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Journal of Hazardous Materials B121 (2005) 187–194

Journal of Hazardous Materials

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The use of non-uniform electrokinetics to enhance in situ bioremediation of phenol-contaminated soil

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Received 19 June 2004; received in revised form 3 February 2005; accepted 17 February 2005
Available online 16 March 2005

Abstract

In situ bioremediation is an attractive and often cost-effective technology for the cleanup of organics-contaminated sites, but it often requires extended treatment time under field conditions. This study explored the feasibility of using non-uniform electrokinetic transport processes to enhance in situ bioremediation. A bench-scale non-uniform electrokinetic system with periodic polarity-reversal was developed for this purpose, and tested by using a sandy loam spiked with phenol as a model organic pollutant. The results demonstrated that non-uniform electrokinetic processes could accelerate the movement and in situ biodegradation of phenol in the soil. Bidirectional operation enhanced the phenol biodegradation more effectively than unidirectional operation. At the same time, a smaller polarity-reversing interval induced a higher and more uniform removal of phenol from the soil. The results also showed that reversing the polarity of electric field applied could maintain the soil pH and moisture, but it increased the consumption of electricity.

Keywords: In situ bioremediation; Non-uniform electrokinetics; Electrokinetic transport; In situ biodegradation; Phenol

1. Introduction

In situ bioremediation is often a cost-effective technology for the cleanup of sites contaminated with organic pollutants. It neither alters the intrinsic soil properties nor does it involve transport of contaminants off site [1]. However, the remediation rate is usually very slow. The slow rate of in situ bioremediation results primarily from the limited opportunities of interactions between contaminants and degrading bacteria under in situ conditions [2]. Previous studies had supported this by demonstrating that the well-mixed ex situ systems are often orders of magnitude faster than the undisturbed systems [3]. Unfortunately, the energy consumption and costs associated with the well-mixed ex situ operation are considerable.

An alternative solution is to mix the contaminants, bacteria, and even bacterial nutrients under in situ conditions. This may be achieved by using non-uniform electrokinetics and reversing periodically the polarity of the applied direct current electric field (dc field). When a non-uniform dc field is imposed upon soil matrix, it can induce a variety of transport processes, including electromigration, electroosmosis, electrophoresis and dielectrophoresis [4–8]. These mechanisms can transport the contaminants, degrading bacteria, bacterial nutrients, and even pore fluids through the soil matrix, and thus may have the potential to accelerate the mass transfer and the interactions among contaminants, bacteria, and bacterial nutrients during in situ bioremediation. At the same time, reversing the polarity of dc field can change the movement direction, and hence may produce more opportunities for the bacteria and contaminants in soils to contact and interact with each other.

The objective of this study is to investigate the effectiveness of using non-uniform electrokinetic processes to en-

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doi:10.1016/j.jhazmat.2005.02.007

hance in situ bioremediation of soils contaminated with organic pollutants. For this purpose, a bench-scale non-uniform electrokinetic system with periodic polarity-reversal was developed, and tested by using phenol and a sandy loam soil as model organic chemical and soil, respectively. The choice of phenol was based on its prevalence in the environment and its biodegradability in a wide range of concentration [9,10].

2. Background

When a non-uniform dc field is applied to a soil matrix, it can induce various complex electrochemical and geochemical transport processes and phenomena including electroosmosis, electromigration, electrophoresis, dielectrophoresis, and electrolysis reaction. Electroosmosis is the mobilization of pore fluids in an electric field, usually from the anode toward the cathode. Electroosmotic flow is able to drive the free-phase dissolved and even the sorbed organics toward the cathode [11]. Electromigration is the movement of ionic species in a dc field; the cations towards the cathode while the anions towards the anode. Due to the combined effects of electromigration and electroosmosis, the movement of cationic species towards cathode is enhanced whereas the movement of anionic species towards anode is reduced [12]. Since electromigration rate is at least one order of magnitude greater than the electroosmotic flow [13], electromigration generally dominates the mass transport. In addition, electrophoresis is the transport of charged particles such as clay particles or bacterial cells toward the electrode opposite in polarity, while dielectrophoresis is the translational motion of neutral matter and even bacteria toward strongest field region due to polarization effects in a non-uniform field regardless of its direction [8,14].

