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# Application of a new electrolyte circulation method for the ex situ electrokinetic bioremediation of a laboratory-prepared pentadecane contaminated kaolinite

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**Abstract**

Ex situ electrokinetic (EK) bioremediation of a laboratory-prepared pentadecane-contaminated kaolinite was carried out. Extraneous bacteria and ionic nutrients were continuously supplied to the soil specimen by a new electrolyte circulation method, which controlled electrical pH change of electrolyte solution to keep bacterial activity. During the EK bioremediation the anode region showed the highest colony forming unit (CFU) due to electrical attraction between anode and bacteria. Simultaneous increases of CFU and uniform pentadecane removal in most soil regions demonstrated that electro-osmosis as well as electrophoresis affected the bacterial transport in soil. At 3.13 mA/cm$^2$, increase in soil temperature to above 45 °C inhibited bacterial activity, which caused the decrease of removal efficiency. The removal amount of pentadecane increased with initial pentadecane concentration at the same current densities (0.63 and 1.88 mA/cm$^2$) because of the increased amount of weakly bound pentadecane onto the soil surface. The highest removal efficiency (77.6%) was obtained at 0.63 mA/cm$^2$ for 1000 mg/kg pentadecane after 14 days. Consequently, the present methods of EK bioremediation demonstrated superiority over the conventional bioremediation, which had inherent demerits of slow degradation and low removal efficiency.


*Keywords:* Electrokinetic bioremediation; Pentadecane; Kaolinite; Extraneous bacteria; Electrolyte circulation method

## 1. Introduction

The widespread usage and storage of petroleum fuels have made petroleum hydrocarbons the most prevalent soil and groundwater contaminants. The treatment of sites contaminated with long chain alkanes and polycyclic aromatic hydrocarbons involved in petroleum fuels has limitations because of their properties such as low volatility, low mobility, low solubility and low degradability [1,2].

Bioremediation has been applied to various contaminated sites for several decades, because it has many advantages such as permanent elimination of waste, cheaper biological system, positive public acceptance, minimum site disruption, risk elimination with long-term liability and combination with other treatment techniques [3]. In a heterogeneous and/or low permeability soil, however, bacteria cannot sufficiently metabolize contaminants due to the transport limitation of bacteria or nutrients, and so an additional management is required [4,5].

When a direct current (dc) field is introduced across soil deposits through inert electrodes, the EK phenomena such as electro-osmosis, electrophoresis, and electromigration cause the transport of various compounds even in a low permeability soil [6–10]. Therefore, application of EK phenomena to bioremediation, namely EK bioremediation, can uniformly and rapidly supply nutrients, electron donors/acceptors, and bacteria to soil. It is generally known that electrophoresis is an important mechanism for bacterial transport [4,5], which depends upon the surface charge density of individual or

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aggregative bacteria. However, few researches on EK bioremediation have been reported.

In this study, ex situ EK bioremediation of a laboratory-prepared pentadecane-contaminated kaolinite was carried out. A new electrolyte circulation method was employed to improve the process efficiency by supplying bacteria and ionic nutrients continuously and preventing electrical pH change. The effects of electric current density and initial pentadecane concentration were investigated on bacterial behavior and removal efficiency. The process feasibility was evaluated for petroleum hydrocarbon-contaminated sites compared to other conventional bioremediation.

## 2. Materials and methods

### 2.1. Microorganism and electrolyte composition

The microorganism used in this study was a bacterial consortium consisting of several *Pseudomonas* sp. (L Company, Korea). Electrolyte was prepared to supply the bacterial consortium with ionic nutrients and to increase the electrical conductivity of soil [11]. The composition of electrolyte is as follows (g/l): $Na_2HPO_4{\cdot}12H_2O$ 18.1; $KH_2PO_4$ 4.4; $MgSO_4{\cdot}7H_2O$ 0.2; $NH_4Cl$ 1.0; glucose 10.

