Status of Microbial Remediation Technology in Petroleum Contaminated Land

Longfei Xia¹,²,³,⁴
¹Shaanxi Provincial Land Engineering Construction Group Co., Ltd, Xi'an 710075, China
²Institute of Land Engineering & Technology, Shaanxi Provincial Land Engineering Construction Group Co., Ltd, Xi'an 710075, China
³Key Laboratory of Degraded and Unused Land Consolidation Engineering, the Ministry of Natural Resources, Xi'an 710075, China
⁴Shaanxi Provincial Land Consolidation Engineering Technology Research Center, Xi'an 710075, China
383034747@qq.com

Abstract. Leakage accidents occur frequently during the process of oil exploitation, storage and transportation. Effective management of the soil contaminated by it has become the focus of social attention. This paper introduces the oil pollution problem and physical, chemical and biological treatment methods. It focuses on the classification and application status of microbial remediation technology, and discusses the key factors that restrict bioremediation effects such as degrading microbial screening and colonization, and improving the bioavailability of petroleum hydrocarbons.

1. Introduction

As an important industrial raw material, petroleum plays a vital role in the fields of energy, materials and chemicals. However, the abnormal leakage of oil in the mining, processing, storage and transportation will cause soil and water pollution, and then the plant or drinking water in the soil will cause harm to human health, resulting in a series of economic, environmental and social problem.

Since the mining of Zhongyuan Oilfield in Puyang, Henan Province in 2002, more than 30,000 mu of cultivated land has been polluted due to drilling, oil recovery and underground operations. Among them, heavy polluted land accounts for 60% of the total pollution area. This not only brings huge economic losses to the oil field, but also has a very bad impact on the environment [1]. In 2004, Yan'an was polluted by hundreds of acres of farmland due to oil spills; in 2005, a large oil tanker in Qingdao had an accident at Huangzhang Road, and more than 40 tons of crude oil overflowed to cause large-scale pollution; in 2009, a diesel accident occurred in the Weihe River, and the leak occurred. The surrounding wheat fields are polluted by diesel oil. A large amount of diesel oil remains in the soil in the form of residual saturation. If it is not removed in time, it will cause groundwater pollution [2]. In April 2010, the Deepwater Horizon drilling platform in the Gulf of Mexico exploded and caused a fire. The oil well at the bottom of the drilling platform was leaking. After half a month, there was still
After the soil is polluted by oil, the organic matter and carbon content increase greatly, the serious lack of nutrients such as nitrogen and phosphorus and the change of soil pH value destroy the growth of indigenous microorganisms, which in turn affects the intake of nutrients by surface crops and destroys the local ecology. Environment [4]. At the same time, the highly mobile petroleum hydrocarbons will migrate with the soil moisture, pass through the soil aerated zone to the underground aquifer and pollute the groundwater, and then endanger the human health with the transportation and use of groundwater. In addition, some refractory petroleum hydrocarbon components, such as naphthalene, toluene, phenanthrene, anthracene, pyrene, etc., can accumulate in the soil for a long time, enter the human body through the food chain, and affect the liver, kidney and cardiovascular system, causing harm to humans [5].

2. Development status of remediation technology of petroleum contaminated soil

Because of its high fluidity, strong permeability and significant adhesion to soil, petroleum-contaminated soil has the characteristics of complex system, wide range, difficult treatment, long cycle and great harm. At present, the repair techniques of petroleum-contaminated soil mainly include physical methods, chemical methods and biological methods.

