Research on Microbial Remediation Technology Based on Oil Contamination of Oil and Gas Exploration Soil

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Abstract. The paper used the optimized in situ indigenous microbial flora and combined physical and chemical ecological remediation technology to carry out the field remediation of oil residue contaminated soil. The remediation results show that when the average residual oil content in the soil is 2898.25mg/kg, after the 99d microbial ecological remediation technology is implemented, the oil content in the soil can be degraded by more than 99%, which provides technical methods and methods for the remediation of residual oil pollution in the oil field. Feasibility study for popularization and application.

Keywords: Oil and gas exploration, soil, oil pollution, microbial remediation.

1. Introduction
In recent years, with the rapid development of the petroleum industry, soil pollution caused by petroleum has increased day by day. Crude oil spillage and substandard discharge of oily sewage have become the main pollution channels. Studies have shown that harmful substances in oil-contaminated soil will not only cause pollution of surface water and groundwater, but will also be transferred to animals and plants through the food chain, and eventually reach the human body and endanger human health. Therefore, the remediation of oil-contaminated soil is urgent. The commonly used remediation methods for petroleum-contaminated soil include physical, chemical, and microbiological methods, but the former two have the disadvantages of high cost, cumbersome operation, and complex post-processing [1]. Microbiological methods are low-cost, convenient to operate, efficient and environmentally friendly. The advantage of has been more widely used in this field. Figure 1 shows the principal diagram of microbial degradation of petroleum pollution.
In this paper, the microbial ecological comprehensive restoration technology that optimizes the indigenous microbial flora supplemented by a combination of physical and chemical methods is used to carry out degradation and restoration experiments and applications on long-term residual oil pollution in the soil, and has achieved some results [2]. The method has the advantages of simple treatment method, low cost, good repair effect, little impact on the environment, no secondary pollution, and in-situ treatment. Therefore, this research provides technical support for the popularization and application of this technology, which has important practical significance.

2. Materials and methods

2.1. Test materials
The test soil was taken from the farmland around the oilfield, belonging to fluvo-aquic soil, organic matter 22.3g·kg⁻¹, alkali hydrolysable nitrogen 263.6mg·kg⁻¹, available phosphorus 36.3mg·kg⁻¹, and available potassium 59.3mg·kg⁻¹, pH7.8. The petroleum-contaminated soil used for screening and enriching petroleum-degrading bacteria is taken from the abandoned oil production well site of Zhongyuan Oilfield. The components of the selection medium for petroleum degrading bacteria are: crude oil 10000mg·L⁻¹, NH₄NO₃, 300mg·L⁻¹, KH₂PO₄, 200mg·L⁻¹, MgSO₄, 100mg·L⁻¹, FeCl₃, 100mg·L⁻¹. 1L of sterile soil extract.

2.2. Experimental plan for microbial remediation of petroleum contaminated soil
In the thesis, 500g of the test soil was divided into round porcelain pots, and the soil was bioremediated for 8 weeks according to the scheme shown in Table 1. During the restoration, the soil was evenly turned over to maintain air permeability every day.
Table 1. Microbial remediation treatment plan for petroleum contaminated soil

| Repair process number | Soil remediation treatment plan                                                                 |
|-----------------------|-------------------------------------------------------------------------------------------------|
| 1                     | Natural test soil + sterilized pure water (soil moisture content 40%)                          |
| 2                     | Sterilized test soil + sterilized pure water (soil moisture content 40%)                       |
| 3                     | Natural test soil + SZ-1 bacterial suspension + sterilized pure water (soil moisture content 40%) |
| 4                     | Natural state test soil + nitrogen and phosphorus nutrient solution (soil C: N: P=100:10:1) + sterilized pure water (soil moisture content 40%) |
| 5                     | Natural state test soil + SZ-1 bacterial suspension + nitrogen and phosphorus nutrition (soil C: N: P=100:10:1) + sterilized pure water (soil moisture content 40%) |
| 6                     | Sterilized test soil + SZ-1 bacterial suspension + sterilized pure water (soil moisture content 40%) |

2.3. Test method

2.3.1. Screening and enrichment of petroleum degrading bacteria. In the experiment, 5g of petroleum-contaminated soil was added to a 250mL Erlenmeyer flask containing 100mL of petroleum-degrading bacteria screening medium, placed in a constant temperature shaking incubator, and cultured for 10 days at 30℃, 160·min⁻¹. The culture solution is obviously turbid. Take 25mL of the culture solution and add it to a 250mL Erlenmeyer flask containing 75mL of culture medium. Add 4g of petroleum as a carbon source for microbial growth. After the above-mentioned culture conditions are continuously transferred to enrichment culture for 6 times to obtain petroleum-degrading microorganisms, Expand the cultivation and reserve. The microorganisms selected in the experiment are mainly bacteria and fungi.

