Removal of heavy metals and organic contaminants from soils using integrated Upward Electrokinetic Soil Remedial (UESR) technology and biological remediation

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**REMOVAL OF HEAVY METALS AND ORGANIC
CONTAMINANTS FROM SOILS USING
INTEGRATED UPWARD ELECTROKINETIC
SOIL REMEDIAL (UESR) TECHNOLOGY AND
BIOLOGICAL REMEDIATION**

**TAY TSE CHUAN EUGENE
SCHOOL OF CIVIL AND ENVIRONMENTAL
ENGINEERING**

2008

**Removal of Heavy Metals and Organic
Contaminants from Soils Using Integrated
Upward Electrokinetic Soil Remedial (UESR)
Technology and Biological Remediation**

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School of Civil and Environmental Engineering

A thesis submitted to the Nanyang Technological University
in fulfilment of the requirement for the degree of
Master of Engineering

2008

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SUMMARY

This research proposed to develop an integrated process that applied both electrokinetic remediation and bioremediation to remove heavy metals and polycyclic aromatic hydrocarbons (PAHs) from soil.

Soil was initially spiked with cadmium (Cd), lead (Pb), zinc (Zn), phenanthrene and pyrene. The electrokinetic tests were conducted by varying the treatment duration (4 and 8 days), initial soil water content (40% and 60%), and soil type (natural soil and kaolin). After the electrokinetic treatment, all the tests showed that the removal efficiency for Cd was the highest (45% to 72%), followed by Zn (38% to 62%) and Pb (9% to 37%). The test with electrokinetic treatment duration of 8 days and with natural soil (60% initial water content) showed the highest removal efficiency for the heavy metals among the tests. The removal efficiency was 72%, 62% and 37% for Cd, Zn and Pb, respectively.

An increase in the removal efficiency of heavy metals was achieved in the tests with higher treatment duration, and in the tests that had natural soil with higher initial soil water content. As the electrokinetic duration increased, more heavy metals migrated up towards the cathode and out of the soil into the circulating acid effluent or deposited on the cathode. Natural soil with higher initial soil water content improved the removal efficiency of heavy metals. This could be because higher water content increased the electroosmosis effect. In addition, more pore water could result in a higher concentration of heavy metals being mobile in the water phase than adsorbed on the soil surface, thus improving the removal of heavy metals by electromigration.

The removal efficiency for PAHs was much lower compared to heavy metals. The PAHs were removed from the soil by migration and not degraded. For all the tests, the removal efficiency for phenanthrene was higher (10% to 29%) than pyrene (6% to 19%). The test with electrokinetic treatment duration of 8 days and with kaolin

(60% initial water content) showed the highest removal efficiency for the PAHs among the tests. The removal efficiency was 29% and 19% for phenanthrene and pyrene, respectively. An increase in the removal efficiency of PAHs was achieved in tests with higher treatment duration. However, the removal efficiency for PAHs was not significantly affected by the type of soil used.

The costs for selected soil remediation technologies were S\$ 129-245 /m³ for soil heating/vapour extraction technology, and S\$ 198-305 /m³ for chemical oxidation (with potassium permanganate or hydrogen peroxide). The costs for the electrokinetic treatments in this study were lower and ranged from S\$ 4.4-10.9 /m³. There could be potential for the electrokinetic technology to be more cost-effective than conventional technologies for treating contaminated soil.

The bioremediation tests were conducted using white rot fungi, *Pleurotus ostreatus* by varying the treatment duration (7, 14, 21, 35 and 56 days), fungi type (pure fungi and cultivated fungi from commercial mushroom), and fungi inoculum to soil concentration (10%, 30%, 50% and 70%, v/w). The tests showed that it was possible to degrade PAHs with the pure or cultivated white rot fungi. In addition, the tests showed that degradation of PAHs increased over time although that increase became smaller over time. This could be because the production of enzymes responsible for the biodegradation of PAHs decreased over time or the remaining lower levels of PAHs were not available to the fungi for degradation. The final degradation of phenanthrene in the tests with cultivated fungi ranged from 53% to 75%, and from 42% to 69% for the pure fungi. The final degradation of pyrene in the tests with cultivated fungi ranged from 18% to 25%, and from 17% to 21% for the pure fungi.

An increase in the fungi inoculum to soil concentration increased the degradation of phenanthrene and slightly increased the degradation of pyrene. Tests with cultivated fungi showed higher phenanthrene and pyrene degradation than tests with pure fungi. This could be because of the higher concentration of fungi inoculum for the cultivated fungi tests as compared to the pure fungi tests (about 1.8 times higher). The cultivated fungi tend to grow faster than the pure fungi as shown in the incubation stages on agar plates and in liquid medium.

The integrated tests included an initial electrokinetic test with natural soil (60% initial water content) and electrokinetic treatment for 8 days. After the electrokinetic treatment, the treated soil was then used for the bioremediation test. The cultivated fungi and fungi inoculum to soil concentration of 70% (v/w) was used to degrade the PAHs. The final degradation of phenanthrene and pyrene after 56 days was 68.4% and 19.3%, respectively. The test showed that degradation increased over time although that increase became smaller over time, especially for pyrene. This integrated study showed that it was possible to further remove PAHs from soil using bioremediation after the electrokinetic remediation.

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LIST OF SYMBOLS

A	Total cross-sectional area normal to flow direction
E	Electric field strength
E_v	Energy consumption per unit volume of soil
F	Faraday's constant
G_t	Specific gravity of the soil solids at the test temperature
I	Current
M_s	Mass of the oven dry soil solids
$M_{\rho w,t}$	Mass of pycnometer and water at the test temperature
$M_{\rho ws,t}$	Mass of pycnometer, water, and soil solids at the test temperature
V	Voltage
V_s	Volume of soil column
k_e	Coefficient of electroosmotic permeability
n	Porosity of medium
q_A	Electroosmosis flow rate
u_e	Electroosmosis velocity
u_m	Velocity of an ion
z	Ionic charge number
ε	Permittivity of liquid
η	Viscosity of liquid
ρ_s	Density of the soil solids
$\rho_{w,t}$	Density of water at the test temperature
v	Ionic mobility
ξ	Zeta potential

CHAPTER 1 INTRODUCTION

1.1 Background

Land contamination is a serious environmental problem and also poses potential health problems to humans and animals. Contaminants could include heavy metals (arsenic, chromium, zinc, lead, cadmium, nickel, copper, mercury, etc) and organic pollutants (polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phenols, pesticides, etc). Several technologies are available for the remediation of soil contaminated with heavy metals or organic pollutants (Riser-Roberts 1998; Suthersan 1999). These technologies are shown in Table 1.1. The main limitations of the different technologies include the ineffective removal of both heavy metals and organic pollutants, and the need to excavate the contaminated soil for remediation.

For this proposed research, the approach is to remove both heavy metals and organic pollutants from soil using electrokinetic and bioremediation technologies. The advantages of this approach over other technologies are that it can effectively treat soil contaminated with both heavy metals and organic pollutants, and that there is no need to excavate the contaminated soil.

Electrokinetic remediation is one technology used for the removal of heavy metals from contaminated soil (Page and Page 2002; Virkutyte et al. 2002; Reddy and Ala 2005). It can be achieved by applying a direct current via electrodes placed in the soil. The electric field generated causes migration of charged metal ions where the positive ions migrate to the negatively charged cathode and negative ions migrate to the positively charged anode.

Table 1.1 Current technologies for soil remediation

Technology	Description	Advantages	Disadvantages
Soil vapour extraction	Apply pressure to volatilise contaminants	Effective in removing volatile contaminants	Ineffective in removing heavy metals
Thermal desorption	Apply high temperature to volatilise contaminants	Effective in removing volatile contaminants	Ineffective in removing heavy metals
Bioremediation	Add bacteria and nutrients to stimulate degradation of contaminants	Effective in removing some organic contaminants	Ineffective in removing heavy metals
Soil washing	Add chemical agents to wash contaminants from soil	Effective in removing contaminants	Soil has to be excavated for washing
Solidification and stabilisation	Immobilise contaminants in soil using chemical agents	Reduce mobility of contaminants	Soil is usually excavated for mixing with chemical agents

Conventional electrokinetic technology uses horizontal migration of heavy metals to remove heavy metals from contaminated soil. Researchers from the Nanyang Technological University (NTU) have developed an Upward Electrokinetic Soil Remediation (UESR) technology that uses vertical migration of heavy metals (Wang et al. 2006; 2007). The technology applies vertical non-uniform electric fields to cause an upward migration of heavy metals to the top of the soil, where the heavy metals could be easily removed from the soil surface. It is also possible to remove polycyclic aromatic hydrocarbons (PAHs) using the electrokinetic technology. However, further research to improve the heavy metal removal efficiency and

explore the possibility of removing PAHs is needed. One advantage of the UESR process is that the contaminants would be transported upwards towards the cathode and accumulate at the top layer of the soil. This allows easier and less excavation of the top layer of contaminated soil for disposal or further treatment.

