Bioremediation of Crude-Oil Polluted Soil Using Immobilized Microbes

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Abstract: Microbial bioremediation technologies in the past usually cannot degrade the corrosive pollutants in the polluted soil. These microbial bioremediation technologies are often impacted by many environmental factors, and therefore the remediation effect of these bioremediation technologies on the polluted soil is reduced. With the advance of science and technology, more advanced immobilized microbial remediation technologies are developed. These technologies have been growing rapidly due to their advantages over the old microbial remediation technologies. This paper investigates and researches the immobilized microbial and other remediation technologies, and the results show in the process of remediation of polluted soil using immobilized microbes, that the activity characterization of enzyme and the number of bacteria are both relatively higher. Moreover, this paper provides the detailed statistics on various factors that impact the effect in remediating oil polluted soil using immobilized microbial remediation.

1. Introduction
With the rapid economy growth in China, more and more industries rise. As a widely used natural resource of energy, the consumption of crude oil becomes larger and larger, its frequency of being used becomes higher and higher. Although the large consumption of crude oil has significantly helped the economic development, made people’s life more and more convenient, the usage of crude oil seriously polluted the land where human beings live when you look at it from a different angle. With the social development becoming stabilized, and China’s goal of sustainable development, people’s focus gradually shifts toward environmental improvement, especially the improvement of crude oil-polluted land. Based on the research and discoveries of social environmental organizations, the main reason of land pollution in China is the onsite disposal of crude oil after usage. This seriously impacted the quality of land and water supply. Furthermore, polluted soil contains large amount of substance harmful to humans. The increase of crude oil-polluted land has seriously affected people, therefore Chinese government starts encouraging the remediation of crude oil-polluted land through scientific solutions. Currently, the common remediation techniques on crude oil-polluted land include physical remediation, chemical remediation and microbial remediation, etc. Among these three remediation technologies, both physical and chemical remediation have the characteristics of high construction cost, complicated construction techniques and very difficult to implement. Therefore, they are not suited for the actual application of crude oil soil remediation. However, the last microbial remediation technology among these technologies is the advanced remediation technology developed under the guidance of modern advanced science and technology. It has the characteristics of extremely low remediation cost, easy remediation operation and no construction pollution. Therefore, microbial
remediation technology is currently the most mainstream remediation method. This paper conducted detailed analysis and research on the common microbial remediation technologies, thereby concluded the feasibilities of each type of microbial technologies [1].

2. Current microbial remediation technologies on crude oil-polluted land

2.1. Immobilized microbial technology
The principle of immobilized microbial technology is to use advanced physical-chemical method to limit the free microorganisms inside the appropriate medium, thereby increase the amount of microbes in the carrier and adjust its chemical reaction to realize the improvement of land quality and stabilization. This type of land remediation technology is immobilized microbial remediation technology. The feature of this type of land remediation technology is easy decomposition after the immobilization treatment of microbes, enabling multiple usages of immobilization carrier [2]. Common carriers include powders made from nutshells, activated charcoal and organic materials. The statistics of the crude oil degradation effectiveness of each type of immobilized microbial remediation technology for crude oil polluted soil are shown in the table below. It can be seen from Table 1 that the crude oil degradation capability of immobilized microbial remediation technology is far greater than that of other biological remediation technologies. Although immobilized microbial remediation technology is becoming increasingly mature, the disposal of the remainder of immobilization carriers is an issue, and this creates other environmental problems. Therefore, with the crude oil remediation technology becoming mature, the research direction should shift to the area of environmental protection [3].

| Immobilized microbial technology | Crude oil degradation rate |
|----------------------------------|--------------------------|
| SiO₂ material                    | 94%, compared to 65% by mobile bacteria + enhanced microbes |
| Nutshell powder                  | 59%, compared to 59% by mobile bacteria + stimulated microbes |
| Organic material                 | 21%, compared to 19% by mobile bacteria + stimulated microbes |
| Activated charcoal               | 47%, compared to 24% by mobile bacteria + stimulated microbes |

2.2. Enhanced microbial remediation technology
Enhanced microbial remediation technology applies treated highly-effective degradation bacteria to the crude oil-polluted soil layer. This is a type of crude oil land remediation technology that can protect ecosystems. Enhanced microbial remediation technology has very good adaptability. This technology injects specialized degradation bacteria with great adaptability into different layers of crude oil-polluted soil. The degradation efficiency of this technology is very high. This technology, however, is prone to be affected by external environmental factors after the specific degradation bacteria is injected, resulting in the deviations of remediation effects. Therefore, how to increase the anti-interference capability is the development direction of this technology [4].

