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Effect of electric intensity on the microbial degradation of petroleum pollutants in soil

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Abstract

Electro-bioremediation is an innovative method to remedy organic-polluted soil. However, the principle of electrokinetic technology enhancing the function of microbes, especially the relationship of electric intensity and biodegradation efficiency, is poorly investigated. Petroleum was employed as a target organic pollutant at a level of 50 g/kg (mass of petroleum/mass of dry soil). A direct current power supply was used for tests with a constant direct current electric voltage (1.0 V/cm). The petroleum concentrations were measured at 3275–3285 nm after extraction using hexane, the group composition of crude oil was analyzed by column chromatography. The water content of soil was kept 25% (*m/m*). The results indicated the degradation process was divided into two periods: from day 1 to day 40, from day 41 to day 100. The treatment of soil with an appropriate electric field led the bacteria to have a persistent effect in the whole period of 100 days. The highest biodegradation efficiency of 45.5% was obtained after treatment with electric current and bacteria. The electric-bioremediation had a positive effect on alkane degradation. The degradation rate of alkane was 1.6 times higher in the soil exposed to electric current than that treated with bacteria for 100 days. A proper direct current could stimulate the microbial activities and accelerate the biodegradation of petroleum. There was a positive correlation between the electric intensities and the petroleum bioremediation efficiencies with a coefficient of 0.9599.

Key words: electro-bioremediation; electric filed; microbial activity; petroleum

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Introduction

Petroleum and its derivatives are typical organic pollutants in soil, and are difficult to be degraded (Brüggemann and Freitag, 1995). Accidental spillage and improper use and handling at processing sites have led to the contamination of underlying soils (Kim et al., 2005; Virkutyte et al., 2002). Current bioremediation of contaminated soil has shown to be a practical method, which is safe and cost-effective (Braddock et al., 1997; Franzmann et al., 2002). Therefore, bioremediation is widely recognized as a feasible treatment technology to eliminate soils contaminants and organic pollutants (Khan et al., 2004). However, this method also has its drawbacks and limiting factors, such as microbial and physicochemical conditions of the soils, soil temperature, pH values, moisture contents (Mulligan et al., 1999). Thus, electro-bioremediation has been employed as an incorporate technology in the treatment of soil contaminated with organic pollutants in recent years. Several studies have shown improved removal of organic pollutants such as aromatic compounds, herbicides and trichloroethylene in electric fields applied to soil (Davis-Hoover et al., 1999; Page and Page, 2002; Jackman et al., 2001). Lear et al. (2004) have studied the response of bacteria exposed to electric current, which caused little changes in the activity and composition in the microbial community near the anode. Alshawabkeh and Bricka (2000) proved that the strength of electric field and the electrode matrix had effects on the pollutants degradation during *in situ* bioremediation. Wick et al. (2004) found low current densities have little effect on bacterial activity either, but they also found that 1.57 A/m^2 direct current (DC) may stimulate their activity (She et al., 2006). In the following year, Wick et al. (2007) reported that there was no negative influence to soil bioremediation when DC was applied; fortunately, there were beneficial effects on the microbe transference and nutrients dissolution. Uses of electric field to the phenol-contaminated soil enhanced the desorption of bacterial cells from soil, and it may have a potential for the acceleration of mass interactions among organic pollutants, bacteria and nutrients (Luo et al., 2006). Petroleum is a mixture of many organic compounds. Most of them are nonpolar, which are non-conductors in electric field. As a result, electricity may be impossible to stimulate the mobilization of petroleum, and electrokinetics may be not involved in the biodegradation.

Here, we present a study to explore the possible mechanisms involved in the electrokinetics in biodegradation in

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the petroleum contaminated soil. The petroleum biodegradation with electricity was carried out to reveal relationships between the electric intensity and the petroleum biodegradation efficiency. The possible mechanisms for petroleum biodegradation are also discussed.

1 Materials and methods

1.1 Soil, sample, and bacteria

The soil sample was collected from Shenyang Experimental Station of Ecology, China from 0 to 20 cm deep, and sieved (2 mm). The preliminary experimental results showed that the indigenous microorganism had a great influence on petroleum degradation rate. Therefore, the soil samples were sterilized three times by alternately using an autoclave (121°C for 45 min) and a drier (105°C for 30 min). Some properties of the soil are listed in Table 1. The organic content is 66.3 g/kg after adding petroleum into the clean soil.