Bioremediation is one technology commonly used for the removal of organic pollutants from contaminated soil and can be achieved by using microorganisms. Research has shown the feasibility of bioremediation by white-rot fungi for the degradation of several organic pollutants.

For the removal of organic contaminants from soils, biological remediation using white rot fungi has been shown to degrade recalcitrant compounds such as PAHs. The white rot fungi, *Pleurotus ostreatus*, is capable of producing both non-ligninolytic and ligninolytic type enzymes to breakdown PAHs (Bamforth and Singleton 2005). Treatment could be done by adding the liquid inoculum containing white rot fungi to the contaminated soil. Further research on the use of white rot fungi for the removal of organic contaminants need to be conducted.

Several studies have been conducted on the remediation of heavy metals or PAHs from contaminated soil (Puppala et al. 1997; Li et al. 1998; Lee and Yang 2000; Reddy and Chinthamreddy 2004; Al-Shahrani and Roberts 2005; Li et al. 2000; Reddy and Saichek 2002; Weng et al. 2003; Kim et al. 2005). However, limited studies were done on the simultaneous removal of heavy metals and PAHs from contaminated soil (Hakimipour 2001; Maturi and Reddy 2006; Wang et al. 2007).

1.2 Objective and Scope

The main objective of the proposed research was to develop an integrated process that applied both electrokinetic and bioremediation technologies to remove both heavy metals and PAHs from soil. The electrokinetic treatment was able to remove most of the heavy metals and some amount of PAHs from the soil. The bioremediation process using white rot fungi could be used to further degrade the remaining PAHs and acted as a polishing step.

The scope of this study included the following tasks:

- a) To study the feasibility of simultaneous removal of heavy metals and PAHs from soil using the UESR technology;
- b) To study the effect of treatment duration on the electrokinetic remediation of heavy metals and PAHs;
- c) To study the effect of initial soil water content on the electrokinetic remediation of heavy metals and PAHs;
- d) To study the effect of soil type on the electrokinetic remediation of heavy metals and PAHs;
- e) To study the feasibility of removal of PAHs from soil using bioremediation with white rot fungi;
- f) To study the effect of treatment duration on the bioremediation of PAHs;
- g) To study the effect of fungi type on the bioremediation of PAHs;
- h) To study the effect of fungi inoculum to soil concentration on the bioremediation of PAHs; and
- i) To study the feasibility of further removal of PAHs from soil using bioremediation after the electrokinetic remediation process.

1.3 Structure of this Report

This report contains 5 chapters that explain the research work conducted. Chapter 1 introduces the soil contamination problem, and the electrokinetic and bioremediation technologies. Chapter 1 also states the objective and scope of this study. Chapter 2

reviews the electrokinetic soil remediation principles and systems, and the enhancement methods used for the treatment of heavy metals and organic contaminants. Chapter 2 also reviews the mechanism involved in the biodegradation of PAHs using *Pleurotus ostreatus* and the effect of heavy metals. Chapter 3 describes the experimental and reactor set-up, soil and fungi preparation, sampling procedures and analytical methods used in this study. Chapter 4 presents the experimental results obtained and the discussion of the results. Chapter 5 summarises the findings of this study and gives some recommendations for further studies. This report also includes an Appendix section that shows pictures of the experimental set-up and presents the experimental data obtained for the study.

CHAPTER 2 LITERATURE REVIEW

2.1 Electrokinetic Remediation

Electrokinetic treatment of contaminated soil can be achieved by applying direct current via electrodes placed in the soil. The electric field generated causes migration of charged ions where the positive ions migrate to the negatively charged cathode and negative ions migrate to the positively charged anode. The migration relies on three mechanisms including electroosmosis, electromigration and electrophoresis.

2.1.1 Electrokinetic Mechanisms

Electroosmosis is the movement of soil moisture or groundwater from the anode to the cathode of an electrolytic cell (Virkiute et al. 2002). When an electric field is applied, the mobile cations in the diffuse double layer move towards the cathode and drag the pore fluid along, resulting in electroosmosis (Alshawabkeh et al. 1999). Page and Page (2002) summarised the electroosmosis phenomena as shown below. The electroosmosis flow rate, q_A is described by

$$q_A = -k_e EA \quad (2.1)$$

where k_e = coefficient of electroosmotic permeability (or conductivity), E = electric field strength or negative potential gradient, and A = total cross-sectional area normal to flow direction.

The most commonly accepted description of electroosmosis flow is the Helmholtz-Smoluchowski theory. The Helmholtz-Smoluchowski equation gives the electroosmosis velocity as

$$u_e = -\varepsilon\xi E / \eta \quad (2.2)$$

where ε = permittivity of liquid, ξ = zeta potential, and η = viscosity of liquid. The electroosmotic flow rate is then given by

$$q_A = -\varepsilon\xi EnA / \eta \quad (2.3)$$

where n = porosity of medium. Therefore,

$$k_e = \varepsilon\xi n / \eta \quad (2.4)$$

Electromigration is the transport of ions and ion complexes to the electrode of opposite charge (Virkutyte et al. 2002). Cations would migrate to the cathode and anions would migrate to the anode. Probstein and Hicks (1993) summarised the electromigration phenomena as

$$u_m = v_zFE \quad (2.5)$$

where u_m = velocity of an ion, v = ionic mobility, z = ionic charge number, F = Faraday's constant and E = electric field strength.

Electrophoresis is the transport of charged particles or colloids under the influence of an electric field (Virkutyte et al. 2002). Electrophoresis is the mirror image of electroosmosis since a charged particle moves relative to a stationary liquid (Probstein and Hicks 1993). The migration of charged particles by electrophoresis is less important in compacted soil.

In this study, the removal of heavy metals from soil involves the three electrokinetic mechanisms. However, PAHs are electrically neutral and less likely to be removed by electromigration and electrophoresis. Electroosmosis is probably the main mechanism for the removal of PAHs from soil (Reddy and Saichek 2002; Reddy and

Saichek 2003). Electroosmosis can cause the pore water in the soil to move towards to the cathode and helped to transport the solubilised PAHs.

2.1.2 Electrolysis of Water

At the electrodes, the electrolysis of water produces OH^- ions at the cathode and H^+ ions at the anode according to the following reactions:

The reactions increase the pH at the cathode and decrease the pH at the anode, resulting in the propagation of an acid front towards the cathode and a basic front towards the anode. This causes an effect on the soil zeta potential, solubility and adsorption of contaminants, ionic state and charge (Probstein and Hicks 1993).

2.1.3 Horizontal and Vertical Electrokinetic System

Most of the electrokinetic remediation system used in previous studies and field trials employed a horizontal system, where contaminants migrated horizontally between two electrodes inserted vertically into the soil. Few studies employed a vertical system, where contaminants migrated vertically between two electrodes.

Roulier et al. (2000) developed an integrated soil remediation technology called LasagnaTM, where electrodes can be installed vertically or horizontally for electrokinetic transport of contaminants. For this study, horizontal electrodes were installed by hydraulic fracturing to treat soil contaminated with trichloroethylene (TCE). The study showed that it was possible to apply electrical potential gradients of 10 to 40 V/m to soil between horizontal electrodes, resulting in electroosmotic flow that caused the vertical migration of contaminants between the electrodes.

Wang et al. (2006) studied the use of an upward electrokinetic soil remedial (UESR) technology to remove heavy metals from kaolin. The UESR technology used

vertical non-uniform electric field to cause heavy metals to migrate to the top of the soil. The study showed that the heavy metal removal efficiency increased with an increase in current density and treatment duration. When 0.01 M nitric acid was used as the cathode chamber effluent, more of the heavy metals were dissolved in the acid and removed from the soil.

2.1.4 Enhancement Methods

Laboratory and field studies have shown that it was possible to remove inorganic or organic contaminants from soils using electrokinetic treatment. However, there were some limitations to the use of the electrokinetic process and enhancement methods were needed to overcome these limitations. The limitations include the migration of the basic front and the formation of metal hydroxides between the OH⁻ ions generated at the cathode and the metals cations, which increases the soil resistance and electrical current (Page and Page 2002; Virkutyte et al. 2002). The enhancement methods mainly focused on controlling the pH at the electrodes and maintaining or bringing contaminants into solution (Page and Page 2002).