Importantly, when a dc field is applied to wet soils through inert electrodes, electrolysis of pore fluids occurs at both electrodes [15]:

$$\text{At the anode: } 2H_2O \rightarrow 4H^+ + O_2(g) + 4e^-$$

$$\text{At the cathode: } 2H_2O + 2e^- \rightarrow 2OH^- + H_2(g)$$

The oxidation at anode generates an acid front, while the reduction at cathode produces a base front. These acids and bases will advance through soil matrix by diffusion, electromigration and electroosmosis, and thus may change soil pH [13]. The change in soil pH will affect not only the rate and even direction of electroosmotic flow [15,16], but also the sorption and biodegradation of organics in soils. Therefore, pH control is of significance for electrokinetic treatment. Applying conditioning agents and constructing modified reactors are the two common strategies in this field [7]. However, reversing the polarity of electric field seems a simple solution, since the H^+ and OH^- generated at electrodes may be neutralized automatically by this way.

As an ionizable organic chemical, phenol can exist in both neutral and ionized form depending on the pH of environmental media. The concentration of ionized phenol will increase with pH. The sorption of phenol by soil media depends partly upon its form. The neutral phenol is expected to undergo more sorption than the ionized form [17]. In highly alkaline soils, phenol exists mainly in its ionized form and has demonstrated to be poorly absorbed. In addition, the ionized and neutral phenol are mobilized mainly by electromigration and electroosmotic flow, respectively. Therefore, it can be reasonably inferred that the movement of phenol in soils may vary with its dissociation degree, ultimately with the soil pH.

3. Materials and methods

3.1. Soil

A sandy loam was used in this study, with various characteristics as described in previous research [18]. The soil was obtained from the topsoil layer (0–30 cm) of a woodland near Tsinghua University, passed through a 2 mm sieve, sterilized by an autoclave three times, dried at 105 °C, and then stored in a desiccator for the tests.

3.2. Bacteria

A mixed culture of phenol-degrading bacteria was used. The bacteria were isolated from a soil contaminated with petrochemicals by using basic mineral media with phenol as the sole carbon source (designated as MP media here). The MP media consisted of 3.0 g K_2HPO_4, 1.5 g KH_2PO_4, 1.25 g $(NH_4)_2SO_4$, 10 mg NaCl, 100 mg $MgSO_4$, 1.0 mg $FeSO_4{\cdot}7H_2O$, and 500 mg phenol per liter of deionized water. The pH was adjusted to 7.0. All the chemicals used were of analytical grade.

The bacterial cells were grown in MP media on a shaker at 30 °C and 150 rpm, harvested in the exponential growth phase by centrifugation, washed twice, and then resuspended in sterilized deionized water to obtain highly concentrated bacterial suspension for the tests.

3.3. Testing system

A schematic diagram of the testing system was shown in Fig. 1. It consisted of a soil cell, a pair of electrodes, an electrode control system, an electric current and voltage real-time monitoring system, and a dc power supply. The soil cell was made of Perspex with an inner size of length 24 cm × width 12 cm × height 10 cm. Column-shaped graphite electrodes, length 12 cm × diameter 0.5 cm, were used to generate non-uniform electric field. The electrode control apparatus was capable of reversing the polarity of electric field, thus allowing changing the operation mode during the tests. The monitoring system could monitor electric current and voltage on-line and store them into a personal computer for later

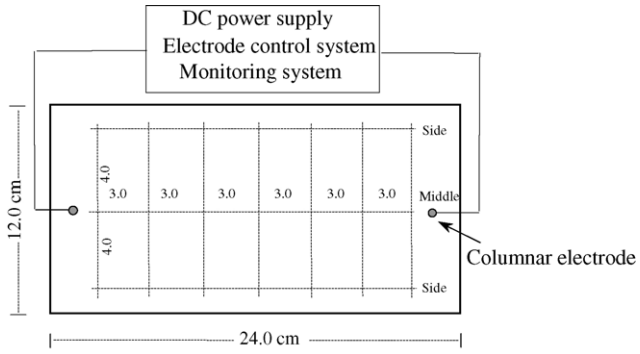

Fig. 1. Schematic of experimental set-up and sampling positions.

analysis. The power supply could provide a constant dc electric voltage in a range from 0 to 60 V for the electrokinetic tests.