The artificial contaminated soil was prepared using petroleum at a target concentration of 50 g/kg (mass of petroleum/mass of dry soil). The crude oil obtained from Daqing is light oil. Its compositions include 68.04% alkanes, 24.30% aromatic hydrocarbons and 7.65% resin-asphaltene. The density of the crude oil is 0.882 g/cm^3 at 20°C. Its solidifying point is 25.8°C and viscosity is 18.9 mPa·sec at 50°C.

The petroleum-degrading bacteria were isolated from petroleum-contaminated soil (Niu et al., 2006). They were B22, B2020, B64, B101, B2008, B2007, B2001 and B2002, which mainly included *Arthrobacter globiformis*, *Clavibacter xyli*, *Curtobacterium flaccumfaciens*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Bacillus* sp. Bacteria were cultured in the medium on a shaker at 28°C. After 72 hr, bacteria were harvested in exponential growth phase by centrifugation, and re-suspended in sterilized deionized water to obtain a highly concentrated bacterial suspension (5.5×10^5 cfu/mL). The processes were all passed the sterility test. To provide a homogeneous distribution of bacteria in the contaminated soil, the bacteria concentration was 100 cfu/mL, and the proper amount of bacteria was 0.01 mL/g in soil.

1.2 Apparatus

The electrokinetic reactor (Fig. 1) was made of square-shaped Perspex (100 cm length × 100 cm width × 25 cm height). Twenty-five cylindrical graphite electrodes, which were 25 cm long each (the distance between two adjacent electrodes was equal), formed an electrode matrix. A controlling system was consisted of an electric current and voltage real-time monitor system. A DC power supply was used with a constant direct current electric voltage. The electrode control apparatus was capable of reversing the polarity of the electric field in turn by the mode of rows/columns every 5 min; therefore, the electric field was completely symmetrical. An electronic thermometer was inserted into the soil. Temperatures were monitored on-line. A constant voltage gradient of 1.0 V/cm was used for all the tests.

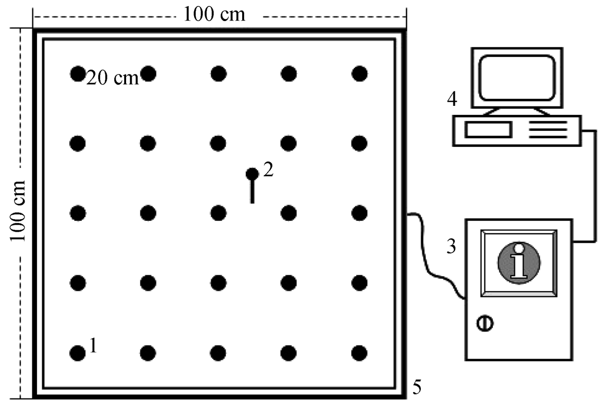

Fig. 1 Design of the electro-bioremediation cell used to treat petroleum-contaminated soils. (1) electrode; (2) thermometer; (3) control system; (4) monitor system; (5) soil cell.

1.3 Experiment

First, the contaminated soil was sterilized and its moisture was adjusted to 30% (*m/m*). Then it was artificially spiked with petroleum by blending petroleum (5%, *m/m*) and a mixed solution of petroleum-degrading bacteria. Afterwards, the soil was balanced for a week.

Dry soil of 150 kg was placed into the electrokinetic reactor by layers. The contaminated soil was divided into three parts for three tests: the soil was treated with both of electricity and bacteria, bacteria only, no electricity or bacteria. The test without electricity or bacteria was used as a control. Soil moisture was kept at 30% (*m/m*) and all the tests were carried out at room temperature (23 ± 2°C). Samples were collected with interval of 20 days within 100 days. The group compositions of crude oil were analyzed on day 40 and day 100.

1.4 Analysis

The electric intensity (E_p) can be calculated as follows (Jackson, 1999):

$$E_p = \left\{ \frac{2b(y^2 + b^2 - x^2)}{[(x^2 + b^2) + y^2][(x^2 - b^2) + y^2]} \right\} i + \left\{ \frac{(-4bxy)}{[(x^2 + b^2) + y^2][(x^2 - b^2) + y^2]} \right\} j \qquad (1)$$

Table 1 Physical and chemical properties of the tested soil

Particles size	pH	CEC (cmol/kg)	OC (g/kg)	TN (g/kg)	TP (g/kg)	AN (mg/kg)	AP (mg/kg)
< 2 μm: 24.8% 2–50 μm: 62.6% 50–2000 μm: 12.6%	6.5	23.5	26.3	1.56	3.12	92.7	53.6

CEC: cation exchange capacity; OC: organic carbon; TN: total nitrogen; TP: total phosphorus; AN: available nitrogen; AP: available phosphorus.

where, b represents the distance between two electrodes; i and j are vectors, which mean the direction of the electric field. Two electrodes of the electrode matrix were set as the field intensity (Fig. 2).

