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The contrast study of the in-situ remediation methods of the petroleum-contaminated soil

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ABSTRACT

The Contrast study of the in-situ remediation methods of the petroleum-contaminated soil was carried out in Zhongyuan Oilfield. Three remediation methods, plant-microbial method, microbial method and plant method, were applied to identify the degradation effect of petroleum. The highly efficient petroleum-degrading bacteria screened from the study area and the rye grass is chosen as remediation plant according to the experiment in laboratory. The results showed that the number of petroleum-degraded microbial after remediation is more than the situation before remediation. That is because the microbial liquid and the rhizosphere effect of plants can promote the growth and propagation of microbial. The plant-microbial method is optimal for the petroleum degradation. The petroleum was degraded fully in the remediation layer and a little part of nutrients leaked into the layer below the remediation layer. In addition, the soil temperature, oxygen content and nutrients should be regulated to promote the degradation of petroleum. The in-situ plant-microbial method provides more economic and efficient technical methods for the remediation of contaminated soil and has profound practical significance for the sustainable development of human beings.

KEYWORDS

Petroleum-contaminated soil;
Plants- microbial;
In-situ remediation;
Degradation effect;
Regulative factors.

INTRODUCTION

Petroleum has been one of the most important energy for human. With the increasing demand for petroleum products, the leakage of petroleum and its products into the soil leads to more and more serious soil pollution and further do harm to biotic and human beings. The petroleum-contamination of soil has been an international environmental problem. Previous researches have shown that over 80 percent of crude oil fallen to the ground was trapped in topsoil[1]. The cumulative crude oil would destroy the soil structure, influence soil permeability and have a negative effect on the growth of crops[2]. Meanwhile, the petroleum pollutants residues in plants by root absorption are dangerous to human health through food chain[3]. There are a lot of studies on the treatment of petroleum pollutions. Bioremediation is not new but it is more con-

cerned as a high-efficient and low-cost clean technology. The researches on bioremediation are very active in recent ten years. The bioremediation technology has been applied in the practical remediation of contaminated soil in some countries and has achieved significant results[4,5]. Sun et al considered that the degradation effect of petroleum-degrading bacterial screened from petroleum-contaminated soil was very effective[6]. Ouyang thought that festuca elata had prominently promoted the petroleum degradation by microbial liquids[7]. Li et al proved that the suitability of teosinte planted in petroleum -contaminated soil[8]. Lin et al believed that the uptake and metabolism of planes play an important role in PAHs degradation[9]. Liu et al had studied the results of contaminated soil remediation by pot experiment in Zhongyuan Oilfield[10]. Most of the above-mentioned researches focus on laboratory test and the researches on in-situ remediation test have been seldom reported. The author carried out in-situ remediation of petroleum-contaminated soil by plant remediation method and has achieved remarkable results[11]. Hence, the paper is based on the former researches and combined with screened plants by Liu[10] in terms of soil characteristics in Zhongyuan Oilfield. The study on plants-microbial in-situ remediation of petroleum-contaminated soil is carried out to provide scientific basis for bioremediation of petroleum-contaminated soil.

EXPERIMENTAL PROCESSES

The study area

The test area is located next to extraction well yard in Zhongyuan Oilfield. A blowout accident has happened in 1991 and polluted the surrounding farmland. According to the investigation, the leakage of crude oil and high-salinity water reached to 200m^3~300m^3 and the area of polluted farmland was more than 6667m^2. The lithology of polluted soil was khaki silt with small amount of gravel. The wet density of soil is 1.72 g/cm^3 and natural water content is 16.3 percent. The pH value is 7.4. In the soil the salt content is 1243mg/kg~18650mg/kg and the background value of petroleum content is 767.7 mg/kg~5028.8 mg/kg with the mean value is 2898.25mg/kg. In the depth region of 25-50cm, the petroleum content is 313.6mg/kg, the pH is 8.5 and the salt content is 1243mg/kg.

Materials

Highly efficient petroleum-degrading bacteria screened from petroleum-contaminated soil in the test area include Pseudomonas, Micrococcus, Actinomayces, Penicillium, Mucor, Aspergillus, et al. The bacteria are cultured for 5 days and the content of bacteria is 10^{11}-10^{15} per milliliter. The well-cultured bacteria liquid is stored in six 25L plastic drums which is 25 liter before remediation experiment.

The compounding nutritive solution includes $MgSO_4$, NH_4NO_3, $CaCl_2$, $FeCl_3$, KH_2PO_4, K_2HPO_4.