3.4. Testing procedure

The soil was spiked with phenol in a ratio of 200 mg-phenol/kg-dried-soil by mixing phenol solution into the sterilized soil and adjusting moisture to 17.5% (w/w) (a water saturation of 53%). About 1.2 kg of the moist soil was then tamped into the soil cell in layers, vibrated the cell, and compacted for 12 h at a pressure of 0.1 kg/cm^2 so as to minimize the void space in the soil. The extruded pore fluids were removed from the surface layer using bibulous paper. A soil bed with a volume of length 24 cm × width 12 cm × height 2.5 cm was prepared for each test.

The phenol-degrading bacteria were inoculated into a specific location (local inoculation), or into the whole soil specimen (whole inoculation). Local inoculation was performed using a pipette to deliver 500 μL aliquots of highly concentrated cell suspension (1.8×10^{12} CFU/ml) at approximately 0.5 cm intervals across the width of soil specimen. The pipette tip was inserted into the compacted soil so that its end was 0.5 cm below the surface, and then expelled the cell suspension gently while pulling the pipette out. Whole inoculation was conducted by mixing bacterial suspension (1.2×10^{10} CFU/ml) directly into the soil during preparing soil specimen. Before the reactor assembly was conducted, a fraction of soil specimen was obtained to determine the actual initial content of phenol, soil pH and moisture, since a portion of phenol and water could volatilize during the preparation.

Two electrodes were oppositely installed into the soil bed with a distance of 20 cm from each other. Power supply, electrode control apparatus, and monitoring equipment were then connected to the electrodes. Once the assembly was completed, the electrokinetic reactor was enclosed with a perspex cover to prevent the soil bed from excessive evaporation of water and phenol. A constant voltage gradient of 1.0 V/cm was then applied through the electrodes in one way (unidirectional operation) or two ways (bidirectional operation), and the voltage and electric current through the soil bed were recorded every 15 min by the real-time monitoring system.

Three separate groups of tests were conducted. The first was to investigate the mobilization of phenol and the change of soil pH and moisture when adopting different operation mode. The resulting information was used to determine the position at which the bacteria were injected and the operation parameters for the subsequent tests. The second and the third were to evaluate the in situ biodegradation of phenol coupled with non-uniform electrokinetics under local and whole inoculation, respectively. Control tests, with no electric field applied or no bacteria inoculated, were run in parallel.

At the end of test, the soil bed was destructively sampled using a U-shaped sampler and a spatula to determine the phenol, phenol-degrading bacteria, soil pH and moisture. In order to reflect the spatial variation regarding the variables, sampling lines were arranged along the middle line on which the two electrodes were located, and along the sideline with a distance of 4 cm from the middle line. Seven spots, with distances of 1, 4, 7, 10, 13, 16 and 19 cm from the (initial) anode, were sampled on each line, as shown in Fig. 1.

Methylene chloride was used to extract phenol from the soil samples. About 1.0–1.5 g of soil sample was mixed with 10 ml of methylene chloride in a Teflon-sealed glass vial, extracted by sonication, and filtered through 0.45 μm membranes. The filtrate was then analyzed by high performance liquid chromatography (HPLC; Hewlett Packard 1050) at 275 nm using a C18 reverse phase column (Agilent, Zorbax extend-C18) and a mobile phase containing methanol and 2% (v/v) acetic acid (60:40, v/v). The HPLC was calibrated using four external standards prior to performing chemical analyses.

About 0.5 g of soil sample was taken to examine the bacteria distribution across the soil bed. Colony forming unit (CFU) was counted by preparing serial dilutions of soil suspensions in sterile 0.1% (w/v) sodium pyrophosphate, plating onto MP-agar plate, and incubating at 30 °C for 2 days.