Physical methods include gas phase extraction techniques, electrical repair techniques, and the like. Soil gas phase extraction is a kind of in-situ remediation technology widely used in the process of petroleum-contaminated soil remediation. It mainly reduces the content of petroleum contaminants in the soil by volatilizing the high concentration of volatile pollutants in the soil. The negative pressure generated by the vacuum pump drives the gas through the contaminated soil pores, desorbs the volatile organic compounds in the unsaturated areas of the underground soil and collects them on the ground for treatment. Wang et al. [6] applied this technology to in-situ remediation of petroleum-contaminated soil in field test sites and found that the petroleum removal rate reached 88% at a suitable aeration depth. Liu Shasha et al [7] further mastered and improved the technology by carrying out soil gas phase extraction and remediation technology demonstration work at a petroleum contaminated site in Guangdong. After three months of repair, the total petroleum hydrocarbon removal rate in the soil reached 64.88%. The function of the post-contaminated site is restored. The gas phase extraction technology mainly removes the high concentration of volatile pollutants in the soil by pumping, but the low concentration and difficult volatile pollutants in the soil still exist. Soil electric remediation technology is a restoration technology that was developed and valued after the 1990s. This method inserts the electrode into the contaminated soil, and the charged ions and particulate matter in the pores of the soil can undergo a directional migration movement in the direction of the electric field after application of direct current. Compared with other soil remediation technologies, it has the advantages of high economic efficiency, protection of on-site ecological environment and safety, and is suitable for the treatment of dense soil with low permeability coefficient. The main disadvantage is that the non-polar organic pollutants lacking charge can not be removed efficiently by electric repair technology, and the chemical insolubilization of insoluble organic pollutants is required to cause secondary pollution [8]. Foreign researchers have studied a variety of high-efficiency soil electrical repair techniques and have been successfully applied in practical engineering, such as Electro-Klean TM electrokinetic separation technology, Lasagna TM technology [9].

The main problem with chemical methods is that the addition of surfactants destroys the soil micro-ecology and is costly. Taking the leaching method as an example, the leaching method is to elute the pollutants in the soil with a fluid, or to wash the organic pollutants in the soil with a surfactant for the oil-contaminated soil. The main eluent used in organic contaminated soil cleaning technologies such as volatile organic compounds is a surfactant. Many researchers [10-12] have used different surfactants to clean and repair soil contaminated with volatile organic compounds of different components, and achieved remarkable results. Fountain et al. [13] used different surfactants for
leaching during the repair of the Hill AFB site in the United States, successfully reducing the concentration of TCE (trichloroethylene) pollutants, which is highly representative in the field of surfactant repair technology in the United States. Researchers at Eckenfelder in Germany have successfully studied how to recover and reuse surfactants in soil-contaminated cleaning solutions [14], laying the foundation for further improving the efficiency of surfactants in contaminated soil treatment.

Soil bioremediation technology originated in the 1980s. Compared with physical and chemical methods, bioremediation technology has the advantages of simple operation, complete degradation of organic matter, no secondary pollution, and in-situ treatment. It has been highly valued by academic, industrial and government departments in various countries. At the forefront of technical research, it is also one of the preferred soil bioremediation technologies recommended by the US Environmental Protection Agency. Phytoremediation means that plants can stimulate microbial activity by directly absorbing and degrading organic pollutants and releasing various secretions to enhance their biotransformation and enhance the mineralization of organic pollutants in plant roots to degrade organic pollution in soil. Things. The method has the advantages of low energy consumption, low cost, simple operation and sustainable development strategy, and is suitable for repairing large-scale contaminated soil. However, most plant roots are concentrated in the surface layer of the soil, and it is impossible to repair the deep pollutants in the soil, and it is affected by the season and the ambient temperature. Therefore, the phytoremediation technology has certain limitations. Zhang Juanjuan et al [15] studied the use of alfalfa plus optimized microbial inhibitors and regulated soil temperature and nutrients to degrade petroleum hydrocarbons in petroleum-contaminated soil. After 99 days of repair, the degradation rate of petroleum hydrocarbons in soil reached 95%. Soil animals play an important role in soil ecosystems and maintain the stability of soil ecological structure. Soil animals can break, digest and absorb some organic matter, and convert the pollutants in the soil into good manure. However, the types and contents of organic substances that can be used are limited, which is suitable for lightly polluted soil. Some animals in the soil, such as cockroaches, can promote the growth and transfer of microorganisms, making the microbial remediation effect more obvious. Therefore, the combination of animal remediation and microbial remediation is more beneficial to the soil remediation process. Microbial remediation is the most widely used in bioremediation of petroleum-contaminated soils. The method removes toxic and harmful pollutants in the soil by utilizing or strengthening the ability of metabolic decomposition of toxic substances specific to environmental microorganisms, and decomposes organic pollutants into other harmless substances. Cao Guannan [16] and other dominant strains B-3, which are enriched and separated from diesel-contaminated soil samples, are Tetra thiobacter kashmirensis. It was cultured in a diesel medium with a mass concentration of 3.78 g/L, and the degradation rate was as high as 60.98%. The research results provide a theoretical basis for the bioremediation of diesel-contaminated soil. Recent studies have shown that in order to improve the degradation rate of pollutants in the soil, a combination of various repair techniques can be adopted. For example, the combination of electric repair technology and bioremediation technology has become an efficient and environmentally friendly composite repair technology. Li Tingting et al [17] used electric-microbial combined repair technology to remove petroleum contaminants. After applying electric field, the number of petroleum-degrading bacteria increased, and the oil degradation rate was 2.4 times that of the control group.