2.3.2. Remediation of oil-contaminated soil. The experimental design oil pollution level is 15g·kg⁻¹. After mixing the soil with the 2mm aperture sieve thoroughly with the petroleum, 4kg (calculated as dry soil) per pot is placed in a container with a size of 20cm x 26cm in diameter. No organic solvent is used when mixing the soil with the crude oil. Let it stand for 3 days, make the oil and soil fully mixed to reach a stable state, and then carry out repair test. In the experiment, microbial remediation refers to the complete mixing of 1000 mL of microbial culture solution in the soil in the same container; phytoremediation refers to planting 50 cotton seeds, 50 sunflower seeds, 50 highdan grass seeds, and 100 bermudagrass seeds in the container. Seed; Plant and microorganism joint restoration refers to sown 50 cotton seeds, 50 sunflower seeds, 50 highdan grass seeds, and 100 bermudagrass seeds in a container that is completely mixed with 1000mL of microbial culture solution. In the experiment, 7 days after the emergence of cotton, sunflower and sorghum, 8 plants were kept in each pot. After 14 days of emergence, 4 plants were fixed in each pot; bermudagrass was not treated. Set a blank test group as a control [3]. The experiment was carried out from April to October, growing under natural conditions, keeping the soil water content at about 25%, and regularly measuring the oil content in the soil of each pot. Each treatment was repeated 3 times.

3. Results

3.1. The remediation effect of microorganisms on petroleum contaminated soil

The physiological process of the metabolism of petroleum pollutants by microorganisms is generally completed by contacting and adsorbing petroleum, secreting extracellular enzymes, absorption of petroleum pollutants, and intracellular metabolism [4]. The key to its degradation of petroleum is the oxidation of petroleum by oxidase. Fungi and bacteria mainly complete the oxidative metabolism of petroleum pollutants through the action of extracellular enzymes and intracellular enzymes (such as
mono/dioxygenase, etc.). The remediation effect of microorganisms on petroleum contaminated soil is shown in Figure 2.

![Figure 2. Microbial remediation effect on petroleum contaminated soil](image)

It can be seen from Figure 2 that the oil content of contaminated soil decreased rapidly in the first 15 days, and the oil degradation rate of the control and microbial treatment was not much different. After that, the oil degradation rate of the control increased little, while the oil degradation rate of the microbial treatment increased significantly [5]. The oil degradation rate increased and slowed down, and the degradation rate reached 67.0% at 120d, while the control was only 25.4%, the former is about 2.6 times that of the latter. According to the analysis, the petroleum degradation rate of the control polluted soil is mainly formed by the volatilization of low-molecular-weight petroleum hydrocarbons under natural conditions and the loss of detection rate caused by the physical adsorption of the soil; after 90 days, the increase in the petroleum degradation rate of the polluted soil is slowed down by adding microorganisms. It is caused by the gradual consumption of components in oil that are easily used by microorganisms. It can be seen that the added microorganisms can degrade petroleum.

### 3.2. Removal rate of oil in soil by microbial ecological restoration

Through the above-mentioned on-site remediation, we can understand the microbial ecological remediation technology, and the in-situ remediation of soil oil pollution has good effectiveness. After adding the optimized bacteria liquid and various nutrients and additives to the restoration area, the removal rate of petroleum residual oil in the soil is over 85%, and the removal rate reaches 99.37% when the restoration reaches 99 days. However, from the data point of view, there is a certain difference between the repair effect of the film and the uncoated film. The removal rate of the film is slower. This is because the film blocks the oxygen in the air from entering the soil after the film is covered, which hinders microorganisms [6]. Oxidizes the oil in the soil and slows its removal rate. Therefore, as long as the environment (such as: temperature, humidity, etc.) conditions permit, it is not necessary to cover the large area in the future. In addition, it can also be seen from the data that the heterogeneity and clumps of petroleum residual oil in the soil make the data obtained have some abnormal values, indicating that the degradation of petroleum clumps in the soil is relatively slow in a short period of time during the restoration process. But overall, the effect of microorganisms on the degradation and restoration of petroleum residual oil in the soil is still significant.
4. Discussion