Rye grass is chosen as remediation plant according to the results of soil phytoremediation experiment in petroleum-contaminated soil in Zhongyuan Oilfield by Liu Jichao.

The Additives added to the soil include wheat husk, corn stock, urea and compound fertilizer.

Analytical methods

UV-spectrophotometry is used to determinate the content of petroleum hydrocarbons. bacterial cultivation refer to Methods for Studying Soil Microbial[12] and the methods introduced in ref[13-15]. Preliminary identification of bacteria adopt the methods introduced in the Common manual of systematic bacteriology[16].

Test procedure

The test area is divided into three sections. No.1 (666.7 m^2) is the microbial remediation area, No.2 (266.7 m^2) is the rye grass-microbial remediation area, and No.3 (400 m^2) is the rye grass remediation area. The steps are as follows:

Step1, The farmland should be ploughed twice, 3-rotary tillage, and at last leveled before remediation.

Step 2, the prepared wheat husk and corn stock (1.05kg/m^2), urea (0.075kg/m^2) and compound fertilizer (0.0375kg/m^2) are spread on the surface soil evenly. And then rotary tillage should be carried out by tractor and the depth of rotary tillage carefully controlled is about 25 cm.

Step 3, the well-cultured bacteria liquid and the prepared nutritive solution are sprayed evenly on No.1 and No.2 test area at 0.07 L/m^2 by atomizer respectively.

Step 4, the farmland are ploughed many times in order to make the the aforementioned added substances

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homogenized.

Step 5, No.1 test area is covered with plastic film. The rye grass seeds are sowed in No.2 and No.3 test are.

Step 6, Sampling of soil should be applied regularly to identify the content of petroleum hydrocarbons, the soluble salt, NH_4^+, NO_3^- and the pH value. The quincunx sampling method at the same depth is introduced to collect soil samples at different positions and blend the samples together for testing in every test area.

RESULTS AND DISCUSSION

The distribution of bacteria in soil

The bacteria which has the potential ability of degradation of petroleum contaminants exists in the natural soil. These bacteria are tamed and cultured in the laboratory and added to the soil. It can increase the number of petroleum-degradation bacteria obviously to enhance the ability of degradation. Meanwhile, it can overcome the problems such as low-viability and competition relationship with indigenous microorganisms of exogenous bacteria. The distribution of the bacteria number in every test area after 99 days' remediation is showed in TABLE 1.

TABLE 1 demonstrated that the bacteria number in No.1 and No.2 test area is more than that in No.3 test area and before remediation significantly. That is because the added bacteria liquid contains large amount of petroleum-degradation bacteria which can degrade contaminants which can't be transformed or transformed slowly after tamed in soil. The number of petroleum-degradation bacteria is increased by the adaptability of bacteria[17]. The total bacteria in No.3 test area is nine times the number before remediation. This illustrates that the effects of rhizosphere of plants, the added nutrient solution and fertilizer promote the growth and reproduction of bacteria.

The degradation rate of petroleum

The soil samples are collected at seeding time, in 3 days, 10 days, 18 days, 28 days, 36 days, 44 days, 56days, 76 days, 99 days later respectively. The testing results are showed in TABLE 2 and figure 1.

The data in TABLE 1 shows that the plants-microbial remediation method and microbial remediation method have obvious remediation effectiveness and the degradation rate after 99 days' remediation reaches to 67.38 percent and 57.34 percent respectively. In the plant-remediation test area the degradation rate is 43.27 percent. This phenomenon reflects that the added bacteria liquid fasten the speed of remediation and the rye grass has a function of remediating petroleum-contaminated soil.

TABLE 1 : Distribution of bacteria in soils samples after 99 days' remediation

Test area	bacteria		fungi		actinomycete	
	total	Petroleum degrading bacteria	total	Petroleum degrading bacteria	total	Petroleum Degrading bacteria
Before remediation	7.1×10^5	3.6×10^3	7.6×10^5	2.8×10^3	1.8×10^4	1.8×10^2
No.1	6.2×10^7	4.5×10^5	8.1×10^6	3.2×10^4	1.4×10^5	1.7×10^3
No.2	5.6×10^8	6.4×10^7	2.8×10^7	1.8×10^5	3.1×10^5	7.1×10^3
No.3	2.8×10^6	1.9×10^4	1.4×10^6	7.2×10^3	4.2×10^4	4.1×10^2

TABLE 2 : Chang of degradation rates with time in oil contaminated soils of different plots (percent)