### 2.2. Shaker-flask culture for bacterial growth and pentadecane degradation

Bacterial growth and pentadecane degradation were observed in a 250 ml flask containing 100 ml of culture solution, of which composition was same as the electrolyte including 5% (v/v) pentadecane (3845 mg/l). Three growth media were prepared to avoid the loss of culture solution and pentadecane content in measuring bacterial growth and pentadecane removal each week. The flask cultivation was carried out in a shaking incubator at 30 °C and 150 rpm. The bacterial growth was determined by measuring the optical density at 600 nm using an UV spectrophotometer (Hewlett Packard 8562A, USA). Prior to pentadecane analysis, the bacterial activity was eliminated by adding NaOH into the culture solution to increase pH to above 12. After extracting pentadecane with *n*-hexane at 25 °C and 150 rpm in a shaking incubator, its content was measured by HPLC (details in Section 2.5).

### 2.3. Specimen preparation

Pentadecane (Sigma, USA), one of the long-chain alkanes contained in diesel fuel, was selected as a model pollutant, because EK bioremediation is appropriate for low volatile pollutants. The model soil used in this EK experiment was kaolinite (Sanchung, Korea) screened with the No. 50 sieve (US standard screen). Kaolinite has a relatively low cation-exchange capacity so that several parameters such as electrical potential gradient, bacterial transport, and soil pH under dc field can be evaluated precisely. After autoclaving of 450 g kaolinite at 121 °C for 15 min the soil water content was controlled to be 25% with deionized water of 150 ml. Initial concentrations of pentadecane artificially contaminated were 1000, 5000, 10,000 and 20,000 mg/kg dry soil. The specimen was loaded into the EK test cell, which was constructed with a horizontal rectangular polyacrylate (20 cm × 4 cm × 4 cm) with stainless steel electrodes coated by platinum immersed in the electrode reservoirs (2.5 cm × 4 cm × 4 cm) as shown in Fig. 1. A peristaltic pump was used to circulate the electrolyte solution at a rate of 4.2 ml $min^{-1}$ to control ionic concentrations and sudden pH change in soil and bioreactor [12,13]. Bioreactor contained 1 l of the electrolyte solution.

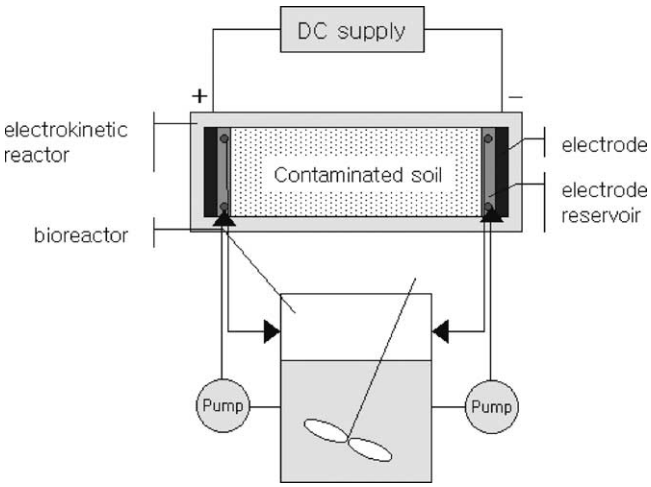


Fig. 1. Schematic diagram of the experimental EK bioremediation.

### 2.4. EK bioremediation

Before the start-up of EK bioremediation, the bacterial consortium was cultivated up to 0.5 of optical density (600 nm) in a bioreactor. After filling electrode reservoirs with the harvested bacterial solution, the EK bioremediation was operated with a constant current for 2 weeks. The bioreactor was operated at 250–300 rpm and 30 °C.

In a preliminary EK experiment, current densities to increase soil temperature to between 25 and 35 °C at which microorganisms grew rapidly were found to be about 0.31–3.13 mA/$cm^2$. Therefore, the ranges of current densities applied in the present experiment of ex situ EK bioremediation were between 0.31 and 3.13 mA/$cm^2$ using a dc power supply with maximum output of 300 V.