2.1.4.1 Treatment of Heavy Metals

Puppala et al. (1997) studied the effect of the use of acetic acid to neutralise the cathode electrolysis reaction and the use of an ion selective membrane to prevent hydroxide ions generated at the cathode from moving into the soil. Synthetic soil was spiked with Pb and a direct current density of 50 or 200 $\mu\text{A}/\text{cm}^2$ was applied. The study showed that the use of acetic acid and membrane showed better Pb removal efficiency compared to the unenhanced treatment. However, it was essential to decrease the catholyte pH to 4 or less when using acetic acid. The use of the membrane resulted in higher energy used and the membrane was relatively expensive, thus increasing the cost of remediation.

Li et al. (1998) studied the electrokinetic removal of copper from soil using cation selective membrane. The cation-permeable membrane was placed in front of the cathode in a 0.01 M potassium nitrate solution. The membrane prevented hydroxyl

ions from migrating to the anode and allowed heavy metals ions to pass through and precipitate in the solution. After the electrokinetic treatment for 3 days, 90% of the initial 300 ppm Cu was removed after 3 days.

Lee and Yang (2000) studied the electrokinetic remediation of lead spiked kaolinite by controlling the electrolyte pH at the cathode and anode. The electrolyte (nitric acid at pH 2) was circulated from the cathode compartment to the anode compartment to avoid a decrease in electroosmotic flow by excess H^+ and heavy metal precipitation by OH^- . The removal efficiency of lead was 25.5% for no circulation after 22 days, 67.5% for 1.1 ml/min circulation after 34 days and 50.6% for 4.4 ml/min circulation after 34 days.

Li and Li (2000) studied the use of cathode and anode conditioning agents for the electrokinetic treatment of Pb contaminated kaolinite and illitic soils. The anode was flushed with 0.4 M NaAc at pH 3.8 and the cathode was flushed with acetic acid at pH 4. A rinsing fluid (nitric acid at pH 3) was circulated in an added porous layer at the cathode to reduce the effect of OH^- . Over 80% of Pb from the soil between the anode and the porous layer was removed.

Reddy and Chinthamreddy (2004) investigated the use of different electrolyte or purging solutions for Cr, Ni and Cd contaminated glacial till soil. The anode and cathode purging solutions used were 0.1 M EDTA, 1.0 M acetic acid, 1.0 M citric acid, 0.1 M NaCl/0.1 M EDTA, and 0.05 M sulphuric acid/0.5 M sulphuric acid. The use of acetic acid in the cathode reservoir was the most effective in removing all 3 metals simultaneously. Removal efficiency was 57% for Cr, 48% for Ni and 26% for Cd after 690 hours at a constant voltage gradient of 1 V/cm.

Zhou et al. (2004) studied the effect of different enhancement agents on the electrokinetic remediation of Cu contaminated red soil. The enhancement agents include a mixture of organic acids such as HAc-NaA, HAc-NaA with EDTA and lactic acid with NaOH. The study showed that enhancement by lactic acid with NaOH resulted in the highest Cu removal efficiency of 81% after 900 hours of electrokinetic treatment.

Al-Shahrani and Roberts (2005) investigated the effect of catholyte pH control on the electrokinetic treatment of caesium contaminated kaolin by using different acids (nitric acid, sulphuric acid, acetic acid and citric acid). The study showed that the use of strong acids such as nitric acid and sulphuric acid was more effective than weak acids such as citric and acetic acid. For the experiment using sulphuric acid to control the catholyte pH, 79% of caesium was removed from a 30 cm long kaolin sample after 10 days at 1 V/cm.

Giannis and Gidarakos (2005) studied the use of electrokinetic remediation and soil washing for Cd contaminated soil. In the first study, the soil was saturated with tap water and acetic acid, hydrochloric acid and EDTA were used as purging solutions at the cathode and anode. There was no significant removal of Cd (< 24%). In the second study, citric, nitric and acetic acids were used as soil washing and purging solutions. This resulted in Cd reduction of about 85%, 70% and 25% for citric, nitric and acetic acid, respectively.

Kim et al. (2005) studied the use of ion exchange membranes (IEM) in electrokinetic treatment of Cd and Pb contaminated kaolinite. A cation exchange membrane and an anion exchange membrane were used. By using the IEM-enhanced electrokinetic treatment, the removal efficiency was increased from 43% to 81% for Cd and increased from 27% to 54% for Pb after 100 hours. However, fouling phenomena was observed at the cation exchange membrane due to metal hydroxide precipitation. This could be overcome by placing an auxiliary solution cell between the soil and cathode so that the metals can precipitate in the solution.

Ottosen et al. (2005) studied the use of enhancement solutions to improve the remediation of Cu, Zn and Pb contaminated soil. Different enhancement solutions such as ammonia, citric acid or ammonium citrate are added to the soil to enhance the desorption of the heavy metals. The study showed that ammonium citrate could mobilise Cu, Zn and Pb but optimisation of pH and concentration of ammonium citrate was necessary.

Pazos et al. (2005) studied the use of polarity exchange for the electrokinetic treatment of Mn contaminated kaolin clay. At short time intervals, the polarity was reversed between the cathode and anode so that H^+ was generated at the cathode, which dissolved the metal precipitate at the cathode. Once the metals were dissolved, the polarity was switched back. The method improved the Mn removal efficiency from 14% in 7.6 days without polarity exchange to 72% in 7.9 days with polarity exchange. The power consumption increased from 108 kWh to 230 kWh when the polarity exchange method was used.

Reddy and Ala (2005) studied the electrokinetic remediation of a low permeability field soil contaminated with heavy metals. The field soil was further spiked with lead and mercury. Different extracting solutions were used including 0.2 M ethylenediamine tetra acetic acid (EDTA), 0.2 M diethylene triamine penta acetic acid (DTPA) and 0.2 M potassium iodide (KI) as the cathode solution, and 10% hydroxypropyl- β -cyclodextrin (HPCD) as the anode solution. The study showed that EDTA was more effective for removal of a variety of metals while KI was more effective for removal of mercury from the field soil.

Chang and Liao (2006) developed a circulation-enhanced electrokinetic (CEEK) system to neutralise the pH of the working solution and prevent the formation of the acid and base front. Results showed that the CEEK system could stabilise the current and keep the pH of the working solution neutral. The graphite electrode was found to be better as it can achieve lower electricity consumption.

2.1.4.2 Treatment of Organic Contaminants

Li et al. (2000) studied the effect of adding cosolvent to the anode conducting fluid for the electrokinetic treatment of glacial till soil contaminated with phenanthrene. The cosolvents used included n-butylamine, tetrahydrofuran and acetone. The study showed that n-butylamine significantly enhanced the desorption and electrokinetic migration of phenanthrene and 43% of phenanthrene was removed after 127 days.

The phenanthrene removal was not significant for acetone and minimal for tetrahydrofuran.

Park et al. (2000) studied the electrokinetic remediation of phenanthrene contaminated clay using three different surfactants, APG, Brij30 and SDS. The study showed that using surfactants as an anode electrolyte enhanced desorption of phenanthrene and mobility in soil. The surfactant, APG, showed a high removal efficiency compared to the other surfactants.

Reddy and Saichek (2002) studied the use of different surfactants and cosolvents for the electrokinetic treatment of kaolin contaminated with phenanthrene. The following surfactants/cosolvents were used: 1% Witconol 2722, 3% Tween 80, 40% ethanol, and 4% Witco 207 in 40% ethanol. The study showed that substantial phenanthrene desorption and solubilisation occurred near the anode although phenanthrene removal was limited. Surfactant/cosolvent solutions that had at least a 3% surfactant concentration and/or a 40% cosolvent concentration generated phenanthrene removal and/or migration.

Reddy and Saichek (2003) studied the use of surfactant and cosolvent for the electrokinetic treatment of two different low-permeability soils, kaolin and glacial till, contaminated with phenanthrene. A surfactant (3% Tween 80) and a cosolvent (40% ethanol) were used. The study showed that phenanthrene was more strongly bound to glacial till due to its higher-organic content than kaolin. The surfactant and cosolvent was able to cause contaminant desorption, solubilisation and/or migration in both soils. The researchers concluded that more studies should be conducted to improve the phenanthrene removal efficiency.