Test area	0 days	3 days	10 days	18 days	28 days	36 days	44 days	56 days	76 days	99 days
No.1	0	11.7	15.43	18.35	25.12	29.27	27.96	46.37	54.78	57.34
No.2	0	12.4	17.89	22.34	29.47	33.13	38.34	50.12	63.21	67.38
No.3	0	5.1	5.89	10.03	20.17	25.14	27.19	41.45	39.26	43.27

The physiological process of bacteria for degrading petroleum pollutants is adsorbing petroleum and then metabolize in cells. The key of petroleum degradation is that the oxidation of petroleum by oxidase[18]. The degradation rate of petroleum hydrocarbon in No.1 and No. 2 test area after 3day' remediation is 11.7 percent whereas that in No.3 test area is 5.1 percent. This result shows the high speed of remediation by bacteria liquid and the adapted period is 1-3 days. After 76

days' remediation, the degradation rate of petroleum increased slowly in No.1 and No. 2 test area because that the useful ingredient in petroleum exhausted gradually.

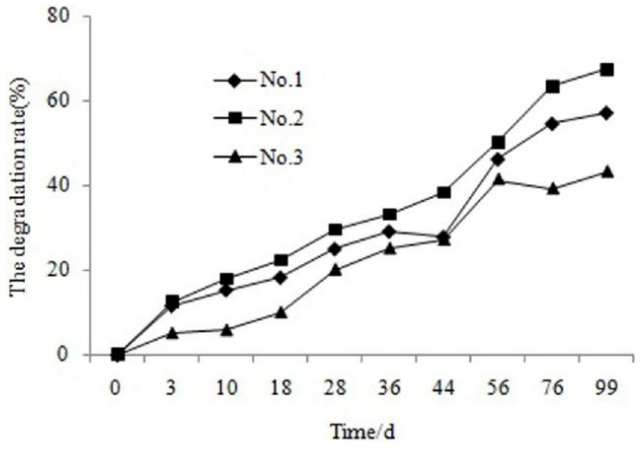

Figure 1 : Chang of oil degradation rates with time in soil of different plots

Plant rhizosphere is an important microorganism system and has special physicochemical properties. The remediation function of plant rhizosphere is mainly reflected in absorption, transformation and decomposing organic contaminant[19]. Between 3 days to 18 days after remediation, the degradation rate of petroleum hydrocarbon in No.3 test area remained more than 5 percent. That is because the addition of nutrient solution, fertilizer, wheat husk, corn stock and plowing activities provide the nutrient and oxygen for indigenous microorganisms in order to degraded petroleum hydrocarbon. There has an obvious increase in degradation rate of petroleum hydrocarbon 28 days later and the result demonstrated the remediation function for petroleum-contaminated soil by plants.

In addition, there were catastrophic phenomena in degradation rate in No.1 and No.3 test area occurred in 44th day and 56th day respectively. The reason is that the the heterogeneity and complexity of soil lead to the nonuniformity of sampling.

Effect on subsoil

The soil samples are collected at 50cm depth underground and tested in laboratory for the contents of petroleum hydrocarbon, soluble salt, NH_4^+, NO_3^- and Cl^- after 99 days' remediation. The results are shown in TABLE 3.

TABLE 3 showed that the content of petroleum hydrocarbon has not increased in the soil below remediation layer. It showed that the petroleum was not diffused downward and degraded in remediation layer. The content of soluble salt, NH_4^+, NO_3^- and Cl^- with a little change showed that only a small part enter into the lower layer along with the water. This conclusion has an important meaning for the requirement and adding method of nutrient in the remediation process.

TABLE 3 : Contents of petroleum hydrocarbon, soluble salt, NH_4^+, NO_3^- and Cl^- at 50-cm after 99 days' remediation

Test area	Petroleum Hydrocarbon (mg/Kg)	Soluble salt (mg/Kg)	NH_4^+ (mg/Kg)	NO_3^- (mg/Kg)	Cl^- (mg/Kg)
Before remediation	313.6	1243	9.18	31.23	932.33
No.1	310.78	1289	11.83	32.48	1017.02
No.2	294.37	1301	7.46	29.27	901.87
No.3	311.67	1278	12.34	30.18	890.78

Regulative factors for remediation technology

The in-situ remediation technology in this paper was optimized utilizing of in-situ microbial community, combining the plant method with geological environment and using the microscopic effects to change the macro environment. The key in the application of the technology contained that the association, interdependence, interaction and regulation between microbial and plants. The main regulative factors are temperature, water, oxygen, nutritive elements and the improvement of geological environment et al.