The experimental condition is summarized in Table 1: the effect of high current density on the removal efficiency was investigated by experiments 1 and 2. The relationship between contamination level and removal amount was examined at 20,000, 10,000 and 5000 mg/kg pentadecane (experiments 2, 3 and 4). Three different current densities were tested to find an optimum value for 5000 mg/kg pentadecane (experiments 4, 5 and 6). The process feasibility was evaluated

Table 1
Experimental condition

| Experiment number | Initial pentadecane concentration (mg/kg dry soil) | Current density ($mA/cm^2$) |
|---|---|---|
| 1 | 20000 | 3.13 |
| 2 | 20000 | 1.88 |
| 3 | 10000 | 1.88 |
| 4 | 5000 | 1.88 |
| 5 | 5000 | 0.63 |
| 6 | 5000 | 0.31 |
| 7 | 1000 | 0.63 |

for the low concentration of 1000 mg/kg pentadecane (experiment 7).

A control EK treatment (5000 mg/kg pentadecane at 0.63 $mA/cm^2$) by a non-inoculated bioreactor was carried out to see the washing effect without contribution of biodegradation.

### 2.5. Analysis

Pentadecane concentration was analyzed by HPLC (Waters, USA) with $C_{18}$ symmetry column and RI detector. At the end of the experiments, the soil specimen was promptly removed from the EK cell, and sliced into 5 or 10 segments of uniform thickness. Each segment was dried at room temperature for 4 days, and 5 g of the dried soil powder was mixed with 10 ml of *n*-hexane in a 50 ml serum bottle. The extraction was performed at 25 °C and 180 rpm in a shaking incubator for 2 days. After centrifuging the suspended soil at 6000 rpm for 10 min, clear supernatant was injected into the HPLC system. The flow rate of eluent was 1.0 ml $min^{-1}$, which consisted of acetonitrile and ethanol (3:2, v/v). Colony forming unit (CFU) in soil solution was calculated from relative light unit measured by Profile™ (New Horizons, USA).

## 3. Results and discussion

### 3.1. Shaker-flask culture for bacterial growth and pentadecane degradation

As shown in Fig. 2A, the optical density with the addition of pentadecane was higher than that in the absence of pentadecane. This is because the bacterial consortium used pentadecane as an additional carbon source and fine emulsions containing bacteria, pentadecane, and water were formed. The pH decrease below 5 in all bioreactors was caused by some organic acids produced through the bacterial glucose oxidation and pentadecane degradation [14]. The surface tension with pentadecane was lower than that without pentadecane because of bacterial biosurfactant production (Fig. 2B). The color of culture solution became milky as the amount of fine emulsion increased. The dissolution type was Winsor type III, which could be made under an excess oil phase [15]. After 3 weeks, the removal efficiency of pentadecane was 26% (Fig. 2C). Consequently, the obtained results demonstrated

