DEGRADATION OF BENZO[A]PYRENE(BAP) IN A CLAY SOIL BY ELECTRO-BIOREMEDIATION

HUANG D.N.[1,2], **LI W.**[1], **FU J.X.**[1], **WANG D.**[3] **and AI Z.S.**[2]

[1] School of Municipal and Environmental Engineering, Shenyang Jianzhu University, Shenyang,110168, China, [2] Institute of Applied Ecology, Chinese Academy of Sciences,Shenyang,110016, China,
[3]Liaoning Environmental Monitoring Center,Shenyang, 110161,China
E-mail:7nan@163.com

ABSTRACT

Electro-bioremediation is an innovative technology for the cleanup of organic-contaminated soil. This study attempted to test the feasibility of electrokinetic process for the bioremediation of soil contaminated with benzo[a]pyrene (Bap) as a model polycyclic aromatic hydrocarbon (PAH) at laboratory scale. The electric field caused great change in soil pH and moisture content near the anode; while using electrode polarity reversal, pH and moisture content could be finely controlled. Over 40% of the initial 50 mg/kg Bap in clay soil could be removed under electric field with electrode polarity reversal in 40 d, around 88% higher than that with bacteria treatment only. The electrokinetic stimulates the bacteria growth and accelerates the decontamination efficiency of Bap. Hence, the present study provides a promising electrokinetic technology for bioremediation of PAH contaminated soils.

Keywords: bioremedation, electrokinetic, benzo[a]pyrene, clay soil

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of environmental contaminants which are mostly derived from the incomplete combustion of organic matter like gasoline and diesel fuel, oil spills, former gas plant facilities, etc.(Meckenstock *et al.*, 2004). Pollution of these compounds has recently been paid a close attention because of their potential toxicity, mutagenicity, carcinogenicity and ubiquitous distribution. Moreover, due to their hydrophobicity, low volatility and resistance to biological degradation, most PAHs are adsorbed strongly on soil particles and sediments, which make them less available for biological uptake, resulting in serious soil contamination problems(Boonchan *et al.,*2000). 16 PAHs are recognized as priority pollutants by US EPA. Among them, benzo[a]pyrene (Bap), a five-ring PAH, was the first one identified as a carcinogen. It is generally used as an indicator for monitoring PAH-contaminated wastes (Saraswathy & Hallberg, 2002).

The use of bioremediation technology for decontamination of PAHs in soils has been studied for many years. However, the long-time treatment period is a main shortcoming of this approach. Such slow remediation rate commonly due to the limited opportunities of interactions among pollutants, microorganisms, and nutrients (Wick *et al.,*2007). Particularly, in some low permeability soil, the mobilities of bacteria and contaminants are further believed to be inhibited. Thus, an associated operation of electro-bioremediation has been employed in the treatment of soil contaminated with PAHs in recent years. Several researches have revealed that the removal of some organic pollutants such as trichloroethylene (TCE), pentachlorophenol, and PAHs, can be improved by a proper application of electric fields (Harbottle *et al.,*2009; Niqui-Arroyoa *et al.,*2006; Xu *et al.,*2010). Lear *et al*. found that electrokinetic has no serious negative effect on 'soil microbial health', thus they regarded electrokinetic as a viable soil remediation technology (Lear *et al.,*2004).

However, soil characters such as pH and moisture content have a significant effect on biodegradation and contaminant behaviour, and can be rapidly altered by an applied electric field (Aca & Alshawabkeh, 1993). The changed soil character may also affect the health of the soil microbial community and its response to decontamination of pollutants. Too high electric field may have detrimental effects on the bacteria. Jackman *et al*. found a current density of 200 A m^{-2} inactivated acidophilic bacteria (Jackman *et al., 1999*). Lear *et al*. reported that a even a low electric current(3.14 A m^{-2})in soil detrimentally impacted communities near the anode(Lear *et al.,*2004). Further, the change in moisture content and contaminant distribution might inhibit the contaminant removal efficiency.