In addition, soil pH was determined using a soil-to-water ratio of 1:2.5 and water content was determined using the methods described by Lu [19]. All the analysis was performed in triplicate, and the result was calculated as the average.

4. Results and discussion

4.1. The change in soil pH and moisture

The applied dc field induced the change in soil pH and moisture depending on the operation mode, as shown in Figs. 2 and 3. Unidirectional operation dramatically changed the soil pH and water content. After one-directional running for 14 days, the soil pH near the anode dropped to pH 2.6 while near the cathode increased to about pH 12 from the initial pH 7.7; the soil moisture near the anode dropped by 10% while near the cathode increased by 11% (Fig. 2). The extreme pH condition and the soil consolidation due to water loss were considered not favorable for the biodegradation of organic pollutants in soils. As contrast, when reversing the po-

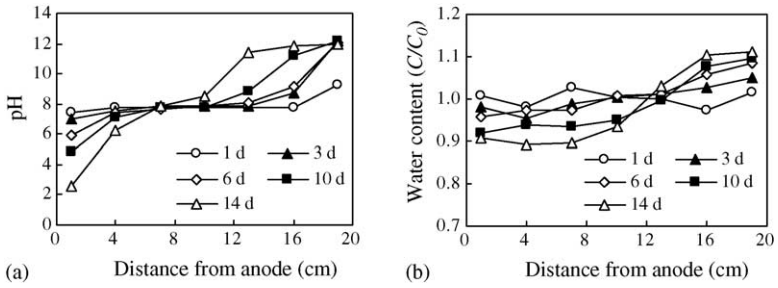

Fig. 2. The change in soil pH (a) and moisture (b) under unidirectional operation.

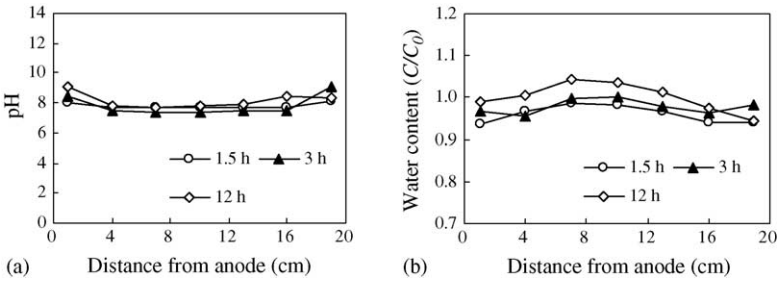

Fig. 3. The change in soil pH (a) and moisture (b) under bidirectional operation for 10 days at different polarity-reversal intervals.

larity of electric field at intervals of 1.5, 3 and 12 h, the soil pH was maintained in a range from 7.4 to 9.0, and the soil moisture changed no more than 5% (Fig. 3), indicating that the soil properties could be kept undisturbed by reversing the polarity of the applied electric field at an interval of no more than 12 h. Therefore, bidirectional operation may be favorable for in situ bioremediation.

4.2. The mobilization of phenol in the soil

A saturated sorption amount of about 240 mg-phenol/kg-dried-soil was previously reported for the soil [18]. In this study, the actual initial content of phenol was about 165 mg/kg, much less than the saturated sorption amount. Mobilization of the phenol at this content requires desorption of phenol from soil particle surface since most of the phenol are absorbed at this content [11]. At the beginning of test, the phenol in the spiked soil was uniformly distributed. Under a constant electric gradient of 1.0 V/cm, however, the phenol was concentrated to a specific region depending upon the treatment time and the operation mode, as shown in Figs. 4 and 5. These findings demonstrated that the applied dc field could desorb and mobilize the phenol through the soil matrix.