To determine if the electric intensity has relationship with efficiency of bioremediation, three sampling lines were set up, and they were perpendicular to the middle line in which the two electrodes (A and B) were located. Samples were taken from the centerline, and 5 and 10 cm from the centre towards both electrodes, respectively. A total of 15 sampling points were obtained around two electrodes which were marked out using triangle, as shown in Fig. 3. Soil samples were taken using a self-made sampler once for every 20 days for analysis. No replicas were taken to preserve the surface of the soil as intact as possible.

The petroleum in soil was extracted using hexane as described by Urum et al. (2003, 2004). The mixture of 10 cm^3 of n-hexane and 5 g contaminated soil was shaken for 30 min and the solvent n-hexane was removed by evaporation. This process was repeated three times. The solution from the third extraction had the same absorbance (3275–3285 nm) as that of pure n-hexane (zero absorbance). All the solutions from extraction were combined, collected into a 50 cm^3 volumetric flask and adjusted to 50 cm^3 with n-hexane. The resulted solution was centrifuged for 20 min at 3000 r/min. Petroleum concentrations were determined by Infrared Oil Content Analyzer (Model JDS-106U, Jilin, China).

The group compositions of crude oil were analyzed by column chromatography using alumina as adsorbent, and petroleum ether, benzene and mixture liquid of methanol and chloroform (1:3, V/V) as eluents to separate alkanes, aromatic hydrocarbons and resin-asphaltene, respectively. The contents of group compositions were determined by Infrared Oil Content Analyzer (China National Petroleum Corporation, 1995).

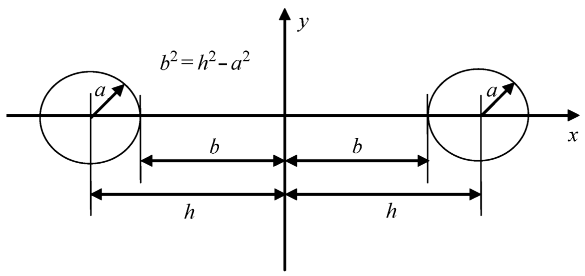

Fig. 2 Schematic of the electric intensity calculation. a: electrode radius; b: distance between two electrode; $h \gg a$; $b \approx h$.

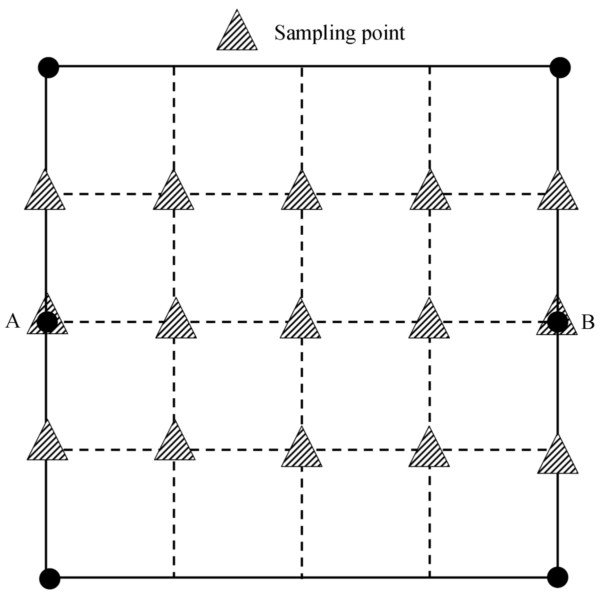

Fig. 3 Schematic of sampling positions. A and B are electrodes.

2 Results and discussion

2.1 Removal of petroleum

The removal of petroleum from contaminated soil was measured and the effect of electric fields on petroleum biodegradation was elucidated. As shown in Fig. 4, the maximum removal of petroleum 45.5% was achieved in electro-bioremediation at the end of the experiments, and the value was 1.6 times of an average of general biodegradation. The removal efficiency in electro-bioremediation was slightly faster (13.2%) than that in biodegradation within the first 40 days; whereas it became significantly high after 40 days. The petroleum removal efficiency with electricity was twice of that without electricity supply.