2.3. Biostimulation technology
Optimizing natural environment through human interference can stimulate soil microorganisms in the land to help them better ferment and degrade crude oil contaminants to a certain extent. This technology is called biostimulation feedback technology. According to investigation, it is revealed that this technology can stimulate the evaporation of low molecular weights gasses in the soil and the freeradical chain reaction of soil microorganisms to high molecular weights gasses [5]. Different
biostimulation technologies have different values for crude oil degradation and decomposition, showing a gradual downward trend. Different biotechnology methods, such as soil permeability technology, the addition of abundant chemical substances such as appropriate amount of electron acceptors, can improve crude oil degradation and decomposition to varying degrees. This technology doesn’t introduce new pollutants and its operation is simple and convenient. However, there are relatively few high-quality soil microorganisms and their growth rate is slow in general. This technology’s efficiency of decomposing and degrading the crude oil pollutants is low [6].

2.4. Integrated electrokinetic - microbial technology
The survey data shows that using effective degrading bacteria to degrade crude oil pollutants while increasing the electric charge and electric field to decompose and degrade is of great significance for the degradation and elimination of the crude oil pollutants. On the basis of using DC voltage, the crude oil pollutants can be quickly decomposed, which will also help improve the soil quality and increase the degradation rate of crude oil pollutants [7].

2.5. Integrated plant-microorganism technology
By utilizing the complementary relationship between plants and soil microorganisms, the technology of enhancing the root system of plants and oxidatively degrading crude oil and other substances is called the integrated plant-microorganism remediation technology. When the quantity of crude oil is too small to support the survival and growth of soil microbials, the effect of conventional microbial remediation technology is insignificant. The oxygen adsorbed by green plants and some chemical substances produced by their rhizomes can promote the decomposition and degradation progress by soil microorganisms to a certain extent. Therefore, it is possible to combine conventional biodegradation technology and the integrated plant-microorganism remediation technology to utilize their respective advantages to improve soil remediation capabilities. Under the same soil environment, the effect of integrated plant-microorganism remediation technology is obviously better than the conventional biodegradation technology [8]. The disadvantages of the current integrated plant-microorganism remediation technology are: limited application range by using plants only to remediate and optimize soil quality; the growth of plants is usually affected by climate, environment, and soil quality; the soil remediation effect is not good by using only one type of plant; the recycled dead leaves and branches need to be treated, etc. Therefore, the Chinese government and relevant departments must focus on three aspects in the future research: Firstly, paying attention to the common working principles between soil microorganism and green plants, inspecting and repairing them; secondly, optimizing the quality of plants by modifying the DNA sequence using biochemical methods, choosing high-quality green plants for cultivation, and enhancing their vitality and ability to adapt to the environment; thirdly, improving remediation technology, high-quality seed selection can maximize the integrated remediation effect of soil microorganisms and plant [9].

3. Factors affecting the microbial remediation technology on crude oil-contaminated soil
Generally speaking, the remediation technology of microorganisms on crude oil-contaminated land is a method of human interference that uses advanced science and technology to inject various microorganisms and degrading bacteria deeply into the soil layer polluted by crude oil to accelerate the degradation rate of crude oil inside the soil layer to achieve the goal of improving the quality of soil layers and maintaining the local ecological environment. The actual implementation principle of microbial remediation technology on crude oil-contaminated land is to inject microorganisms into the soil layer contaminated by crude oil, and use the microorganisms to breathe oxygen for the oxidation reaction to promote the decomposition rate of crude oil in the polluted soil layer. The actual process is shown in Figure 1:
Figure 1. Remediation process of microbial technology on crude oil pollution