3. Microbial remediation technology classification and application
Microbial remediation refers to the microbial use of its life metabolism to ultimately degrade organic pollutants into carbon dioxide and water to achieve the purpose of repairing contaminated soil. The microbial remediation technology can be divided into natural attenuation method, biological stimulation method, bio-enhancement method [18] and enzymatic reaction method according to the type of preparation added to the soil during the treatment process and the source of the microorganism. The classification, advantages and disadvantages of microbial remediation techniques are shown in Table 1.
Natural attenuation relies on natural processes in the soil or groundwater system to remove or weaken pollution. When the environment is contaminated by chemical substances, the natural attenuation of pollutants occurs in most contaminated areas. However, due to the weak degradation ability of natural decay processes, it usually takes 5 to 25 years to complete the purification of the environment [25].

| Method                        | Strain                                      | Advantage                                                                 | Shortcoming                                                                 |
|-------------------------------|---------------------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Natural attenuation [19-20]   | Protozoa or microorganisms in the environment | In the natural environment, no need to consume manpower, material resources, financial resources | Degradability is weak, it takes a long time, and the repair efficiency cannot be guaranteed. |
| Biological stimulation [21-23]| Native microorganism                         | No effect on soil physical and chemical properties, easy to operate       | Refractory petroleum hydrocarbons still exist and cannot guarantee the efficiency of repair and safety during the repair process. |
| Biofortification [24]         | Screened out a highly efficient degrading bacterium or a variety of microbial flora | Degradation of pollutants quickly and thoroughly, no secondary pollution to the environment | Limited exposure to microorganisms due to their physical, chemical and biological properties |
| Enzyme reaction               | Extracted from microorganisms               | Broaden reaction conditions and accelerate degradation rate and efficiency | The operation is complicated, and the current experimental research needs to be improved. |

Biostimulation repair technology strengthens the degradation of pollutants by degrading bacteria in indigenous microorganisms by regularly adding nutrients such as nitrogen sources and phosphorus sources to the soil [26-29]. Wang Congying et al [30] of the Institute of Soil Science, Chinese Academy of Sciences [30] studied the degradation of 13 PAHs in soil by adding \((\text{NH}_4)_2\text{HPO}_4\) to polycyclic aromatic hydrocarbons (PAHs) contaminated soil. After 13 weeks of aerobic culture, the content of each PAHs decreased to varying degrees, with the highest degradation rates of 2 and 4 rings, 24% and 38%, respectively. This is due to the high content of organic carbon in the contaminated soil, while the N and P nutrient sources are relatively insufficient. After adding \((\text{NH}_4)_2\text{HPO}_4\), the growth conditions of the microorganisms are optimized, and the activity of the indigenous microorganisms is activated, thereby promoting the degradation of PAHs. The nutrient sources N and P can also be added to the contaminated soil in the form of fertilizer. Recent studies have shown that the addition of organic and inorganic fertilizers can increase the degradation rate of petroleum hydrocarbons to varying degrees. Ayotamuno et al. [31] applied fertilizer in situ dosing to repair petroleum-contaminated agricultural soils. The degradation rate of petroleum hydrocarbons in treated samples reached 50% and 65%, which is a more effective method for petroleum hydrocarbon degradation. Margesin et al. [32] studied the degradation of petroleum hydrocarbons by InipoEAP22 lipophilic fertilizer and NPK inorganic fertilizer. It was found that fertilizer can significantly improve the degradation rate of petroleum hydrocarbons and the activity of lipase, and the higher the initial concentration of pollutants, the fertilizer The more significant the effect.