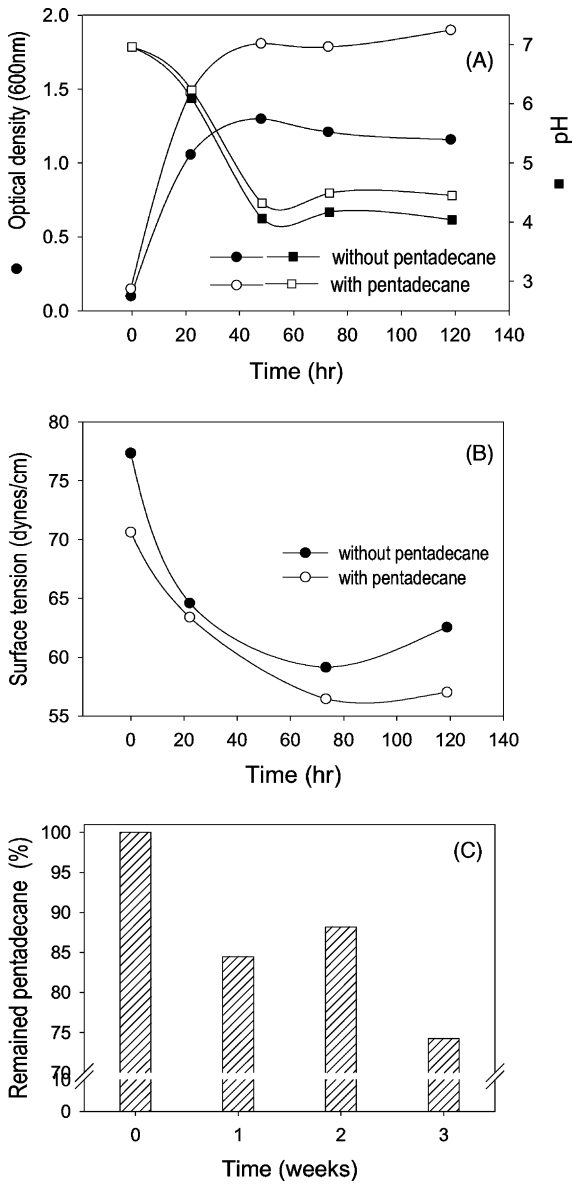


Fig. 2. Change in bacterial growth (A), surface tension (B) and pentadecane degradation (C) in shaker-flask culture. (●, ■) Without pentadecane and (◯, □) with pentadecane.

that the bacterial consortium was effective on pentadecane removal in EK bioremediation. The surface tension decrease was so small that pentadecane dissolution would hardly occur in EK bioremediation.

### 3.2. Electrical potential gradient and soil temperature

Fig. 3A shows the change of electrical potential gradient during EK bioremediation. As ions were electrically supplied to soil [16], the electrical potential gradient continuously decreased. As ionic concentrations reached the steady state by electrolyte circulation, the electrical potential gradients were maintained constantly. At the steady state, the electrical potential gradient was proportional to current density.

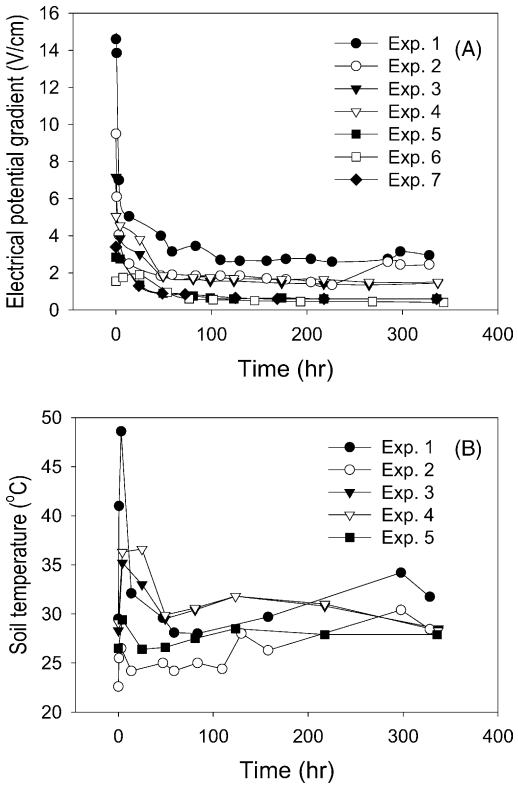


Fig. 3. Change in electrical potential gradient (A) and soil temperature (B) with initial pentadecane concentration and current density. (experiment 1 (●): 20,000 mg/kg pentadecane and 3.13 mA/cm$^2$; experiment 2 (○): 20,000 mg/kg and 1.88 mA/cm$^2$; experiment 3 (▼): 10,000 mg/kg and 1.88 mA/cm$^2$; experiment 4 (▽): 5000 mg/kg and 1.88 mA/cm$^2$; experiment 5 (■): 5000 mg/kg and 0.63 mA/cm$^2$; experiment 6 (□): 5000 mg/kg and 0.31 mA/cm$^2$; experiment 7 (♦): 1000 mg/kg and 0.63 mA/cm$^2$).