The results showed that the degradation process could be divided into two periods with day 40 as a turning point. The bacteria maintained high activity within 40 days. After the turning point, the removal efficiency decreased due to the consumption of nutrients and the slowdown of biological metabolism. However, with additional electricity, bacteria in soil had persistent activity in the bioremediation process. Similar results could also be found in other studies (Kim et al., 2005; Shi et al., 2008). Therefore, the removal efficiency of petroleum from soil could be accelerated by applying a proper electric intensity onto bacteria.

To our knowledge, there are few reports about restoration petroleum polluted soil under electric field. Lee et al. (2006) used electrokinetics to enhance transportation of

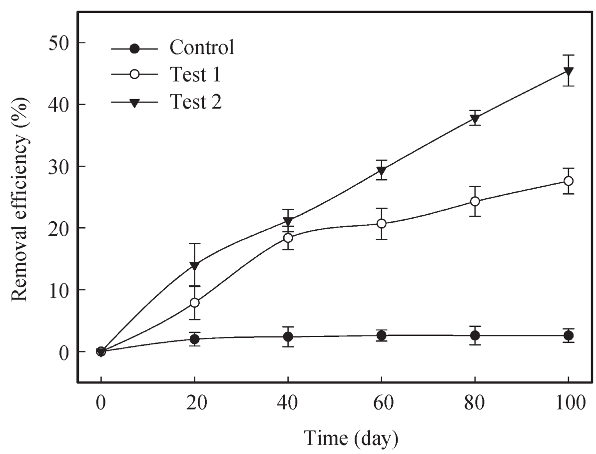

Fig. 4 Comparison of petroleum removal in treatments of utilizing petroleum-degrading bacteria (test 2) and utilizing electro-bioremediation (test 1) during 100 days.

nutrient phosphorus in the petroleum polluted soil. Our research is the first study to investigate the relationship between electric intensity and the biodegradation in the petroleum-contaminated soil. Earlier studies have shown that the application of 200 A/m^2 to soils led to a mortality of sulphur-oxidizing bacteria (Jackman et al., 1999). In recent years, however, several studies reported that there was neither negative nor positive effects observed by applying current (3.14 A/m^2) on soil bacteria (Wick et al., 2007; Harbottle et al., 2009; Lear et al., 2004). In our study, there are strong indications that sufficient strength electric intensity stimulated the activity of the microbe and accelerated the biodegradation.

However, when bacteria were absent into petroleum-contaminated soil (test 3), the rate of petroleum removal approached zero, even if an electric field was applied. It was not significantly different for the petroleum concentration in soil with the distances from anode. This indicated that petroleum hardly transports in electric field as a mixture of nonpolar organic compounds. The removal of organic pollutants from the soil might be a result of biodegradation, volatilization and the migration of degrading bacteria. Petroleum is a complex mixture of organic compounds, it mainly contains alkanes, aromatic hydrocarbons and resin-asphaltene. A large proportion of petroleum is alkanes which are non-polarity hydrocarbons, therefore, it hardly migrates in soil under electric current. On the other hand, resin-asphaltene is polar mixture, but it has strong absorption and hardly migration either. It is well known that the volatility of petroleum is low. All the analyses showed that the only factor influencing petroleum removal from the soil was biodegradation. In addition, control test provided another evidence that when soils were free of degrading bacteria, the removal rate of petroleum approached zero.

Therefore, when the organic carbon content in the soil for the treatment was changed from 60.4 to approximately 50.2 g/kg at the end of experiment, the activities of petroleum degradation microbe increased in response to environmental change.

2.2 Group composition of crude oil

The group composition of oil was analyzed on day 50 and day 100, in order to determine which composition of oil was readily to be degraded (Fig. 5). The test results show that the proportion of alkanes was decreased about 15.68% and 9.61% with and without electricity, respectively, and the resin-asphaltene was hardly degraded in all situations. Furthermore, the rates of alkane biodegradation showed no significant difference with or without electricity at the beginning of the 50 days. In contrast, the rate of alkane degradation under electric current was 1.6 times higher than that with bacteria treatment only by the end of the study (100 days). The results demonstrated that the electric-bioremediation has positive effect on alkane degradation. However, neither electric-bioremediation nor biodegradation showed a significant effect on resin-asphaltene.