Under unidirectional operation, the phenol in the anode region was gradually reduced while in the cathode region was increased before 10 days, indicating that the phenol was mobilized to the cathode region over this period. After 10 days, however, it was quite the contrary; the phenol was moved gradually to the anode region. Moreover, the peak phenol content was observed at 1 cm away from the cathode after 1 day; over the period from 3 to 10 days, the peak values were all at 4 cm from the cathode; however, the peak con-

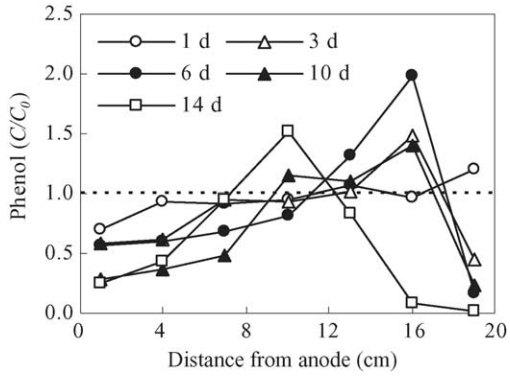

Fig. 4. The temporal variation of phenol in the soil under unidirectional operation.

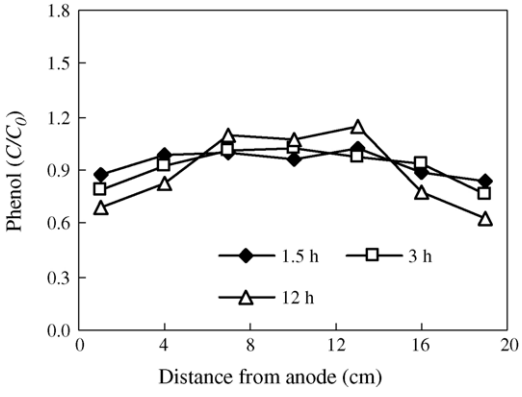

Fig. 5. The effect of polarity-reversal on the movement of phenol in the soil (10 days).

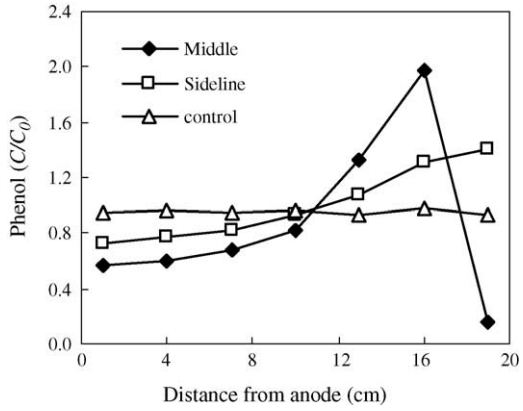

Fig. 6. The spatial variation of phenol in the soil under non-uniform electrokinetics (6 days).

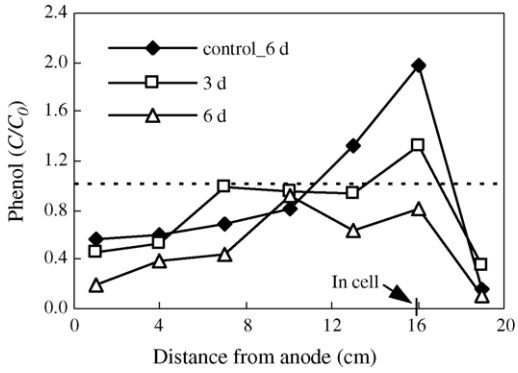

Fig. 7. The biodegradation of phenol in the soil under unidirectional operation (local inoculation).

tent was at 10 cm from the cathode after 14 days (Fig. 4). These results suggested that, under unidirectional operation, the phenol was moved much faster after 10 days. This might result from the change of soil pH during the test. With treatment time increased, the soil pH near the anode gradually decreased while near the cathode increased (Fig. 2a). Therefore, the ionized phenol in the cathode region would increase, suggesting that an increasing amount of phenol might be moved by electromigration to the anode. At the same time, the electroosmotic flow towards the cathode region would decrease as a result of soil pH drop in the anode region [10,20], and thus a decreasing amount of phenol would be dragged towards the cathode region by the electroosmotic flow.

Under bidirectional operation, however, no accumulation of phenol was observed in spite of the slight increase in the middle cell after 10 days (Fig. 5). Reversing the polarity of electric field can change the movement direction, and hence may cause the phenol to move back and forth through the soil.