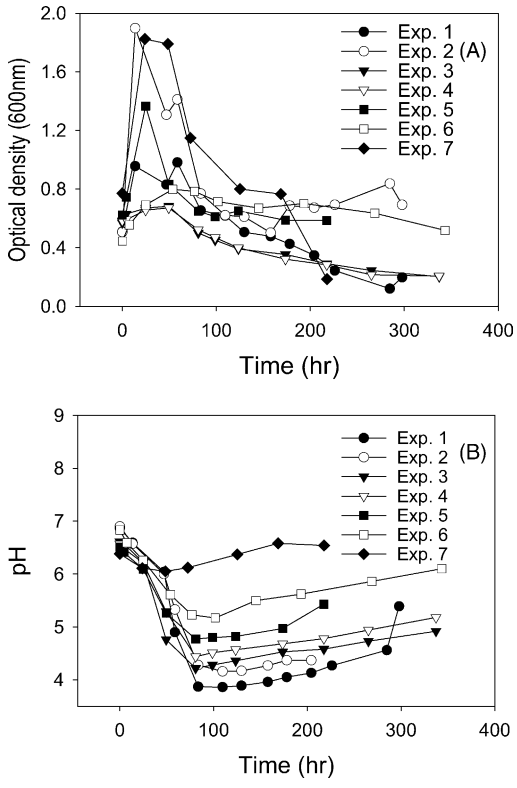


Fig. 4. Change in bacterial growth (A) and pH (B) in bioreactor with initial pentadecane concentration and current density during EK bioremediation. (experiment 1 (●): 20,000 mg/kg pentadecane and 3.13 mA/cm$^2$; experiment 2 (○): 20,000 mg/kg and 1.88 mA/cm$^2$; experiment 3 (▼): 10,000 mg/kg and 1.88 mA/cm$^2$; experiment 4 (▽): 5000 mg/kg and 1.88 mA/cm$^2$; experiment 5 (■): 5000 mg/kg and 0.63 mA/cm$^2$; experiment 6 (□): 5000 mg/kg and 0.31 mA/cm$^2$; experiment 7 (♦): 1000 mg/kg and 0.63 mA/cm$^2$).

As shown in Fig. 3B, the soil temperatures were changed with electrical potential gradient, because it depended upon electric field strength and the resistivity of the medium. At current densities between 0.63 and 1.88 mA/cm$^2$, the soil temperatures were changed between 23 and 37 °C, which were proper for bacterial growth. At 3.13 mA/cm$^2$, however, the soil temperature increased to above 45 °C initially when the bacterial activity was severely inhibited.

### 3.3. Bacterial growth in bioreactor

As shown in Fig. 4A, optical density in bioreactor rapidly increased initially, because the bacterial growth rate was accelerated by oxygen generated at anode by electrolysis reaction, which implied the improvement of pentadecane degradation in soil as well [17]. The microorganism used in this study was not single strain, but bacterial consortium consisting of several *Pseudomonas* sp. and thus the optical density was changed irregularly. Even in the same cultivation conditions such as medium composition, temperature, shaking speed, etc., the bacterial growth was sometimes different according to the instantaneous condition of inoculated bacteria such as strain composition and growth phase. Nevertheless, the bacterial quantity in bioreactor was appropriate for pentadecane degradation in soil.

The pH in bioreactor decreased with the increases of current density and initial pentadecane concentration (Fig. 4B), because oxygen generation rate through electrolysis reaction depended upon total current concerning the production of organic acids through glucose oxidation and pentadecane degradation [14]. The difference in pH among 20,000, 10,000, and 5000 mg/kg pentadecane at 1.88 mA/cm$^2$ was clearly observed. The highest pH in 1000 mg/kg pentadecane was due to its lowest concentration.

In a general EK process pH values of anolyte and catholyte are changed to below 2 and above 12, respectively. However, the electrolyte circulation method used in this study neutralized an acid ($H^+$) and a base ($OH^-$) in a bioreactor [12,13] and thus electrolyte pH and bacterial activity were not influenced by electrolysis reaction.

