, longer half-life, wider suitable pH and nearly twice higher degradation efficiency of anthracene.

## 4. Conclusion and Prospect

At present, microbial remediation of petroleum-contaminated soils is mainly at the stage of isolation, cultivation and degradation characteristics. The mechanism of degradation and survival mechanism is not fully understood, the characteristics of bacteria need to be improved, and the technological parameters need to be optimized. There is a big gap between theoretical research and practical application. Future innovative research can focus on the following aspects: (1) Strengthen the screening, breeding and research mechanism of high-efficient degrading bacteria, explore the degradation mechanism and survival mechanism of microorganisms; (2) Petroleum degradation is accomplished by the co-metabolism of multiple microorganisms, so we should strengthen the study of bacterial flora construction and find the appropriate mixing proportion of different degrading bacteria in order to achieve better petroleum. Removal effect; (3) Increase basic research and technological research and development for practical remediation projects to promote the engineering application of bioremediation technologies for petroleum-contaminated soils; (4) Focus on the cross-cutting and infiltration of related disciplines, pay attention to the use of new technologies, new methods and joint remediation technologies; (5) Strengthen the risk assessment of bioremediation of petroleum hydrocarbon-contaminated soils.

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