3.1. Effect of different Microorganisms on soil remediation
According to previous investigations in the field of microorganisms, there are hundreds of types of microorganisms that can effectively decompose crude oil in contaminated soil. Among them, pseudomonas bacteria, alkali-producing rod bacteria, nucleobacteria, colorless rod bacteria, trichoderma fungi, penicillium fungi, aspergillus fungi, etc. have significant effects. In the remediation of soil contaminated by crude oil, these fungal microorganisms have outstanding effects. The use of microorganisms is subject to conditions. Only fungal microorganisms that are active in enzymes, decompose quickly, and have high environmental adaptability can better take effect in the remediation of contaminated soil. And biological experts suggest that the actual effect of using multiple fungal microorganisms together far exceeds the decomposition effect of a single fungal microorganism [10].

3.2. Effect of the type of soil and crude oil being repaired on the remediation
The physical and chemical properties of the soil, such as weight, moisture, density, water content, porosity, and material contents, will directly affect the quality of crude oil decomposition. According to survey data, to decompose crude oil pollutants, the most suitable hydrogen ion concentration index is between 6.8-7.8, the most suitable temperature is 13-28 Celsius degrees, and the most suitable soil moisture content is 68-79%. The physical-chemical properties of the crude oil pollutants play a vital role in crude oil regeneration. According to theoretical data, currently crude oil hydrocarbons above C20 is what most difficult to decompose in China, a very small percentage of soil microorganisms can decompose them; on the contrary, crude oil hydrocarbons in the C8-C20 range can be easily decomposed and oxidized. Different crude oil substances have different degradation rates. The specific manifestations are as follows: the degradation rate in the decreasing order is linear saturated hydrocarbons, branched chain saturated alkanes, colloidal crude oil and asphaltene crude oil [11].

4. Remediation of soil physical and chemical properties by immobilized microorganisms

4.1. Improvement of soil organic matter by immobilized microorganisms
The organic matter compositionin the soil during the 35D crude oil-contaminated soil remediation process with various remediation methods is shown in Figure 3. It can be seen in the figure that the remediation with proper addition of immobilized microorganism can increase organic matter composition; As for straw, it has organic matter such as fiber and semi-fiber, and microorganisms use the organic matter contained in the straw as a carbon reservoir to absorb the rich nutrients in the straw. In addition, the straw can effectively increase the content of microorganisms, which makes crude oil degrading bacteria more flexible, and plays a role in efficiently decomposing saturated hydrocarbons of oxidized crude oil. In the process of soil quality improvement, the composition of organic substance becomes less and less, which realizes the basic requirements for microorganism conservation and respiration. At the initial stage of the remediation optimization, the organic soil remediated and maintained by immobilized microorganisms and mobile migratory bacteria gradually increased. The reason is that because some weak microorganisms cannot adapt to the surrounding environment and climate, their remains supplement the organic components in the soil. With the increase of remediation time, most of the microorganisms have gradually adapted to the surrounding environment, climate,
humidity, etc., this increases the flexibility and survival rate of soil enzymes, and gradually improves microorganisms metabolic capability. Microorganisms breed more actively using the organic substances in the soil repeatedly, resulting in a gradual reduction in the composition of organic matter in the soil. The treatment time is about 20-25d. When the flexibility and survival rate of microorganisms reach the peak, the breathing and ventilation demand of soil will directly reduce the organic matter composition in the soil. During this period, the decomposition and oxidation of saturated hydrocarbons in crude oil vary in a relatively larger range, as is shown in Figure 2; the composition of organic substance varies in a relatively smaller range between 24 and 30 days, because the harmful substances generated during the degradation and decomposition of the saturated hydrocarbons of the crude oil hinder the growth of microorganisms. Compared with other soils, the organic content of the soil using straw is significantly increased. During the remediation and degradation, the magnitude of change of the organic substance component is relatively smaller compared with the former, mainly because of the small size of the microorganisms and the sufficient organic components. It can be seen from Figure 2 that the amplitude change of the organic matter composition is inversely proportional to the decomposition rate. Compared with other remediation methods, the control and management of organic matter by the immobilized microbial remediation method is specifically displayed in that the organic matter contained in the soil always maintains an ascending state during the remediation process, and the degradation rate is fast, and the organic substances in the soil can be fully utilized. These are the fundamental reasons for the increased decomposition and degradation rate of saturated hydrocarbons in crude oil.