### 3.4. Microorganism in soil

Fig. 5 shows the change of bacterial population in anode, middle, and cathode regions of soil specimen during EK bioremediation. Anode region showed the highest

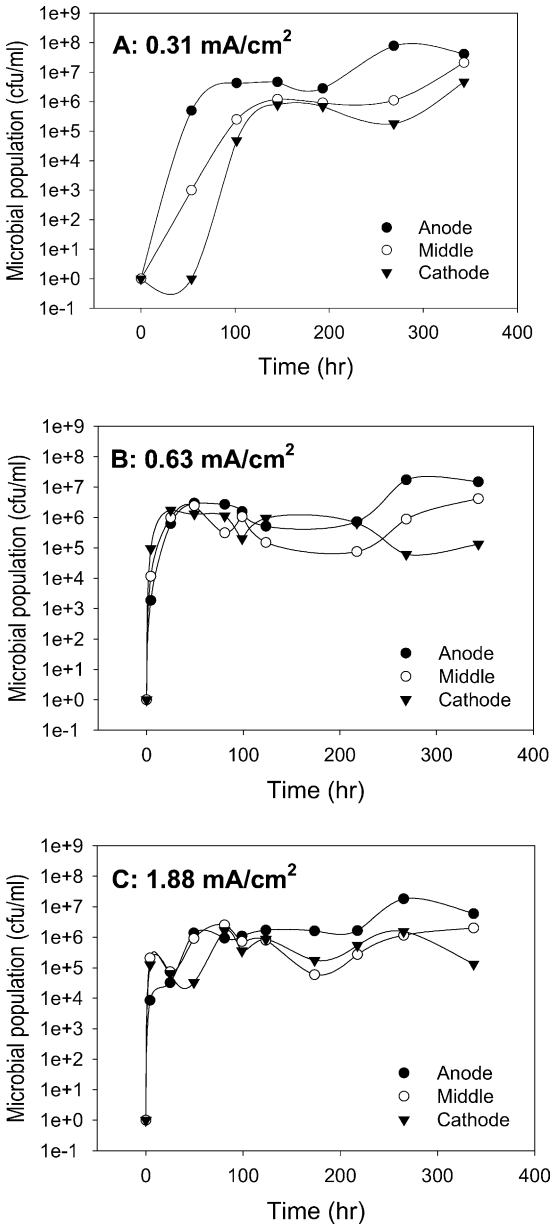


Fig. 5. Change of bacterial population in anode (●), middle (◯) and cathode (▼) regions of soil specimen during EK bioremediation of 5000 mg/kg pentadecane-contaminated kaolinite. (A: 0.31, B: 0.63 and C: 1.88 mA/cm$^2$).

CFU because of electrical attraction between anode and bacteria. The bacterial transport flux by EK phenomena depended upon electric field intensity and thus CFU increase rate at 0.31 mA/cm$^2$ was smaller than those at 0.63 and 1.88 mA/cm$^2$. The CFU at 0.31 mA/cm$^2$ was higher than the others due to a longer residence time in soil.

It was generally known that microbial transport under dc field mainly depended upon electrophoresis [5]. In this study, however, increases of CFU were observed over all soil regions both at 0.63 and 1.88 mA/cm$^2$, which indicated that electro-osmosis as well as electrophoresis was the major mechanism for bacterial transport in soil [18]. At the low current density

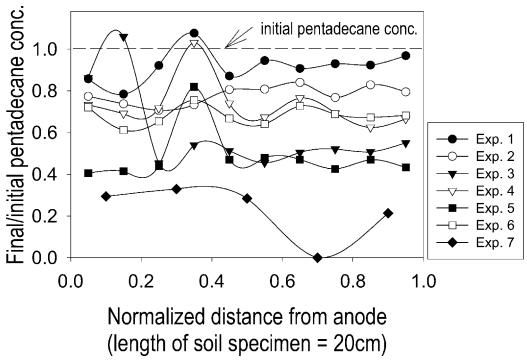


Fig. 6. Final/initial pentadecane concentration with initial pentadecane concentration and current density after 2 weeks of EK bioremediation. (experiment 1 (●): 20,000 mg/kg pentadecane and 3.13 mA/cm$^2$; experiment 2 (◯): 20,000 mg/kg and 1.88 mA/cm$^2$; experiment 3 (▼): 10,000 mg/kg and 1.88 mA/cm$^2$; experiment 4 (▽): 5000 mg/kg and 1.88 mA/cm$^2$; experiment 5 (■): 5000 mg/kg and 0.63 mA/cm$^2$; experiment 6 (□): 5000 mg/kg and 0.31 mA/cm$^2$; experiment 7 (♦): 1000 mg/kg and 0.63 mA/cm$^2$).