In addition, in a non-uniform dc field, the movement of phenol varied spatially, as shown in Fig. 6. The phenol in the middle region was concentrated to 4 cm away from the cathode with a maximum increase of 98% after unidirectional operation for 6 days, while in the side region the phenol was accumulated at 1 cm away from cathode with a maximum increase of 40%. This may result from the spatial variation of electric field density throughout the soil bed. The electric field density is greater in the middle region than in the side region [8], and thus greater driving forces can be induced in the middle region.

4.3. The in situ biodegradation of phenol under local inoculation

From the results of the first experiment, in which the phenol could be concentrated to a region of about 4 cm away from the cathode under one-directional operation for 3–10 days (Fig. 4), it was decided to add the phenol-degrading bacteria into that region in attempt that the bacteria would be moved in a direction opposite to the movement of phenol. The bacteria could be transported by electrophoresis to the anode region due to their negative surface charge [21], and hence more opportunities might be produced for the phenol to be attacked by the inoculants under such conditions. The results (shown in Fig. 7) showed that, after unidirectional operation for 3 and 6 days at 1.0 V/cm, the phenol in the middle region dropped on average by 21 and 50%, respectively. Compared with the control test in which no bacteria were inoculated, the phenol in the regions of 4–10 cm away from the cathode decreased 42% after 6 days; however, about 80% of the initial phenol still remained in this region. These suggested that, under local inoculation, unidirectional operation could not effectively enhance the in situ biodegradation of phenol in the soil.

Three factors associated with unidirectional operation might contribute to the higher local phenol remains. The first was the sharp change in soil pH and moisture (Fig. 2), which would affect adversely the biodegradation process in the soil. The second was the concentrating effect (Fig. 4), which could hinder the bulk of phenol from approaching the inoculants. The third was the limited chance to interact between the inoculants and the phenol since, under unidirectional operation, the locally injected bacteria were moved as a whole to the anode region [5,21,22], and hence might be dispersed inadequately throughout the soil.

However, reversing the polarity of electric field could maintain the soil pH and moisture (Fig. 3) as described above, and also could manipulate the movement of both phenol (Fig. 5) and bacterial inoculants [21] in the soil, which might be favorable for in situ biodegradation of phenol. In order to demonstrate this, the biodegradation of phenol under bidirectional operation was tested by injecting the phenol-degrading bacteria at the middle location of 10 cm away from the cathode and reversing the polarity of electric field every 12 h. Two bidirectional operation modes were tested: continuous and intermittent operation. The intermittent operation was performed by switching the power off for 12 h between two adjacent polarity-reversals. The intermittent operation allowed

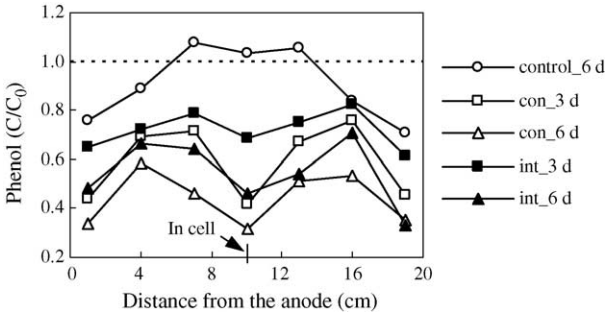

Fig. 8. The biodegradation of phenol in the soil under bidirectional operation (local inoculation).

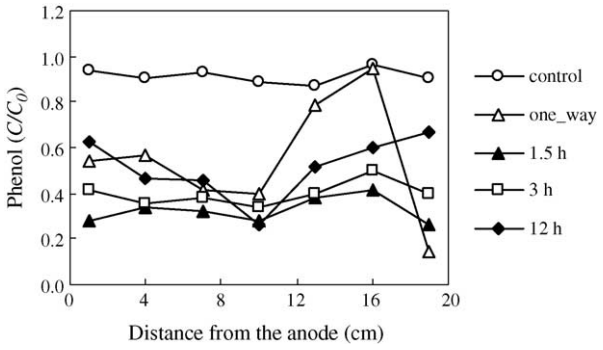

Fig. 9. The biodegradation of phenol in the soil under whole inoculation (10 days).

providing adequate time for the bacteria and phenol to interact before they were mobilized again.