It is well-known that different petroleum degrading

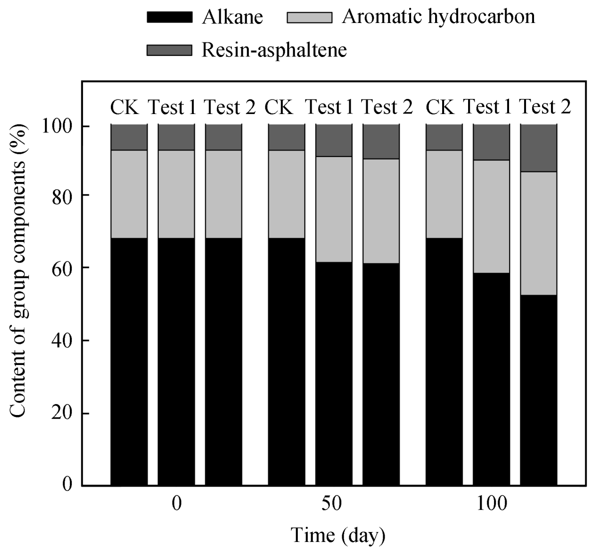

Fig. 5 Group components analysis of the oil. Test 1: utilizing electro-bioremediation; Test 2: utilizing petroleum-degrading bacteria; CK: control.

bacteria strains have different degradation abilities to hydrocarbon oil. Typically, long chain saturated hydrocarbons could easily be degraded by microbe while short chain alkane and aromatic hydrocarbon were difficult to be biodegraded, resin-asphaltene was hardly degradated (Ding et al., 2001). This is because that microbes selectively consume the components of the crude oil (Etoumi, 2007). The present study showed the similar results (Fig. 5), except that there is no persistent effect hampering the biodegradation. When electric current is applied to bacteria, the rate of alkane degradation was higher than treated by bacteria only. These findings demonstrated that a liquid alkane in petroleum is more sensitive to the electrokinetic treatment. Therefore, electro-bioremediation may be an effective technology and an alternative bioremediation method.

2.3 Relationship between electric intensity and petroleum removal efficiency

Figure 6 shows the correlation between the petroleum removal efficiency and the distance from the electrodes. The experimental results showed that the average petroleum removal rate was 31.6% around the electrode (0 cm) and the rate was two folds of that in the middle between the two electrodes (10 cm). Therefore, the nearer to the electrode, the higher the petroleum removal efficiency would be. This is because that the shorter the distance from the electrode, the stronger the electric intensity was (Fig. 7).

It was proved that employing an electric field to phenanthrene-contaminated soil could enhance the biodegradation (Niqui-Arroyo et al., 2006). Interestingly, there is an intimated correlation between the electric intensity and the petroleum removal efficiency. The results in this study showed that the efficiency of the biodegradation near the electrode region/area was higher than that far from the electrode region/area (Fig. 6). Furthermore, from control test we found that electric intensity have

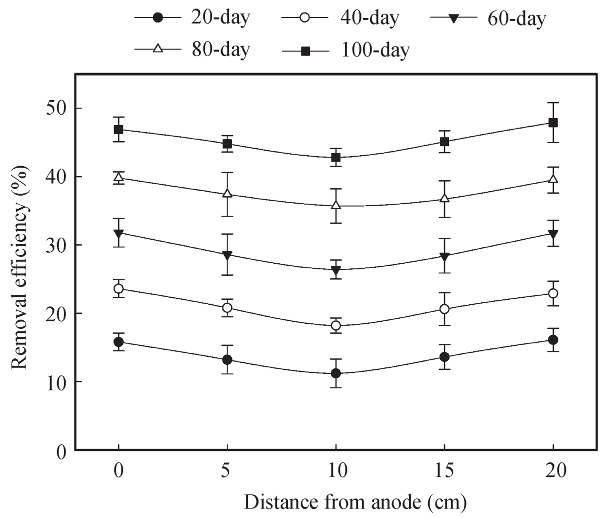

Fig. 6 Distribution of the petroleum removal during 100 days.

insignificant effect on petroleum migration. In order to test the reliability and practicalness of the methodology, real polluted soil, which was collected from Liaohe oilfield, was treated with electricity. The results showed that the average petroleum removal rate was 7.7% around the electrode within 10 days. Furthermore the rate was higher than that in the middle of the two electrodes in the electric field. Accordingly, the possible reason was that there was a stronger field intensity near the electrodes, and the field intensity might stimulate the bacterial activities, and then accelerate the petroleum removal efficiency. Suni and Romantschuk (2004) also suggested that the electrokinetics may accelerate the biodegradation in soil.