of 0.31 mA/cm$^2$, the CFU increased in order of anode, middle and cathode regions mainly by electro-osmosis.

### 3.5. Pentadecane removal

In the case of EK soil flushing, extractants such as surfactant, solvent, absorbent, etc., are supplied from anode reservoir and moved towards cathode reservoir through soil pore and thus the removal efficiency near anode is higher than the other soil regions [19]. However, in the present EK bioremediation pentadecane was evenly removed in most soil regions (Fig. 6), because the bacterial consortium was transported in both directions: electro-osmosis transported the bacterial consortium from anode to cathode along bulk water flow while the direction of transport by electrophoresis was from cathode to anode. Similar results were reported by Wick et al. [18]. Moreover, small pH difference between anolyte and catholyte generated similar pH in all soil regions, which did not make any difference in bacterial activity with location of soil specimen (data not shown).

Table 2 shows summary of pentadecane removal after 2 weeks of EK bioremediation. At 3.13 mA/cm$^2$ the soil temperature increased to above 45 °C initially when the bacterial activity was severely inhibited and so a very low removal (8.2%) at 3.13 mA/cm$^2$ was obtained compared to 22.1% at

Table 2
Summary of pentadecane removal after 2 weeks of EK bioremediation

| Experiment number | Removal amount (mg/kg dry soil) | Removal efficiency (%) |
|---|---|---|
| 1 | 1640 | 8.2 |
| 2 | 4420 | 22.1 |
| 3 | 4030 | 40.3 |
| 4 | 1335 | 26.7 |
| 5 | 2585 | 51.7 |
| 6 | 1590 | 31.8 |
| 7 | 776 | 77.6 |

1.88 mA/cm$^2$ for the same concentration (20,000 mg/kg) of pentadecane. The removal amount of pentadecane increased with initial pentadecane concentration at the same current densities (0.63 and 1.88 mA/cm$^2$) because of the increased amount of weakly bound pentadecane onto the soil surface. The optimum current density for 5000 mg/kg pentadecane was 0.63 mA/cm$^2$ presumably because of appropriate environmental conditions for the bacterial growth. The low concentration (1000 mg/kg) of pentadecane at 0.63 mA/cm$^2$ allowed the highest removal efficiency (77.6%).

In a non-inoculated control EK treatment of 5000 mg/kg pentadecane at 0.63 mA/cm$^2$, only 320 mg/kg was removed from the soil specimen and thus it could be deduced that less than 320 mg/kg for 1000 mg/kg pentadecane would be removed by washing effect alone. The result of present control experiment is similar to that of Saichek and Reddy [19] who reported that EK flushing without extractants hardly removed phenanthrene for about 6 months. Considering the similar difficulties in transport through soil pores because of large carbon numbers of both pentadecane and phenanthrene, we could easily make sure that the amount of pentadecane carried by electro-osmotic flow and found in the effluent stream from the soil system was very small, which implies the biodegradation in the soil specimen was dominant mechanism of removal.

Boopathy [20] and Hess et al. [21] reported that in a laboratory-prepared bioremediation of 550 and 1100 mg/kg diesel-contaminated soil, 81 and 31% of initial concentrations were degraded for 310 and 96 days, respectively. Consequently, the present EK bioremediation could greatly shorten the period of operation to achieve the same removal efficiency for similar type of contaminant compared to other conventional bioremediation. A short treatment period is very important for the cost-effectiveness in a field application of remediation technologies.