The results (shown in Fig. 8) showed that, under continuous operation, the phenol in the middle region dropped on average by 41 and 56% after 3 and 6 days, respectively, while under intermittent operation, just dropped by 28 and 45%, respectively. Compared with the control test in which no bacteria were inoculated, the phenol dropped on a whole 51 and 40% after 6 days under continuous and intermittent operation, respectively. These findings demonstrated that, under local inoculation, reversing the polarity of electric field could accelerate the in situ biodegradation of phenol in the soil. In addition, intermittent operation produced a lower phenol removal of 19% than continuous operation, but it consumed only about half of electricity since the electric field was applied only for half of the total operation time. Therefore, intermittent operation might be valuable under local inoculation.

4.4. The in situ biodegradation of phenol under whole inoculation

In order to minimize the effect of bacterial diffusion and biofouling that might occur under local inoculation, the phenol-degrading bacteria were injected into the whole soil bed by mixing the bacterial suspension with the soil during soil preparation. At the beginning of test, the inoculants and the phenol were both evenly distributed in the soil, and hence a maximum chance of contact between them might exist. Under no application of electric field, however, the phenol just decreased on average about 9% after 10 days, suggesting that the mass-transfer governed the biodegradation of phenol by the inoculants even if they were both dispersed uniformly throughout the soil. When a constant electric gradient of 1.0 V/cm was applied in one-way for 10 days, the phenol in the middle region dropped on average by 46%, but 86% of the initial phenol still remained in the region of 4–7 cm from the cathode (Fig. 9). These indicated that, under whole inoculation, unidirectional operation stimulated the biodegradation of phenol in the soil, but it still caused the phenol to concentrate to specific regions, just like the cases under local inoculation (Fig. 7) and no inoculation (Fig. 4).

However, when reversing the polarity of electric field every 1.5, 3 and 12 h, the phenol in the middle region dropped on average by 67, 60 and 49%, respectively, after continuously running for 10 days. Moreover, the phenol was relatively uniformly removed at intervals of 1.5 and 3 h; while at an interval of 12 h, the phenol remained higher near both electrodes and lower in the middle cell (Fig. 9). These revealed that polarity-reversal could enhance significantly the in situ biodegradation of phenol in the soil, and the faster the polarity-reversal, the higher the biodegradation rate. An interval of no more than 3 h seemed to be appropriate under whole inoculation.

More importantly, the phenol in the side region (4 cm away from the middle region) also decreased (about 54%) under continuous operation for 10 days at an interval of 3 h (Fig. 10), suggesting that these sideline regions were still the effective range in the non-uniform electric field. However, the phenol-degrading bacteria were distributed mainly in the middle region, especially in the two electrodes regions (Fig. 11). Under continuous operation for 10 days at an interval of 3 h, the bacteria in the middle region were about 1.6 times higher than that in the sideline regions. This might result from the dielectrophoretic movement of bacteria in a non-uniform dc field. Under bidirectional operation, the phenol could not be mobilized significantly (Fig. 5), but the bacteria might be moved

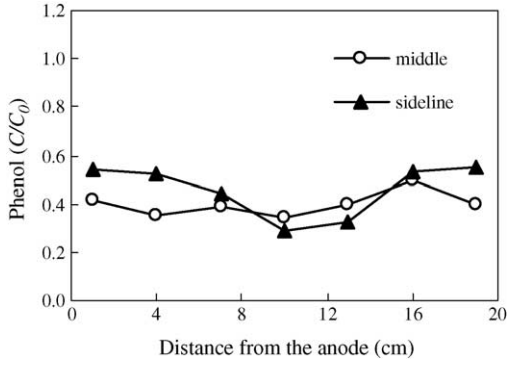

Fig. 10. The spatial distribution of phenol in the soil under bidirectional operation at an interval of 3 h (whole inoculation, 10 days).