Saichek and Reddy (2003) studied the use of surfactant and cosolvent for the electrokinetic treatment of kaolin contaminated with phenanthrene. Previous studies showed that surfactants and cosolvents would help to solubilise phenanthrene but the actual amount removed was limited. For this study, a surfactant (3% Tween 80) and a cosolvent (40% ethanol) were used, and pH was controlled at the anode using 0.01 M NaOH to improve the soil-solution-contaminant interaction and removal

efficiency. The study showed that controlling the pH improved contaminant solubilisation and migration to the anode. However, subsequent changes in the soil and/or solution chemistry could cause the deposition of contaminant in the middle or cathode soil regions.

Weng et al. (2003) investigated the use of electrokinetic process to remediate clay soil contaminated with trichloroethylene (TCE) using synthetic groundwater as the processing fluid. TCE removal efficiency was 75.8% for 3 days and 86.4% for 5 days of electrokinetic treatment using a voltage gradient of 1 V/cm. TCE removal efficiency was 91.3% for 3 days of electrokinetic treatment using a voltage gradient of 2 V/cm.

Kim et al. (2005) studied the electrokinetic-Fenton process for the remediation of kaolinite contaminated with phenanthrene. The study showed that adding H_2O_2 to the anode solution generated intermediate anions with Fenton-like reaction, which affected the electrical current. The phenanthrene degradation yield was proportional to the transfer rate of the acid front and the H_2O_2 stability. Therefore, using H_2O_2 and a dilute acid as an anode purging solution was possible for treating phenanthrene contaminated soil.

Luo et al. (2005) studied the use of non-uniform electrokinetic treatment to mobilise phenol and 2,4-dichlorophenol (2,4-DCP) in kaolin and a natural sandy loam soil. The study showed that electrokinetic treatment can enhance the desorption and movement of phenol and 2,4-DCP to the anode in unsaturated soils. The movement can be controlled by regulating the pH of the soil and the electric field.

Ribeiro et al. (2005) studied the removal of atrazine from natural and spiked soil using electrokinetic remediation. The study showed that atrazine migrated towards the anode due to reverse electroosmosis and about 30% to 50% of atrazine was removed from the soil within 24 hours.

Yang et al. (2005) investigated the removal of phenanthrene from kaolinite using a surfactant-enhanced electrokinetic treatment. The surfactants can help to solubilise

phenanthrene, and a nonionic surfactant, alkyl polyglucosides (APG) and an anionic surfactant, Calfax 16L-35 was used. The removal efficiency of phenanthrene increased with increased APG surfactant concentration as most of the APG moved towards the cathode as micelles containing phenanthrene and accumulated in the effluent. The removal efficiency of phenanthrene was low with increased Calfax 16L-35 surfactant concentration as the surfactant was accumulated near the anode. This might be due to the surfactant adsorbing onto the soil. The study showed that a non-ionic surfactant might be more suitable for surfactant-enhanced electrokinetic treatment.

Park et al. (2007) studied the removal of phenanthrene from kaolin by using surfactant-enhanced electrokinetic treatment. The surfactants used included APG (alkyl polyglucoside), Brij30 (polyoxyethylene-4-lauryl ether) and SDS (sodium dodecyl sulphate). The results showed that APG showed the highest removal efficiency, with 65% degradation after 4 days.

2.1.4.3 Treatment of Heavy Metals and Organic Contaminants

Hakimipour (2001) studied the simultaneous removal of lead, nickel and phenanthrene from clayey soils. A chelating agent, EDTA and a surfactant were introduced into the soil in various sequences and different periods to optimise the contaminant removal. Ion exchange textiles were also used to control the impacts of extreme pH at the electrodes. The study showed that average simultaneous removal was 74% for phenanthrene, 85% for lead and 84% for nickel.

Maturi and Reddy (2006) investigated the use of cyclodextrins in electrokinetic remediation to simultaneously remove nickel and phenanthrene from contaminated kaolin. Hydroxypropyl β -cyclodextrin (HPCD) at low (1%) and high (10%) concentrations was used as flushing solutions at the anode. A periodic voltage gradient of 2 V/cm was applied for the study and 0.01 M NaOH was added to keep pH neutral at the anode. The study showed that phenanthrene migrated to the cathode and 1% HPCD resulted in higher phenanthrene removal. Nickel also

migrated to the cathode but there was incomplete removal of nickel into solution as nickel precipitated near the cathode due to high pH conditions.

Wang et al. (2007) studied the removal of Cu, Pb, *p*-xylene and phenanthrene from kaolin using an upward electrokinetic soil remediation (UESR) process. The removal efficiency of *p*-xylene and phenanthrene were higher when smaller diameter or larger height cells and distilled water were used. The removal efficiency of Cu and Pb were higher when smaller diameter or shorter height cells and 0.01 M nitric acid solution were used. The results showed that the experiments with duration of 6 days achieved removal efficiencies of 67%, 93%, 62% and 35% for phenanthrene, *p*-xylene, Cu and Pb respectively.

2.1.5 Hybrid Electrokinetic System

Electrokinetic remediation could be combined with other remediation technologies to increase the removal or degradation of contaminants. The electrokinetic technology was usually used to increase the migration of nutrients or contaminants. The main options of hybrid systems include combining the electrokinetic process with bioremediation or phytoremediation.

Maini et al. (2000) combined bioleaching and electrokinetics for the remediation of copper contaminated soil, which was amended with sulfur. Bioleaching occurred when the indigenous sulfur-oxidising bacteria converted reduced sulfur compounds to sulphuric acid, acidifying the soil and mobilising the metal ions. The acidified soil was then treated by electrokinetics and 86% of copper was removed in 16 days. The combination of bioleaching and electrokinetics increased the cost effectiveness by reducing the power requirement by 66%.

Rabbi et al. (2000) studied the use of electrokinetic process to inject benzoic acid cometabolite to enhance the biodegradation of trichloroethene (TCE) in Loess soil. With electrokinetic, the cometabolite would migrate through the soil and the microbial inoculum added to the soil would be able to degrade TCE. The study

showed that homogeneous penetration of additives is not achieved unless the rate of additive injection is greater than its rate of consumption.

Jackman et al. (2001) studied the combination of electrokinetic remediation and biodegradation to treat soil contaminated with 2,4-dichlorophenoxyacetic acid (2,4-D). Under an electric field, 2,4-D migrated towards the anode, where it passed the inoculated region with bacterium that has chromosomally encoded degradative genes for 2,4-D. The study showed that it was possible to use electrokinetic treatment to move organic contaminants to a biodegradative zone where bacteria can mineralise the contaminants. The inoculated bacteria were metabolically active under the electrokinetic conditions.

O'Connor et al. (2003) investigated the combination of electrokinetic remediation and phytoremediation to treat one soil contaminated with Cu and another soil contaminated with Cd. A constant voltage of 30 V was applied to the soil with perennial ryegrass sown. The study showed that metals migrated from the anode to cathode during the 80-98 day experimental period. There was an enhancement of plant Cu uptake in the cathode area but Cd uptake was less clear-cut. Plant growth was affected at the anode but not in other parts.

Wick et al. (2004) investigated the mobility, viability and activity of PAH-degrading bacteria under electrokinetic conditions. The two bacteria used were *Sphingomonas* sp. L138 and *Mycobacterium frederiksbergense* LB501TG. The electric field applied did not affect bacteria viability and degradation of PAHs. The bacteria strain L138 was transported by electroosmosis and electrophoresis whereas the bacteria strain LB501TG had poor transport. The addition of non-ionic surfactant Brij35 resulted in about 80% enhancement in electrokinetic movement for both bacteria strains.

Luo et al. (2005) studied the use of biodegradation and non-uniform electric field with periodic polarity-reversal to treat phenol contaminated sandy loam. Microbial inoculum was added to the sandy loam and under the electric field, phenol was able to desorb and move in sandy loam, thus resulting in enhanced biodegradation. In addition, reversing the polarity of the electric field would disperse the phenol more

uniformly and increase biodegradation. The electricity consumption increased with polarity-reversal.

Niqui-Arroyo et al. (2006) studied the combination of bioremediation and electrokinetic remediation to treat clay soil contaminated with phenanthrene. Nutrients and bacterium were added to the soil. The study showed that phenanthrene degradation was ten times higher as compared to the treatment without current or microbial activity. The increased degradation could be due to the mobilisation of phenanthrene and nutrients in the soil.

2.2 Bioremediation Using White Rot Fungi

The review by Pointing (2001) on the feasibility of bioremediation by white-rot fungi provided the evidence for the degradation of several organopollutants by the ligninolytic enzymes of white-rot fungi. This study would focus on the degradation of PAHs by using a common white-rot fungus, *Pleurotus ostreatus*.