Regulation of soil temperature

Temperature is one of important factors which effect the growth and survival of microbial. The activity intensity and biochemical action of microbial are closely related with soil temperature. The microbial survive in a certain range of temperature. Extreme cold or hot environment retards bacterial growth or causes microbial death, so the moderate temperature may fasten the biochemical reaction rate in microbial cells. In the test area most of the enhanced microbial community is mesophilic bacteria (13-45°c) and its most suitable temperature for growth is 25-38C.At the beginning of the experiment, the plastic film covered the surface in the No.1 test area in order to keep the soil temperature drop

remaining constant but No.2 and No.3 test areas are uncovering areas. the land surface temperature records in the remediation periods shows that day-and-night temperature difference is small. The max temperature appears between July and September and most of all are above 35°C.most of the min temperature are above 20°C. So the soil temperature is not needed to regulate for the excellent remediation results. But the measures of increasing and maintaining temperature should be adopted in other months. The conclusion was drawn that the optimum temperature for remediation is between late June and mid-September with the soil temperature is maintained above 25°c to guarantee the activity and fecundity of microbial.

Regulation of soil oxygen

Oxygen supplement is one of important regulation factors for the degradation of organic materials by microbial. The oxygen content in soil would effect enzymic activity and respiration in microbial cell and further control the microbial growth and degradation of organic materials. In this study four methods were applied to increase the soil oxygen content. First, fully plough up the soil is to mix the gas in soil with atmospheres well. Second, the water content of soil should be kept at the 20 percent level to supply oxygen. Third, the wheat husk and corn stock, which is cheap and easy-obtained, were added to soil to supply nutritive elements and improve the soil porosity to make the oxygen in atmospheres enter into soil easily. Fourth, the oxygenous nutrient elements, such as K_2HPO_4,KH_2PO_4,$MgSO_4{\cdot}7H_2O$,NH_4NO_3,NO_3^- et al, can supply a lot of oxygen. The above-mentioned measures provide abundant oxygen for petroleum-degraded bacteria in the degradation processes.

Supplement of nutrients

Nutrient elements participate in the formation of microbial cell and provide energy for physiological activities. the main elements in microbial cell are C,H,O,N,P et al. C, H are mainly from organic matters such as petroleum pollutants, and oxygen originates from water, atmosphere and other resources. The trace elements such as nitrogen, phosphorus, sulfur et al are supplied and regulated as nutrient substances. Meanwhile, urea and compound fertilizer contain plenty of nutrient elements and the added wheat husk and corn stock supply other biotin and nutrients.

CONCLUSIONS

1 The petroleum-degraded microbial in the petroleum-contaminated soil are acclimated, cultured and add to test areas at last. The number of petroleum-degraded microbial in No.1, No.2 and No.3 test area is more than the situation before remediation. The results show that the microbial liquid contains a lot of petroleum-degraded microbial which can produce the degradation ability of pollutants to degrade the unconvertible or slowly-convertible petroleum pollutants in soil. In addition, the rhizosphere effect of plants promotes the growth and propagation of microbial in the soil.

2 Plant-microbial method, microbial method and plant method have an obvious remediation effect on petroleum-contaminated soil. The degradation rate of petroleum hydrocarbon is all above 40 percent. And the effect of plant-microbial method is optimal for the degradation rate reaches to 67.38 percent. The adapting period of added microbial liquid is 1-3 days and the remediation function of plants was demonstrated fully after 18 days' remediation.

3 The content of petroleum hydrocarbon below the remediation layer have not increased and a little part of soluble salt, NO_3^-, Cl^- etc have been leaked with water and reached into lower layer. The conclusions have significance of requirement and adding method of nutrients.

4 The optimum remediation period is between late June and mid-September with the max value and the min value of temperature are above 35 and 25°c respectively. Ploughing soil and adding the wheat husk and corn stock, nutrient solution and fertilizer have supplied amount of oxygen for the degradation process. In the meanwhile, the additives in the soil support essential nutrients for petroleum-degraded microbial.

In conclusion, the in-situ plants- microbial remediation technology has remarkable degraded effect of petroleum in the petroleum-contaminated soil. The technology is characterized by simple treatment method, low-cost, in-situ remediation et al. the in-situ

plants- microbial remediation technology have great development potential as an environmental remediation technology. The conclusion can provide more economic and efficient technical methods for the remediation of contaminated soil and has profound practical significance for the sustainable development of human beings.

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