The control of soil pH and moisture content is of importance for electro-bioremediation of organic contaminated soil. When the electric field with periodic electrode polarity reversal is applied, the soil pH can be controlled as the H^+ and OH^- ions could be automatically neutralized and the water can be distributed evenly as the changing direction of electroosmosis flow(Luo *et al.*, 2005).

At present, there are lots of studies on the decontaminants of phenanthrene in the soil by electro-bioremediation technology, but little is known to Bap because of its highly persistence. The objective of this work is to test the effects of enhanced electro-bioremediation technology by reversing the polarity of electric field on the degradation of Bap. Using laboratory microcosms, the study examined the effects of electric current regime and subsequent soil pH and moisture content conditions on a clayey soil artificially contaminated with Bap, and inoculated with Bap-degrading bacteria.

2. Materials and methods

2.1. The tested soil and bacterias

A natural clay soil was used as the experimental soil with some main properties as described in Table 1. The soil samples (0-15cm) were collected from Shenbei New Area, Shenyang, China. The soil was air-dried at room temperature, and passed through a 2-mm mesh sieve. The soil samples were sterilized three times by alternately using an autoclave (121°C for 45 min)and a drier(105°C for 30min).

Bap was selected as the tested organic contaminant. The target concentration of Bap was 50mg kg^{-1}(mass of contaminant/mass of dry soil). Bap was dissolved in acetone before addition to the soil. After blending homogenously, a sample was taken to measure the initial concentration of Bap, as some of the contaminant might have been volatilized along with the acetone. The initial concentration of Bap was about 41mg kg^{-1}dry soil.

A mixed culture of PAH-degrading bacteria was used as the experimental bacteria. By using basic mineral medium with Bap as the sole carbon source with components, the bacteria were isolated from a contaminated soil near steel plant which is long term exposed to the PAH air pollution.

Table 1: Main physical and chemical properties of the tested soil

N(%)	P(%)	Org.C (%)	CEC (cmol•kg^{-1})	pH	Texture(%)		
					Sand	***Silt***	***Clay***
0.08	0.01	1.53	24.3	7.58	30.2	26.2	43.6

2.2. Electrokinetic cell and experiment design

The experiments were performed in a Plexiglass chamber with dimentions 18cm×14cm×5cm. Figure 1 shows the schematic view of the EK test setup. Appropriately 600g of the Bap-spiked soil with the initial moisture content (about 25%, w/w) was carefully stacked into the chamber. Two cylindrical graphite electrodes (6cm height and 1cm diameter) were inserted into the soil at either end of the soil chamber at a distance of about 1cm from the wall of the chamber. De-ionized water was selected as the processing fluid in both electrode chambers. A constant potential difference of 24V(1.5V cm^{-1})was applied during 40 days in all experiments. The soil pH, moisture content, Bap concentration and bacteria counts were analyzed periodically.

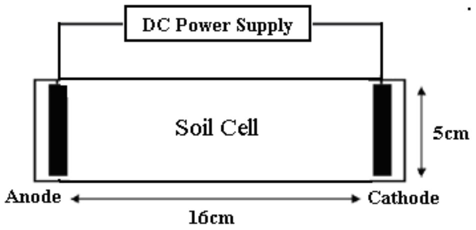

Figure 1: A schematic view of the setup used for electrolinetic experiments

As summarized in Table 2, three experiments, named as Test1, Test2, Test3, were tested to investigate the variation of soil character and Bap removal under different treatment. CK was used as a control test, without electricity or bacteria.

Table 2: A summary of the experiments carried out

Test code	Processing fluid	Electric field (V cm^{-1})	Initial microbial counts ($CFUg^{-1}$)	Polarity reversal time(h)
CK	de-ionized water	0	0	0
Test1	de-ionized water	0	1.89×10^7	0
Test2	de-ionized water	1.5	1.97×10^7	0
Test3	de-ionized water	1.5	1.92×10^7	3

3. Results and discussion

3.1. Influence of electrokinetic on soil pH

Test2 and Test3 were carried out to investigate pH changes under electrokinetic treatments with and without polarity reversal. In Test2, the pH values were ranged from 3.4 to 8.7 at the end of the experiment. The acid or basic pH conditions near the electrode regions were due to the H^+ and OH^- generation in anode and cathode during the electrokinetic process, respectively. An acid front predominated in the whole soil chamber mainly resulted from the smaller size and faster moving of hydrogen ions. The extreme pH near the electrodes was adverse to the bacteria and even slowed down the removal efficiency of contaminants.