4.1. The mutual benefit of plants and microorganisms
This technology can combine the advantages of phytoremediation and microbial remediation, and the mutual benefit between the two strengthens the degradation of rhizosphere organic pollutants. Plants provide a living place for microorganisms, and can transfer oxygen so that the aerobic effect of the root zone can be carried out normally; root exudates and sloughs can provide a lot of nutrients for microorganisms, stimulate the growth and reproduction of various rhizosphere flora, and enhance bacteria Joint degradation; in some cases, plant root exudates can be used as a natural co-metabolism substrate of microorganisms to promote the degradation of pollutants; the organic carbon formed in the root zone can prevent the transfer of organic compounds to groundwater, and can also increase the microbial contamination of pollutants. Mineralization. In some cases, plant root exudates can also be used as natural co-metabolic substrates to promote the degradation of pollutants. In addition, plant roots can extend to different levels of soil, so the degrading bacteria can be dispersed in the soil without mixing the soil. On the other hand, microorganisms convert pollutants into a state that plants can absorb and utilize, reduce the toxicity of pollutants to plants, and improve plant tolerance.

4.2. Electric-microbe combined technology
Research has found that applying an electric field while using highly efficient degrading bacteria to degrade petroleum pollutants can significantly increase the degradation rate of petroleum pollutants. Under the action of a weak DC electric field, petroleum pollutants can move faster, and soil physical and chemical properties are also improved, so that the degradation rate of petroleum pollutants can be accelerated. Under the same soil environmental conditions, the electrokinetic-microbe combined technology has better remediation effects on contaminated soil than simple bacteria or natural attenuation. In the process of electrokinetic-microbial combined treatment, the mechanism of action of degrading bacteria is still unclear. Therefore, the technology is still in the indoor experimental stage.

4.3. Ectopic repair technology
Ex-situ remediation technology refers to the remediation technology that excavates oil-contaminated soil from the original place and transports it to a designated location for disposal. There are two main methods. (1) Bioreactor method: the contaminated soil is excavated and dispersed in water and sent to a reactor device inoculated with microorganisms for processing. The reactor can separate domesticated microorganisms and other additives such as nutrients from the soil, Surfactants, etc. are thoroughly mixed, which can well control the degradation conditions, thereby improving the efficiency of microbial degradation of petroleum. After reaching the treatment target, the soil is discharged, dehydrated and transported back to the original place. (2) Soil composting method: The soil composting method is to excavate the oil-contaminated soil and transport it to the treatment site, add leaves, hay, wheat straw, wood chips, manure and other agricultural wastes to the oil pollutants, and adjust the pH with lime to maintain oxygen, the optimum value of water and pH provides nutrition and optimum environment for microorganisms to improve their metabolism and activity, and promote the degradation of petroleum pollutants. The degradation process requires the introduction of domesticated petroleum highly degrading microorganisms or engineered bacteria to improve the effect. This remediation technology is low in cost, easy to operate and high in efficiency, and is now a common method for the treatment of petroleum-contaminated soil.

4.4. Bioaugmentation technology
Bioaugmentation technology is a technology that inoculates highly efficient degrading bacteria into contaminated soil after domestication, and can significantly promote the decomposition of petroleum substances under the premise of optimizing the microbial environment. This technology has the characteristics of high-efficiency degradation bacteria and strong adaptability, which can overcome the limitations of biostimulation technology itself. Under the same soil environmental conditions,
inoculation of efficient degrading bacteria can significantly enhance the effect of soil restoration. Although this technology has a significant effect on the degradation of petroleum pollutants, it is extremely susceptible to the influence of soil physical and chemical properties and environmental conditions, and has a limited effect in the application of petroleum contaminated soil remediation. Therefore, how to enhance the degradation ability of microorganisms and explore more efficient bio enhancement measures will become a hot issue in future research and development.

5. Conclusion
In the process of treating oil-contaminated soil, physical remediation, chemical remediation and bioremediation are combined. In high-concentration oil-contaminated soil, physical and chemical methods should be used first, and then bioremediation methods should be used to achieve bioremediation and traditional methods. The organic combination of methods builds an efficient repair system. Improve the technology, optimize the process, build a bacterial colony immobilization model, improve the oil degradation rate through the synergy between the dominant degrading strains, and improve the optimization degree of the joint symbiosis of exogenous strains and indigenous strains. It can also be constructed through genetic engineering Efficient degradation bacteria. It is foreseeable that the microbial remediation technology will become a research hotspot in the field of oil-contaminated soil protection in my country with its huge economic and environmental advantages.

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