![Figure 2. Variation of crude oil hydrocarbon decomposition rate with time for various methods of remediation of crude oil-contaminated soil.](image1)

![Figure 3. Changes in soil organic matter content (w (Organic matter)) during the 30-day process of remediation of crude oil-contaminated soil using various remediation methods](image2)

4.2. Effect of immobilized microorganisms on soil moisture

From the data in Figure 4, it can be concluded that the soil moisture change during the remediation of immobilized microorganisms and straw is slower than that of mobile bacteria, which shows that the above two methods have the effect of increasing soil moisture retention. This is consistent with the result that the soil organic matter and the soil moisture is proportional. During the 9 days of remediation, the soil moisture was reduced from the original 18.6% to 9.4% by immobilized microorganisms, from 19.8% to 8.6% by mobile bacteria, and from 16.7% to 8.9% by straws. The original bacteria decreased from 16.9% to 9.8%. During the remediation period, the immobilized microbial remediation method can always keep the soil humidity in a rising state, promoting the breeding and reproduction of microorganisms, and increasing the decomposition capability of crude oil saturated hydrocarbons.
4.3. Effect of immobilized microorganisms on soil PH
It can be seen from Figure 5 that the soil layer treated by the immobilized microbial remediation technology, the pH value of the soil decreased slightly between 0-6 days, its pH value increased by 0.15 between 6-13d. There are two reasons for this phenomenon: 1. In the treatment of contaminated land, the acid produced by organic matter reduces the pH of the soil. 2. The nitrogen contained in the microbial straw used in the remediation technology is absorbed by the soil. After 14 days, because the microorganisms selected in the microbial remediation technology have strong adaptability, these microorganisms have a relatively high self-regeneration ability. The microorganisms continuously generate organic acids, which causes the soil to absorb too much organic acid, resulting in the decrease of the soil pH value.

4.4. Controlling effect of immobilized microorganisms on soil nitrogen content
It can be seen from Figure 6 that after the immobilized microorganisms are injected into the soil contaminated by crude oil, the nitrogen content in the soil is much higher than the other two methods. After the land remediation is carried out for a period of time, the mobile fungal microorganisms are injected into the soil layer to quickly reduce the nitrogen content in the soil until it can meet its own regeneration needs. After 6 days, the injected fungal microorganisms can already survive in the soil with their strong adaptability. The normal self-regeneration and secretion activities caused the nitrogen content in the soil to decrease greatly. As seen from the figure below, the immobilized fungal microorganisms can decrease the nitrogen content in the soil greatly. The soil layer nitrogen content is significantly reduced from nine grams of nitrogen per kilogram of soil to only one gram per kilogram of soil. The content of nitrogen in the soil treated by mobile bacteria decreased from 8 grams of nitrogen per kilogram of soil to two grams of nitrogen per kilogram of soil, and the mobile bacteria remediation method causes the nitrogen content in the soil to decrease rapidly. Combining Figures 5 and 6, it can be found that crude oil saturated hydrocarbons have a great demand for nitrogen content during the decomposition in order to meet the basic requirements for decomposing fungi’s...
regeneration, it is consistent with the research results of experts and scholars in the field of microbiology; Immobilized microorganisms have higher requirements for nitrogen content, the fastest degradation rate and the best remediation effect. Based on this, nitrogen supply must be increased to increase the decomposition rate\(^{11}\).

5. Summary
Conversion and separation are commonly used in old-fashioned polluted-soil remediation. This method has too many disadvantages, and the actual operation is extremely complicated. Microbial remediation technology developed with the advance of science and technology has absolute advantages in decomposition. Contemporary microbial remediation technology has many advantages such as extremely low construction cost, simple construction method, fast decomposition of crude oil pollutants, and no damage to the ecological environment. However, the emergence of microbial remediation technology is not long, so that the microbial remediation technology is not perfect, and there is still great research space for the synthesis of fungal microorganisms, which are the main research directions of microbial remediation technology in the future.

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