2.2.1 Biodegradation of PAHs using *Pleurotus ostreatus*

The white rot fungi, *Pleurotus ostreatus*, is capable of producing both non-ligninolytic and ligninolytic type enzymes to breakdown PAHs (Bamforth and Singleton, 2005). A non-ligninolytic enzyme, cytochrome P₄₅₀ monooxygenase enzyme, is able to oxidise the aromatic ring of PAHs to form an arene oxide, which could then undergo epoxide-hydrolase catalysed reaction or non-enzymatic rearrangement (Figure 2.1). The compounds formed would be less toxic than the PAHs. Ligninolytic enzymes such as lignin peroxidase, manganese peroxidase and laccase are able to oxidise the PAH rings, forming PAH-quinones and acids, and further mineralised to CO₂ (Figure 2.1).

Figure 2.1 Pathways for the degradation of PAHs by fungi and bacteria (Bamforth and Singleton, 2005)

Bezalel et al. (1996a) studied the degradation of catechol, phenanthrene, pyrene, benzo[a]pyrene, anthracene, fluorene and fluoranthene by *Pleurotus ostreatus*. After 11 days of incubation, catechol, phenanthrene, pyrene and benzo[a]pyrene were mineralised. Anthracene and fluorene were mineralised much slower after 15 days, while fluoranthene was not mineralised. Laccase and manganese-inhibited peroxidase activity were observed but it did not correlate to the degradation of PAHs.

Bezalel et al. (1996b) studied the degradation of phenanthrene by white rot fungus *Pleurotus ostreatus* in basidiomycetes rich medium. After 11 days, 94% of the phenanthrene was metabolised. It was suggested that the initial oxidation of phenanthrene was done stereoselectively by a cytochrome P-450 monooxygenase, followed by epoxide hydrolase-catalysed hydration reactions.

Bezalel et al. (1996c) studied the degradation of pyrene, anthracene, fluorene and dibenzothiophene by *Pleurotus ostreatus*. The study showed that *Pleurotus ostreatus* initially metabolised PAHs by similar reactions as non-ligninolytic fungi, and was

able to mineralise the PAHs. The initial metabolism could be due to a cytochrome P-450 monooxygenase since the PAH metabolism did not correlate with manganese peroxidase, laccase and other peroxidases.

Bezalel et al. (1997) investigated the enzymatic mechanisms that were involved in the degradation of phenanthrene by *Pleurotus ostreatus*. The study showed that cytochrome P-450 monooxygenase and epoxide hydrolase were involved in the initial oxidation of phenanthrene to phenanthrene trans-9,10-dihydrodiol, whereas laccase and manganese-independent peroxidase were not involved.

Eggen and Majcherczyk (1998) investigated the degradation of benzo[a]pyrene in aged creosote-contaminated soil using *Pleurotus ostreatus*. The degradation of benzo[a]pyrene in the aged creosote-contaminated soil increased slightly from 28% after 1 month to 32% after 3 months of incubation. For artificially spiked benzo[a]pyrene, the degradation was higher with degradation increasing from 40% after 1 month to 49% after 3 months.

Bogan et al. (1999) studied the degradation of anthracene, fluoranthene and benzo[a]pyrene in PAH-contaminated soils using *Pleurotus ostreatus*. The white rot fungi were grown on a mixture of cottonseed hulls and alder chips, and the substrate was added to the soil. The addition of fungi-colonised substrate or fungi-colonised substrate plus a non-ionic surfactant significantly increased the degradation of PAHs.

Eggen (1999) studied the degradation of PAHs in aged-creosote contaminated soil for 7 weeks using *Pleurotus ostreatus*, including two commercial sources of spent mushroom compost and one fungal substrate. The degradation of 3-ring PAHs was the same for the two commercial sources of fungi. For 4 and 5-ring PAHs degradation, spent mushroom compost was more effective than the fungal substrate. In one of the experiment, fish oil was used as a surfactant to solubilise PAHs. Fish oil was added to the soil and spent mushroom compost, resulting in degradation of 86% for total 16 PAHs, 89% for 3-ring PAHs, 87% for 4-ring PAHs and 48% for 5-ring PAHs.

Eggen and Sveum (1999) investigated the effect of temperature (8°C or 22°C) and two pre-treatment methods (pine bark with fertiliser or pine bark without fertiliser) on the degradation of PAHs in aged creosote contaminated soil using white rot fungus *Pleurotus ostreatus*. The degradation of PAHs was better with pre-treatment by adding bark before inoculation with white rot fungi. Pre-treatment with bark and fertiliser at 8°C stimulated microbial activity and increased the degradation of PAHs even without adding fungi.

Gramss et al. (1999) studied the capacity of fifty-eight fungi to degrade five PAHs (phenanthrene, anthracene, fluronthene, pyrene and perylene) after 14 days of incubation. The average degradation rate of the PAHs was well correlated with the production of manganese peroxidase, peroxidase and laccase. Additional tests were conducted for the degradation of the PAHs by *Pleurotus ostreatus* over 28 days. The study showed that high degradation rate was associated with the presence of peroxidases.

Novotny et al. (1999) studied the different species of white rot fungi, comparing their colonisation rates, enzyme activities and degradation of anthracene, pyrene and phenanthrene. The three species used were *Pleurotus ostreatus*, *Phanerochaete chrysosporium* and *Trametes versicolor*. The study showed that *Pleurotus ostreatus* was capable of good growth and enzyme production, and significantly degraded PAHs as compared to the other species, especially for anthracene and pyrene. The degradation was 81%-87% for anthracene, 84%-93% for pyrene and 41%-64% for phenanthrene within 2 months.

Schutzendubel et al. (1999) studied the degradation of PAHs in liquid cultures of *Bjerkandera adusta* and *Pleurotus ostreatus*. After 3 days of incubation, *Pleurotus ostreatus* degraded 43% of fluorene and 60% of anthracene. A maximum 15% degradation of phenanthrene, fluoranthene and pyrene were also obtained with *Pleurotus ostreatus*. The addition of milled wood increased the secretion of laccase and manganese-dependent peroxidase but the degradation of PAHs was less. The production of oxidative enzymes by the fungi did not correlate directly to the degradation of PAHs.

Andersson et al. (2000) studied the fungal growth by estimating the phospholipids fatty acid levels in the soil and the degradation of PAHs. Three different species were used including *Pleurotus ostreatus*, *Phanerochaete chrysosporium* and *Hypholoma fasciculare*. For the autoclaved soils, *Pleurotus ostreatus* and *Phanerochaete chrysosporium* were able to degrade the PAHs over 10 weeks of incubation, whereas no degradation was found in non-autoclaved soil. This could be due to competition from the indigenous soil micro-organisms.

Eichlerova et al. (2000) studied the *Pleurotus ostreatus* f6 strain and its basidiospore-derived isolates, and the enzyme activities, loss of organic mass, degradation of PAHs, and colonisation of sterile and non-sterile soil. The fungi were able to degrade the PAHs (pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and dibenzo[a,h]anthracene) but the enzyme production rate had no significant effect on the degradation rate.

Marquez-Rocha et al. (2000) studied the degradation of soil adsorbed pyrene, anthracene, phenanthrene and benzo[a]pyrene using white rot fungus, *Pleurotus ostreatus*. Liquid fungi inoculum was used to inoculate wheat seed, which was then added to the contaminated soil. After 21 days, 50% of pyrene, 68% of anthracene and 63% of phenanthrene were degraded. When 0.15% Tween 40 was added, the following was obtained: 75% of pyrene, 80% of anthracene and 75% of phenanthrene were degraded. When 0.15% Tween 40 and 1 mM H₂O₂ was added, 90% of pyrene was degraded. The study also showed that benzo[a]pyrene may be bioconverted to an oxidised form instead of being mineralised to CO₂.

Novotny et al. (2004) studied the activities of Mn-dependent peroxidase, lignin peroxidase and laccase in different fungi grown in liquid medium or soil, and its effect on the degradation of anthracene and pyrene. After 8 weeks of incubation, high levels of Mn-dependent peroxidase and laccase in soil colonised by *Pleurotus ostreatus* showed the best PAH degradation rates, compared to *Phanerochaete chrysosporium* and *Trametes versicolor*.

Pozdnyakova et al. (2004) studied the oxidation of anthracene and fluoranthene using yellow laccase from *Pleurotus ostreatus* D1 in the presence and absence of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), and a surfactant, AOT. The study showed that laccase degraded about 49% of fluoranthene in the presence of AOT and absence of ABTS at pH 6 after 10 days. Also, laccase degraded about 95% of anthracene at pH 6 after 2 days, and about 96% degradation with ABTS at pH 4 and about 98% degradation with ABTS and AOT at pH 4.