Bio-reinforcement repair technology refers to the technology of improving the bio-remediation ability of pollutants and adding high-efficiency exogenous strains or flora to the pollution control system to achieve efficient removal of target pollutants. Ma Xiaotong et al. [33] of Northwest A&F University used diesel as the sole carbon source to screen and isolate the diesel-degrading bacteria Q41c from the crude oil contaminated soils in Qingyang and Henan Zhongyuan Oilfield by enrichment culture and flat-screening. Q41e, z41b, z41h, and found the optimum pH and temperature for its growth, providing a stock of strain for in situ remediation of diesel contaminated soil. A researcher at Zhejiang University of Technology [34] isolated a diesel-degrading yeast KML from a soil sample that
was not contaminated by diesel oil in Cameroon. It was identified as Yarrowia lipolytica at a suitable temperature, pH and the degradation rate at the nutrient source concentration was 78%. The GC-MS component analysis showed that the strain mainly degraded C9-C25 long-chain hydrocarbons and a small amount of branched hydrocarbons. Zhao Ruixue et al. [35] of Changchun University of Science and Technology [35] used the soil in the vicinity of the oil storage tank of Zhongcheng Chain Satellite Gas Station for long-term infiltration of diesel oil as the source of bacteria. After dieselification was the only carbon source for domestication, the dominant strains degrading diesel were isolated. Ammonium chloride, potassium dihydrogen phosphate and potassium hydrogen phosphate were used as nutrient sources, and the degradation rate was determined to be 47.8%. Zhao Dongfeng et al. [36] of China University of Petroleum inoculated the thermophilic petroleum hydrocarbon degrading bacteria from indoor screening to the soil samples with long-term pollution of oil at 8-8 stations in Karamay Oilfield, Xinjiang for indoor simulated bioremediation experiments. The soil micro-ecology and physical and chemical properties of the different stages of repair have great changes. During the repair process, soil catalase activity increased, urease and dehydrogenase activities decreased. After 75 days of repair, the degradation rate of petroleum hydrocarbons reached 56.31%.

Studies have shown that strains that are highly efficient in degrading petroleum hydrocarbons can be obtained by mutagenesis, and strains with long growth stability and high tolerance can be obtained, thereby improving the degradation rate. Zheng Jinxiu et al [37] isolated two highly efficient oil-degrading strains W1 and W2 from the contaminated soil of Wuhan Qingshan Petrochemical Plant, which are the genus Bacillus and the spore genus. The bacterial suspension was placed in a sterilized culture dish, and the mutagen was obtained by irradiating 40 S with a 30 W ultraviolet lamp at a distance of 30 cm. The experimental results show that the efficiency of mutagenized bacteria is more and more obvious with the increase of oil concentration. In addition, by comparing the growth curves, it can be seen that the strains after mutagenesis have better genetic stability. Lu Yujin [38] enriched and isolated bacteria capable of efficiently degrading diesel oil from oil-contaminated soil, and identified it as Rhodococcus sp. by morphological characteristics and physiological and biochemical characteristics. Ultraviolet irradiation and the addition of a mutagenizing agent, lithium chloride, were induced into a highly efficient degrading diesel strain, and its degradation ability was increased by 16.9% before mutagenesis. Liao Yougui [39] screened out a strain of Pseudomonas, which has the strongest ability to degrade petroleum, from oil-contaminated soil near Hunan's petroleum refinery. After UV mutagenesis, a strain of oil with higher degradation efficiency was obtained. The degradation rate of 3-15 bacteria was 9.1% higher than that before mutagenesis. In addition, the pH value of the liquid medium of the bacteria decreased after mutagenesis, indicating that the ability of the strain to secrete acid-producing substances after mutagenesis was improved. Hua Xiufu et al [40] performed low-temperature atmospheric pressure plasma mutagenesis on Enterobacter cloacae TU, a high-efficiency petroleum-degrading strain selected from oil-contaminated soil in Zhongyuan Oilfield. Compared with wild strains, the mutant strains did not change. It has salt tolerance and is stable for 15 generations. The mutant strain grew stably under the high salt content of 7.5% NaCl, and the degradation rate of petroleum hydrocarbon was 2.5 times that of wild bacteria.