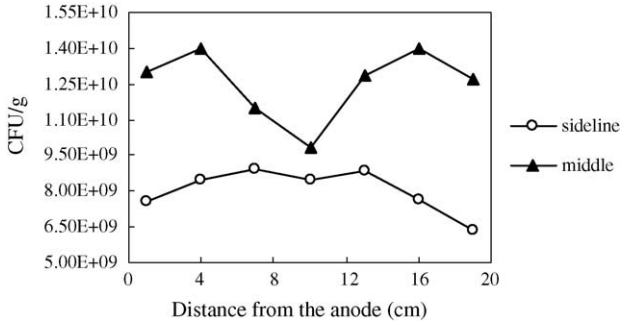

Fig. 11. The spatial distribution of phenol-degrading bacteria in the soil under bidirectional operation at an interval of 3 h (whole inoculation).

by dielectrophoresis to the two electrodes, regardless of the direction of electric field applied [8].

In addition, under intermittent operation for 10 days at an interval of 12 h, the phenol in the soil just dropped on average by 22%, much lower than that under continuous operation (Fig. 12), suggesting that continuous operation might be desirable under whole inoculation.

4.5. The consumption of electricity

The electricity consumption per unit volume of soil was calculated by the following equation [23]:

$$E_u = \frac{1}{V_s}\int_0^t UI\,dt$$

where E_u is the electricity expenditure per unit volume of soil (kW h/m^3), V_s the soil volume (m^3), U the electric potential difference across the electrodes (V), I the electric current (A), and t the treatment time (h). The results showed, when reversing the polarity of dc field every 1.5, 3.0 and 12 h, the electricity consumption in 10 days was 64.5, 63.6 and 59.9 kW h/m^3, respectively, whereas unidirectional operation just consumed 40 kW h/m^3. This implied that polarity-reversal could increase the energy consumption in comparison with unidirectional operation, and the faster the polarity-reversal, the greater the electricity consumption. Polarity-reversal may induce a frequent change in double layer, and consume a relatively high capacitive part of the current due to the double layer discharging or recharging [24]. Therefore, a proper interval should be adopted for the purpose of saving energy.

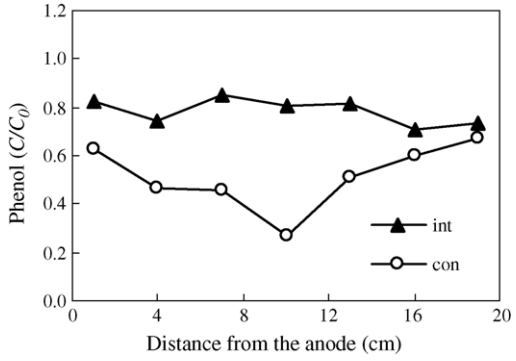

Fig. 12. The effect of polarity-reversal mode on biodegradation of phenol in the soil under whole inoculation (at an interval of 12 h, 10 days).

5. Conclusions

(1) Non-uniform electrokinetic processes can effectively enhance the desorption and movement of phenol in sandy loam depending upon the operation mode. Under unidirectional operation, the phenol was moved and accumulated to specific regions. However, reversing the polarity of electric field could disperse the phenol throughout the soil.

(2) Non-uniform electrokinetics can accelerate effectively the in situ biodegradation of phenol in sandy loam, the efficiency of which is related to the operation mode. Reversing the polarity of electric field could effectively enhance the biodegradation of phenol in the soil depending upon the polarity-reversing interval. A faster polarity-reversal could produce a more uniform and higher phenol removal from the soil. Unidirectional operation could also accelerate the phenol biodegradation, but it caused higher phenol remains in specific regions.

(3) Operation mode has important effect on soil properties and energy consumption. Unidirectional operation induced the extreme soil pH and soil consolidation, whereas bidirectional operation could maintain the soil pH and moisture at large if adopting a proper polarity-reversing interval. However, polarity-reversal increased the electricity consumption; the faster the polarity-reversal, the greater the electricity consumption.

Acknowledgements

This work was supported partly by the National 863 program of China (2002AA601120, 2004AA649220) and partly by the RITE of Japan.

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