Pozdnyakova et al. (2006) studied the catalytic activity of the yellow laccase from *Pleurotus ostreatus* D1 towards PAHs containing 3-5 rings. The following degradation efficiencies were achieved: anthracene (91%), pyrene (40%), fluorine (95%), fluoranthene (47%), phenanthrene (82%) and perylene (100%). The author noted that further studies were needed to explain the catalytic mechanism of yellow laccases.

2.2.2 Effect of Heavy Metals on *Pleurotus ostreatus*

Heavy metals can be beneficial or toxic to white rot fungi in different amounts. Trace amounts of essential heavy metals could be useful for fungi growth while excess metals become toxic to the fungi, inhibiting enzyme activity and fungal colonisation (Baldrian, 2003). The integrated study involved conducting an electrokinetic remediation process first before using bioremediation on the treated soil. This would help to remove heavy metals first and reduce the toxicity to the white rot fungi.

Sanglimsuwan et al. (1993) studied the growth of 21 types of mushrooms and their resistance to heavy metals such as Cu, Cd, Zn, Ni, Co and Hg. The study showed that *Pleurotus ostreatus* has the highest resistance to all the heavy metals. Also, the accumulation of Cu, Zn and Cd in *Pleurotus ostreatus* was studied. The uptake of

the heavy metals in the mycelia increased with increasing heavy metal concentrations.

Baldrian et al. (2000) studied the degradation of benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene and benzo[ghi]perylene contaminated soil using *Pleurotus ostreatus* in the presence and absence of cadmium and mercury. There was no decrease in the degradation of PAHs in soil with 10 to 100 ppm of Cd or Hg. The extent of soil colonisation by the fungi was limited for soil with Cd at 100 to 500 ppm or Hg at 50 to 100 ppm.

Baldrian and Gabriel (2002) studied the addition of copper and cadmium and its effect on the laccase activity of white rot fungus *Pleurotus ostreatus* cultivated in liquid nitrogen-limited medium. The addition of 1mM copper increased the laccase activity 8-fold and the addition of 2 mM cadmium increased the laccase activity 18.5-fold.

Baldrian and Gabriel (2003) studied the different enzyme activities of *Pleurotus ostreatus* growth on wheat straw with and without cadmium. The study showed that increasing Cd concentration decreased the Mn-peroxidase activity and the loss of substrate. The presence of Cd also increased the activities of laccase, endo-1,4- β -glucanase, and 1,4- β -glucosidase.

Baldrian et al. (2005) studied the degradation of wheat straw using *Pleurotus ostreatus* in the presence and absence of Cu, Mn, Pb and Zn. The effect of heavy metals on the production of enzymes was also studied. The study showed that high concentrations of Cu and Zn decreased the substrate colonisation rate, and dry mass loss was lower in treatments containing heavy metals than in the control. Laccase activity was increased by all the heavy metals while Mn-peroxidase activity was decreased by Mn, Cu and Pb.

CHAPTER 3 MATERIALS AND METHODS

This chapter discusses the materials, conditions, experimental set-ups, sampling procedures and analytical methods used in the electrokinetic, bioremediation and integrated studies.

3.1 Electrokinetic Study

The upward electrokinetic soil remediation (UESR) technology was developed by NTU researchers (Wang et al. 2006; 2007) and used vertical non-uniform electric field for the removal of heavy metals and organic contaminants. Conventional electrokinetic treatment uses horizontal electrical field for the removal of heavy metals. One advantage of the UESR technology was that contaminants would be transported upwards towards the cathode and accumulated at the top layer of the soil. This allowed easier and less excavation of the top layer of contaminated soil for disposal or further treatment. The UESR technology was used and developed further for this study.

This study aimed to evaluate the effects of using different treatment duration, soil type and initial soil water content on the removal efficiency of heavy metals and PAHs.

3.1.1 Kaolin and Natural Soil Preparation

Kaolin was obtained commercially (Kaolin Sdn. Bhd., Malaysia) and contained 45%-50% of SiO_2 , 33%-39% of Al_2O_3 and trace amounts of Fe_2O_3 and MgO (Wang et al. 2007). Kaolin was used in this study as it is a low buffering soil and has been used in several studies. However, its homogeneous nature, low organic content and low permeability might not be representative of real soil conditions. Therefore, natural soil obtained from a construction site in the NTU campus was also used in

this study as a comparison. The natural soil was sieved to obtain soil particle size below 2 mm.

Soil polluted with heavy metals often contains organic contaminants such as PAHs. For this study, cadmium, lead and zinc were used to represent typical heavy metals found in contaminated soils. For PAHs, phenanthrene (3-ring) and pyrene (4-ring) were used to represent typical 3-ring and 4-ring PAHs. Natural soil and kaolin were spiked with Cd, Pb, Zn, phenanthrene and pyrene with concentrations between 300 to 500 mg/kg. The PAHs were dissolved in dichloromethane and the solution was added to natural soil or kaolin. The spiked soil mixture was thoroughly mixed using a motor driven mixer (SP-800, Rhino, Taiwan). The mixed soil was placed under a fume hood for 3 days to allow the dichloromethane to evaporate.

After 3 days, heavy metals in their nitrates or sulphates ($\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) were dissolved in deionised water and added to the soil mixture. The spiked soil mixture was thoroughly mixed using the motor driven mixer and stored for 3 days at room temperature until it was used for the tests. The parameters used for the different tests are shown in Table 3.1.

Table 3.1 Parameters for the electrokinetic tests

Test	Soil type	Water content (%)	Test duration (days)
S60D4	Natural soil	60	4
S60D8	Natural soil	60	8
S40D4	Natural soil	40	4
S40D8	Natural soil	40	8
K60D4	Kaolin	60	4
K60D8	Kaolin	60	8
K40D4	Kaolin	40	4
K40D8	Kaolin	40	8

3.1.2 Electrokinetic Reactor Set-up

The electrokinetic reactor consisted of a direct current (DC) power supply; a cylindrical cell containing the spiked soil; the cathode and anode electrodes; the acid circulating pipe tubings; the pumps; the acid reservoir, the effluent and leachate reservoirs; and the electrical wiring (Figure 3.1). The cylindrical cell was made of acrylic and had an inner diameter of 10 cm, an outer diameter of 11 cm, and a height of 16 cm. The cell was marked with the following levels: 0 cm (top); 2 cm; 4 cm; 6 cm; 8 cm; 10 cm (bottom). A removable cover was screwed to the bottom of the cell and had a 1 cm outlet in the middle for leachate flow.

The power supply was obtained by using an AC to DC converter (Nemic Lambda, Model GEN300-2.5) and the voltage was set at 10 V. As the height of the soil in the cell was 10 cm, a constant DC voltage gradient of 1 V/cm was applied for all the tests. Similar voltage gradient have been used in several studies (Reddy and Saichek 2002; Weng et al. 2003; Reddy and Chinthamreddy 2004; Al-Shahrani and Roberts 2005).

The cathode electrode was a stainless steel ring that is perforated and hollow in the middle (Figure 3.2). The anode electrode was a graphite rod that is covered by an insulating PVC tube (Figure 3.2). The anode was only exposed at the end of the insulating tube to prevent direct contact between the two electrodes and also to simulate the electric field of two point charges. The electric field generated by the two electrodes was non-uniform and three-dimensional.

Figure 3.1 Electrokinetic reactor set-up

Figure 3.2 Anode and cathode

3.1.3 Experimental Set-up

Filter paper was placed at the bottom of the cell to prevent soil from leaching out and blocking the leachate outlet. The spiked soil was added into the cell till the 0 cm level. The soil in the cell was uniformly compacted using a pestle. Samples were taken from the cell to determine the initial soil conditions. Filter paper was placed on top of the soil followed by the cathode. The anode covered by the insulating tube was inserted into the soil at the center of the cell.

Electrical wiring was connected to the anode and cathode, and pump tubings were connected to the cell using plastic clips. The pumps were switched on to supply nitric acid (pH 2) to the cathode area and pump out acid from the cathode area. The pump flow rate was adjusted (about 1.5 ml/min) to maintain about 1 cm acid level in the cathode area. After the level was stabilised, the converter was switched on to supply electricity to the electrodes. The acid reservoir was refilled daily to ensure that there was sufficient acid circulating at the cathode area. The electrokinetic treatment was conducted over 4 or 8 days. All tests were done in triplicate.