For some microorganisms that degrade petroleum hydrocarbons, the degradation efficiency is not high due to their slow growth. Therefore, it is a feasible way to construct an enzyme that can degrade petroleum hydrocarbons in microorganisms. Alkanes in petroleum usually become the corresponding primary alcohols by terminal oxygenation, and further oxidized by the action of alcohol dehydrogenase and aldehyde dehydrogenase, and finally enter the β oxidation pathway in the form of fatty acids. Lai Qiliang et al [41] used diesel and petroleum as carbon sources to isolate and culture a deep-seawater seawater in the Indian Ocean, and then isolated a strain of P40 with strong degrading ability of diesel. It was identified with Alc China, Alcanivorax dieselolei B25T and A. dieselolei NO1A. The highest similarity, the similarity is 99.8%. Two alkane hydroxylase gene fragments were cloned from this strain, and it was preliminarily judged that the bacteria might be a deep sea phenotype strain of diesel alkaloids. In the process of petroleum aromatic degradation, catechol dioxygenase is an important enzyme that catalyzes the cracking of the benzene ring. Luo Wanrong [42] amplified the
catechol 2, 3 dioxygenase gene from the degrading strain Sphingomonas ssp. B2-7 by primer design. Wu Yucheng et al [43] extracted catechol 2, 3 dioxygenase gene from soil samples near the refinery and cloned them, and obtained 7 different gene sequences. The analysis indicated that these genes may be derived from Pseudomonas in soil, and the gene richness is related to the number of aromatic degrading bacteria. It can be seen that the diversity of degrading genes in soil provides abundant resources for bioremediation. Hua Xiufu et al [44] used phthalic anhydride to chemically modify the laccase mainly degrading polycyclic aromatic hydrocarbons in white rot fungi, and studied its degradation effect by using ruthenium as a model substrate. The results showed that compared with natural laccase, the modified laccase had higher affinity for the substrate, longer half-life, wider pH, and nearly 2 times higher degradation efficiency.

4. Conclusions and prospects of oil-contaminated soil remediation technology

With the acceleration of China's economic development, the consumption of petroleum resources is also accelerating, and the pollution of the soil is also becoming increasingly serious. Because soil pollution is far-reaching to the environment and the human body, the social needs of soil remediation technology are increasing. Various repair techniques have certain repair effects on oil-contaminated soil, but there are certain limitations and shortcomings. Bioremediation technology has become a research hotspot due to its huge economic and environmental advantages. Molecular ecology technology was used to monitor the growth and reproduction of additional bacteria in the process of arable land restoration and the impact on the degradation rate of petroleum hydrocarbon pollutants. The competition, antagonistic and synergistic behavior of added bacteria and indigenous flora in petroleum-contaminated soil were investigated for petroleum degradation. Reference is made to the construction of microbial flora and microbial agents. At present, the research on bioremediation technology to control oil-contaminated soil is mostly limited to laboratory research, and there are not many applications that are actually applied in industrial processes. According to the pollution situation in China, the development of efficient and low-cost systematic bioremediation technology, using molecular ecology method to study the microbial functional genes of soil important cycle and its abundance and homogenization standardization, combined with soil physical and chemical properties and planting ability evaluation Indicators, systematic evaluation of the effects of bioremediation technology, is particularly important and urgent.

5. References

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