As shown in Fig. 8, the electric intensity vs. the proportion of petroleum removal efficiency had a significant positive correlation with R^2 0.9599. According to the evidences shown in Figs. 6 and 7, it can be concluded

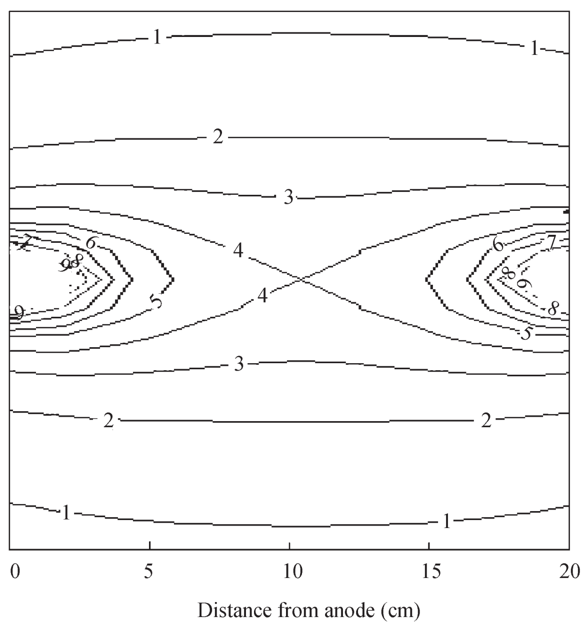

Fig. 7 Distribution of the electric intensity.

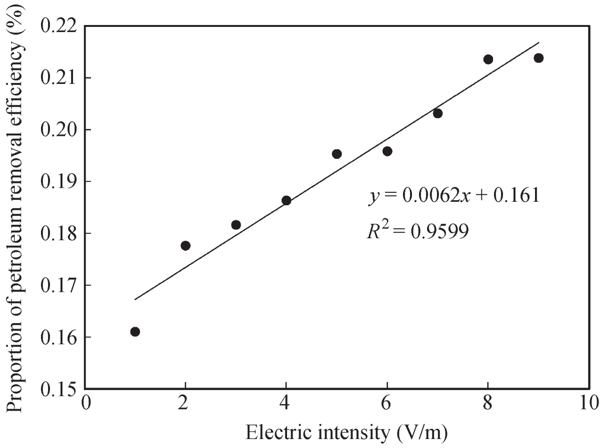

Fig. 8 Correlative analysis of the electric intensity and the proportion of petroleum removal efficiency.

that the petroleum degradation rate and the electric field intensity had a positive correlation. From this study, we speculated that the stronger the electric intensity is, the higher the petroleum removal efficiency is.

The electrokinetic phenomena (electrophoresis, electroosmosis, electromigration) have little effect on petroleum, which consist of nonpolar organic compounds. Based on the experimental results, we assume that electrokinetics could promote the desorption of organic pollutants from soil particles. Moreover, the electrokinetics has a positive impact on the uniformed distribution of nutrients and degrading bacteria to soil matrix. In addition, the electrokinetics may enhance the interactions among organic pollutants, bacteria and nutrients during bioremediation. The character soil is also important for the microbe, because it keeps the soil pH neutral; otherwise, the treatment with electricity and reversing polarity of electrodes will cause a change of soil pH (Luo et al., 2006). The completely symmetrical electric field also exerts the most effective treatment with the greatest electric intensity, which has a positive effect on the oil degradation with bacteria. In conclusion, a proper electric intensity may increase the activities of bacteria and accelerate the biodegradation in soil.

3 Conclusions

This study has shown that the bioremediation of petroleum can be enhanced by electrokinetics. Bacteria may have a persistent effect on the removal of petroleum pollutants from soil in the presence of an appropriate electric current. Although the treatments with an electric field did not change microbe's selectivity in the consumption of the crude oil components during bioremediation, the alkanes were degraded easily. On the other hand, resin-asphaltene was hard to be degraded. The stronger the field intensity was, the higher the removal efficiency was. Two factors are relevant. One is that the proper direct current stimulated the microbial activity. Another is that the proper direct current accelerated the metabolism of the petroleum-degrading bacteria which use oil as the sole carbon source.

Therefore, the rate of removal pollutants and the strength of field intensity were significantly correlated.

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