The removal efficiency for heavy metals was calculated as:

$$\text{Removal efficiency, \%} = \frac{\text{Final amount of heavy metals in effluent and cathode}}{\text{Initial amount of heavy metals in soil}} \times 100 \quad (3.1)$$

This showed the actual removal of heavy metals from the soil due to the electrokinetic effect without the effect of leaching of heavy metals from the bottom of the soil.

The removal efficiency for PAHs was calculated as:

$$\text{Removal efficiency, \%} = \frac{\left(\text{Initial amount of PAHs in soil} - \text{Final amount of PAHs in soil} \right)}{\text{Initial amount of PAHs in soil}} \times 100 \quad (3.2)$$

The calculation for the removal efficiency for PAHs is different from that of heavy metals because PAHs removed from the soil tends to float on the circulating acid and was not transported to the effluent reservoir.

3.1.4 Sampling Procedure

Before starting the electrokinetic tests, soil samples were collected and analysed for water content, pH, PAHs and heavy metal concentration (refer to Section 3.4). During the tests, the current was recorded daily at regular intervals as indicated by the ammeter reading. The effluent volume in the effluent reservoir was recorded daily before draining out. Effluent samples were collected and analysed for pH and heavy metal concentration (refer to Section 3.4).

After the tests, the power supply and pumps were switched off and acid was drained out from the pump tubing. Remaining acid in the cathode area was also drained out into the effluent reservoir. The pump tubing and electrical wiring were then dismantled, and the electrodes were removed. The effluent volume in the effluent

reservoir and the leachate volume in the leachate reservoir were recorded at the end of the tests. Effluent and leachate samples were collected and analysed for pH and heavy metal concentration (refer to Section 3.4).

The soil was extruded out from the cell and cut into 5 layers of 2 cm each. The soil sections were labelled as follows: section 2 (0-2 cm of soil from cathode at top); section 4 (2-4 cm from cathode); section 6 (4-6 cm from cathode); section 8 (6-8 cm from cathode); and section 10 (8-10 cm from cathode). Each layer was weighed and mixed thoroughly before samples were collected and analysed for water content, pH, PAHs and heavy metal concentration (refer to Section 3.4).

The cathode was placed in nitric acid for 3 days to dissolve any metal precipitation on the cathode. The nitric acid solution was then analysed for heavy metal concentration (refer to Section 3.4).

3.2 Bioremediation Study

The bioremediation study involved the degradation of phenanthrene and pyrene using white rot fungi (pure fungi and cultivated fungi from commercial mushroom).

3.2.1 Fungi and Culture Conditions

Commercially available white rot fungi, *Pleurotus ostreatus* or commonly known as oyster mushroom, was bought from local supermarkets. The brand of the oyster mushroom was “Pasar”.

Malt extract agar plates were prepared by adding 33.6 g of malt extract agar powder to 1 L of deionised water and autoclaved at 121°C for 15 minutes. In the clean bench, the autoclaved solution was poured into agar plates and allowed to solidify. The agar plates were then used for the fungi culture.

The mushroom was cut into small pieces of about 2 mm using a sterile knife and tweezer. About 10 pieces of the mushroom was added on the agar for each agar plate

and the agar plates were then sealed with flexible film, Parafilm[®]. The agar plates were kept in an incubator at 25°C for 14 days. After 14 days of incubation in the dark, fungi mycelium would cover the agar plate and can be used to prepare the liquid cultivated fungi inoculum for the bioremediation tests.

The pure fungi, *Pleurotus ostreatus* DSM 11191, was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures). The fungi was spread on the agar plates and kept in an incubator at 25°C for 14 days. After 14 days of incubation in the dark, fungi mycelium would cover the agar plate and can be used to prepare the liquid pure fungi inoculum for the bioremediation tests.

3.2.2 Liquid Inoculum

The following liquid basic medium (BSM) was prepared consisting of 5 g glucose; 0.5 g yeast extract; 0.65 g L-asparagine; 1 g KH₂PO₄; 0.5 g MgSO₄·7H₂O; 0.5 g KCl; 0.01 g FeSO₄·7H₂O; 0.008 g MnSO₄·H₂O; 0.002 g ZnSO₄·7H₂O; 0.05 g Ca(NO₃)₂·4H₂O and 0.003 g CuCl₂·2H₂O in 1 L of deionised water. The liquid medium was autoclaved at 121°C for 15 minutes, and kept at 4°C until further use.

200 ml of the liquid BSM was added to 250-ml Erlenmeyer flasks and autoclaved at 121°C for 15 minutes. 5 pieces of agar plugs (about 1 cm diameter) were cut from the 14-day agar plates with fungi mycelium and added to each flask. The flasks were plugged with cotton plugs and placed on a shaker at 100 rpm for 14 days at room temperature. After 14 days, the liquid fungi inoculum was homogenised using a sterile blender and used for the bioremediation tests.

3.2.3 Soil Preparation

The soil used in this study was natural soil similar to that used for the electrokinetic tests. The soil was autoclaved at 121°C for 40 minutes and kept at room temperature until further use. Natural soil was spiked with phenanthrene and pyrene with concentrations between 200 to 500 mg/kg. The PAHs were dissolved in

dichloromethane and the solution was added to the soil. The spiked soil mixture was thoroughly mixed using a motor driven mixer (SP-800, Rhino, Taiwan). The mixed soil was placed under a fume hood for 3 days to allow the dichloromethane to evaporate.

3.2.4 Experimental Set-up

Deionised water was added to the spiked soil to achieve a moisture content of 15%. 3 g of spiked soil (dry weight basis) was added to 250-ml Erlenmeyer flasks. Liquid fungi inoculum was added to each flask according to the parameters shown in Table 3.2. The concentration of the liquid fungi inoculum was analysed (refer to Section 3.4) and found to be 727 mg/l and 397 mg/l for the cultivated fungi and pure fungi, respectively. The soil in the flasks for the control tests (C0, C10, C30, C50 and C70) was added with autoclaved liquid BSM and did not contain any fungi. The soil in the flasks for the tests F10, F30, F50 and F70 was added with liquid fungi inoculum cultivated from commercial mushroom. The soil in the flasks for the tests P10, P30, P50 and P70 was added with liquid fungi inoculum cultivated from pure fungi. The bioremediation tests were conducted for 56 days. All tests were done in triplicate.

The degradation of PAHs was determined based on the amount of PAHs degraded by the white rot fungi. The degradation of PAHs was calculated as:

$$\text{Degradation, \%} = \frac{\left(\frac{\text{Initial amount of PAHs in soil}}{\text{Initial amount of PAHs in soil}} - \frac{\text{Final amount of PAHs in soil}}{\text{Initial amount of PAHs in soil}} \right) \times 100}{1} \quad (3.3)$$

Table 3.2 Parameters for the bioremediation tests

Test	Type of fungi	Volume of fungi inoculum added to soil (ml)	Fungi inoculum to soil concentration (% v/w)
C0	None	0 (BSM)	0 (BSM)
C10	None	0.3 (BSM)	10 (BSM)
C30	None	0.9 (BSM)	30 (BSM)
C50	None	1.5 (BSM)	50 (BSM)
C70	None	2.1 (BSM)	70 (BSM)
F10	Cultivated	0.3	10
F30	Cultivated	0.9	30
F50	Cultivated	1.5	50
F70	Cultivated	2.1	70
P10	Pure	0.3	10
P30	Pure	0.9	30
P50	Pure	1.5	50
P70	Pure	2.1	70

3.2.5 Sampling Procedure

Soil samples were taken from the flasks on Day 0, 7, 14, 21, 35 and 56. The samples were air-dried for 1 week and stored in a dessicator until extraction and analysis of PAHs concentration.

3.3 Integrated Study

The UESR technology was able to remove most of the heavy metals and some amount of PAHs from the soil. The bioremediation process using white rot fungi could be used to further degrade the remaining PAHs and acted as a polishing step.

3.3.1 Experimental Set-up

This integrated study involved first an electrokinetic test followed by bioremediation tests. The electrokinetic test S60D8 (described earlier in Section 3.1) was first conducted with natural soil (60% initial water content) and electrokinetic treatment for 8 days. After 8 days, the treated natural soil after the electrokinetic test was used for the bioremediation test. The integrated study investigated the further degradation of phenanthrene and pyrene using white rot fungi cultivated from commercial mushroom. The parameters for the electrokinetic test and bioremediation tests are shown in Tables 3.3 and 3.4, respectively.