## 4. Conclusions

Investigations were carried out on EK phenomena coupled with conventional bioremediation to increase the mobility of extraneous bacteria and nutrients. The electrolyte circulation method employed in this study continuously provided the soil specimen with the bacterial consortium and ionic nutrients, and controlled electrical pH changes to keep bacterial activity.

Anode region of soil specimen showed a higher CFU than the other regions because of electrical attraction between anode and bacteria. Increases of CFU and uniform pentadecane removal in most soil regions demonstrated that electro-osmosis as well as electrophoresis was the major mechanism for bacterial transport in soil.

The effects of current density and initial concentration of pentadecane on removal efficiency were examined. A high current density (3.13 mA/cm$^2$) decreased the removal efficiency, because increase in soil temperature to above 45 °C inhibited bacterial activity. The removal amount of pentadecane increased with initial pentadecane concentration at the same current density, because of the increased amount of weakly bound pentadecane onto the soil surface. The optimum current density for 5000 mg/kg pentadecane was 0.63 mA/cm$^2$. The highest removal efficiency (77.6%) was obtained for 1000 mg/kg. From the control experiment, it was found that the biodegradation in the soil specimen was dominant mechanism of removal.

Consequently, the present methods of EK bioremediation demonstrated superiority over the conventional bioremediation, which had inherent demerits of slow degradation and low removal efficiency.

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## References

[1] M.E. Lindsey, G. Xu, J. Lu, M.A. Tarr, Sci. Total Environ. 307 (2003) 215.
[2] U.S. EPA, Publ. EPA/540/2-88/004, Washington, DC, 1988.
[3] J.T. Cookson, Bioremediation Engineering, McGraw-Hill Inc., USA, 1995.
[4] M.F. Rabbi, B. Clark, R.J. Gale, E. Ozsu-Acar, J. Pardue, A. Jackson, Waste Manage. 20 (2000) 279.
[5] M.F. DeFlaun, C.W. Condee, J. Hazard. Mater. 55 (1997) 263.
[6] S. Pamukcu, A. Weeks, J.K. Wittle, J. Hazard. Mater. 55 (1997) 305.
[7] A.P. Shapiro, R.F. Probstein, Environ. Sci. Technol. 27 (1993) 283.
[8] Z. Li, J.W. Yu, I. Neretnieks, J. Environ. Sci. Health, Part A 32 (1997) 1293.
[9] Y.B. Acar, A.N. Alshawabkeh, J. Hazard. Mater. 55 (1997) 322.
[10] R.E. Saichek, K.R. Reddy, Environ. Technol. 24 (2003) 503.
[11] S.S. Radwan, D. Al-Mailem, I. El-Nemr, S. Salamah, Int. Biodeter. Biodegr. (2000) 129.
[12] R.L. Clarke, R. Lageman, W. Pool, S.R. Clarke, US Patent 5,846,393 (1998).
[13] H.H. Lee, J.W. Yang, J. Hazard. Mater. B 77 (2000) 227.
[14] T.N. Vogel, D. Grbić-Galić, Appl. Environ. Microbiol. 52 (1986) 200.
[15] B.J. Shiau, D.A. Sabatini, J.H. Harwell, Groundwater 32 (1994) 561.
[16] J.W. Yang, S.J. Kim, J.Y. Park, Y.J. Lee, H.J. Kim, W.S. Jeong, J. KSEE 25 (2003) 426.
[17] K. Millacheruvu, A.N. Alshawabkeh, Emerging Technologies in Hazardous Waste Management, vol. VIII, Kluwer Academic/Plenum Publishers, 1999.
[18] L.Y. Wick, P.A. Mattle, P. Wattiau, H. Harms, Environ. Sci. Technol. 38 (2004) 4596.
[19] R.E. Saichek, K.R. Reddy, Chemosphere 51 (2003) 273.
[20] R. Boopathy, Biores. Technol. (2004) 143.
[21] A. Hess, P. Höhener, D. Hunkeler, J. Zeyer, J. Contam. Hydrol. 23 (1996) 329.