In Test3, where the polarity reversal was performed, the pH was maintained in a range from 6.9 to 7.3 at the end of the experiment. The even distribution of pH resulted from mutual neutralization of H^+ and OH^- generated from the anode and cathode. Compared with Test2, the anodic and alkaline pH in soil is finely controlled.

3.2. Influence of electrokinetic on soil moisture content

The soil moisture content decreased near the anode and apparent increased near the cathode in Test2. The lowest moisture content (20%) was observed at 2cm away from the anode; and the peak moisture content (25%) was at 2cm away from the cathode. The moisture content near cathode was 28% higher than that near the anode. The change of soil moisture distribution mainly attributed to the migration direction of the electroosmotic flow. The uneven distribution of moisture content might cause the bacteria and contaminants distribute unevenly in soil, further inhibit the contact between contaminants and bacteria and have a negative effect on the bioremediation of contaminants.

In Test3, the distribution of moisture content was even. The polarity reversal can make the changes of directions of the electroosmotic flow, further avoid the dryness in anode (Xu *et al.*,2010). Therefore, the technology of polarity reversal can help the soil moisture maintain at a stable level. It provided suitable conditions for bacteria growth and contaminants removal.

3.3. Bap removal and bacteria counts

An obvious higher Bap removal efficiency was observed in Test2 and Test3 than that in Test1 and CK test during the whole experiment period.

In Test2, the Bap removal efficiency amounted to 33% at the end of the experiments, while the removal in Tes1 with bacteria treatment only just reached to 21%. During the last 10 days, the slope of Test1 curve decreased to near zero. The decreased removal efficiency in Test1 might due to the consumption of nutrients and the slowdown of biological metabolism. With application of electric field, the soil bacteria can maintain persistent activity in the bioremediation process. Some previous researchers have found the similar results (Kim *et al.*,2010). However, the Bap removal efficiency slightly decreased in Test 2 during the last 10days compared to the initial stage. In Test3, the maxmium removal of Bap was 40%，around 1.9 and 1.2 times of that in Test1 and Test2, respectively. Unsimilar to the Test2, the decreased removal efficiency was not observed in Test3 even during the last 10 days.

The changes of bacteria counts during different treatments showed that the Bap-degrading bacteria counts with application of electric field were generally higher than that without DC treatment. The most obvious advantages appeared on 30days, in which the density of the bacteria in Test2 (7.8×10^8 CFU/g) and Test3 (8.9×10^8 CFU/g) were 1.5 and 1.7 times of that in Test1 (5.2×10^8 CFU/g), respectively.

The change of bacteria counts favored the theory that a proper application of electric field might stimulate the growth of bacteria and further enhanced the biodegradation of Bap in contaminated soils. Some previous studies have supported that electric field have a positive effect on the bacteria. Lear(2004) and Kim(2010) respectively revealed that some kind of bacteria number and community structure can be increased after electrokinetic treatments. Our results were well in accordance with the previous studies.

Therefore, application of DC electric field can accelerate the organic contaminant bioremediation process; moreover the electric field with reversing the polarity could further enhance the removal efficiency by maintaining the soil pH and water content at a suitable level to benefit the growth of bacteria.Define the crowding distance.

4. Conclusions

Electrokinetic was found to induce significant pH and moisture content in soil. The pH and moisture content was significantly decreased near the anode when without pH control. Using the polarity reversal, the soil pH was better controlled, ranging from 6.9 to 7.3; while the moisture content in soil distributed evenly.

The proper application of electric field can stimulate the bacteria growth and accelerate bioremediation of Bap in clay soil. Further, with polarity reversal, the Bap removal and bacteria counts were higher than the Electrokinetic treatment without polarity reversal. Therefore, periodically reversing the polarity of electric field was thought to be an effective technology to enhance the biodegradation of Bap in clay soil.

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