65 g of soil from test S60D8 (after electrokinetic treatment) on a dry weight basis was added to 250-ml Erlenmeyer flasks. Liquid fungi inoculum was added to each flask according to the parameters shown in Table 3.4. The soil in the flasks for the control tests (C0 and C70) was added with autoclaved liquid BSM and did not contain any fungi. The soil in the flasks for the test F was added with liquid fungi inoculum cultivated from commercial mushroom. The bioremediation tests were conducted for 56 days. All tests were done in triplicate.

Table 3.3 Parameters for the electrokinetic test in the integrated study

Test	Soil type	Water content (%)	Test duration (days)
S60D8	Natural soil	60	8

Table 3.4 Parameters for the bioremediation tests in the integrated study

Test	Type of fungi	Fungi inoculum to soil concentration (% v/w)
C0	None	0 (BSM)
C70	None	70 (BSM)
F	Cultivated	70

3.3.2 Sampling Procedure

Soil samples were taken from the flasks on Day 0, 7, 14, 21, 35 and 56. The samples were air-dried for 1 week and stored in a dessicator until extraction and analysis of PAH concentration.

3.4 Analytical Methods

3.4.1 Measuring Particle Size Distribution

The soil particle size distribution was determined with reference to the method in ASTM D 422. The distribution of particle sizes larger than 75 μm was determined by sieving and the distribution of particle sizes smaller than 75 μm was determined by a sedimentation process using a hydrometer. Particle sizes larger than 75 μm was reported as sand, particle sizes between 2 and 75 μm was reported as silt, and particle sizes less than 2 μm was reported as clay.

3.4.2 Measuring Specific Gravity

The specific gravity of soil samples was determined with reference to the method in ASTM D 854-02. A water pycnometer was filled with water only, and also filled with water and oven-dried soil. The specific gravity of the soil solids at the test temperature, G_t was calculated as shown below:

$$G_t = \frac{\rho_s}{\rho_{w,t}} = \frac{M_s}{(M_{pw,t} - (M_{pws,t} - M_s))} \quad (3.4)$$

where:

ρ_s = density of the soil solids, g/cm^3

$\rho_{w,t}$ = density of water at the test temperature, g/cm^3

M_s = mass of the oven dry soil solids, g

$M_{pws,t}$ = mass of pycnometer, water, and soil solids at the test temperature, g

$M_{pw,t}$ = mass of pycnometer and water at the test temperature, g

3.4.3 Measuring Water Content

The water content of soil samples was determined with reference to the method in ASTM D 2974-00 and was done on the day of sampling. The weight of the dish, D , was recorded, and about 5 g of wet soil was placed in it and evenly spread. The samples were dried for at least 1 day in an oven at a temperature of 105°C. The weight of the dish with the dry soil, F , was recorded after cooling the samples in a dessicator. The water content was calculated as shown below:

$$\text{Water content, \%} = \frac{(\text{Weight of wet soil} + D - F) \times 100}{\text{Weight of dry soil}} \quad (3.5)$$

3.4.4 Measuring pH

The pH of the effluent and leachate was measured by using a pH meter (Horiba D-24). The pH probe was inserted directly into the solution for measurement. For soil samples, 25 ml of deionised water was added to 5 g of soil and mixed on a shaker at 200 rpm for 1 hour. After shaking, the mixture was centrifuged at 2000 rpm for 5 minutes and the pH probe was inserted for measurement.

3.4.5 Measuring Organic Content

The organic content of soil samples was determined with reference to the method in ASTM D 2974-00. The oven-dried soil sample in a porcelain dish was placed in a furnace at 440°C and held until the soil was completely ashed. The ashed sample was covered with aluminum foil and cooled in a desiccator. The organic content was determined by the loss in mass between the oven-dried soil and the ash over the mass of the oven-dried soil.

3.4.6 Measuring Heavy Metal Concentration

Dry soil samples were acid-digested using a microwave digester (Microwave Digester Anton Paar Multiwave 3000) and analysed for heavy metals using the Inductively Coupled Plasma – Optical Emission Spectroscopy (PerkinElmer Optima DV2000 ICP-OES). The procedures were as follows:

- The soil sample was dried for at least 1 day in an oven at a temperature of 105°C.
- The dried sample was grounded into powder using a pestle.
- Approximately 0.1 g of the powder was added to the microwave vessel, followed by 8 ml of nitric acid with concentration of 69.9%.
- The vessels were placed in the microwave digester and the mixture was digested with a power of 1200 W ramped for 30 mins and held at 40 mins.
- The digested mixture was filtered using a 0.45 µm filter and diluted to 25 ml.
- The filtrate was detected for heavy metals using the ICP-OES.

3.4.7 Measuring PAHs Concentration

The extraction of PAHs from the soil samples was carried out by adding 20 ml of acetonitrile to 2 g of air-dried soil sample. This was followed by extraction on a shaker at 250 rpm for 24 hours. The samples were then centrifuged at 2000 rpm for 5 minutes. The acetonitrile extract was then filtered using a 0.2 µm nylon filter into vials and kept at 4°C until analysis using the High Performance Liquid Chromatograph System (PerkinElmer Series 200 HPLC). The recovery for the PAHs was 93% to 95% using the extraction method.

PAHs concentrations were analysed with the HPLC system using a reversed-phase Chromspher-3 PAH column (100 x 4.6 mm) and an ultraviolet detector set at a wavelength of 280 nm. A mixture of acetonitrile to deionised water (80:20) was used as a mobile phase at a flow rate of 1 ml/min. A typical LC chromatogram is shown in Figure 3.3.

Figure 3.3 A typical LC chromatogram

3.4.8 Measuring Energy Consumption

The energy consumption per unit volume of soil, E_v , is given by:

$$E_v = \frac{E}{V_s} = \frac{1}{V_s} \int VI \, dt \quad (3.6)$$

where E is the energy (kWh), V_s is the volume of soil column (m^3), I is the current (A), V is the voltage (V), and t is the time (h). The voltage was kept constant at 10 V throughout the electrokinetic tests. The current was measured by the ammeter and readings were taken frequently. The volume of the soil column was kept constant with diameter and height of 10 cm.

3.4.9 Measuring Fungi Inoculum Concentration

The concentration of the liquid fungi inoculum was analysed before the bioremediation tests. The weight of the dish, A, and a Whatman filter paper, B, was recorded, and about 30 ml of liquid fungi inoculum was added to the filter paper. The filter paper was placed on the dish and dried for at least 1 day in an oven at a temperature of 105°C. The weight of the dish and filter paper with the dry fungi biomass, C, was recorded after cooling them in a dessicator. The fungi inoculum concentration was calculated as shown below:

$$\text{Fungi concentration, mg / l} = \frac{C - A - B}{\text{Volume of liquid fungi inoculum}} \quad (3.7)$$

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Electrokinetic Study

The results are presented and discussed in the following sections for the soil properties, soil water content, soil pH, effluent pH, concentration of heavy metals in soil, distribution of heavy metals in soil, removal efficiency of heavy metals, concentration of PAHs in soil, removal efficiency of PAHs, changes of current during electrokinetic treatments, and energy consumption.

4.1.1 Soil Properties

The physical and chemical properties of natural soil and kaolin are shown in Table 4.1. According to the United States Department of Agriculture (USDA) system of soil classification by particle size distribution, the natural soil was classified as sandy clay and the kaolin was classified as clay. The natural soil (sandy clay) is likely to have a higher hydraulic conductivity and permeability than kaolin.

Table 4.1 Physical and chemical properties of natural soil and kaolin

Property	Natural soil	Kaolin
Particle size distribution		
Sand	53%	0%
Silt	8%	2%
Clay	39%	98%
Specific gravity	2.46	2.48
Water content	4.1%	1.3%
pH	6.73	6.60
Organic content	2%	0%

Table 4.2 summarises the actual parameters for the different electrokinetic tests. The tests were performed to investigate the effect of treatment duration, soil type and soil water content on the removal efficiencies of Cd, Pb, Zn, phenanthrene and pyrene.

Table 4.2 Actual parameters for the electrokinetic tests

Test	Soil type	Test duration (days)	Initial soil conditions	
			Water content (%)	pH
S60D4	Natural soil	4	62.1	4.31
S60D8	Natural soil	8	62.1	4.31
S40D4	Natural soil	4	39.5	4.45
S40D8	Natural soil	8	39.5	4.45
K60D4	Kaolin	4	60.9	3.97
K60D8	Kaolin	8	60.9	3.97
K40D4	Kaolin	4	41.2	3.90
K40D8	Kaolin	8	41.2	3.90

Table 4.3 Initial concentration of heavy metals and PAHs for electrokinetic tests

Test	Initial concentration of contaminants (mg/kg)				
	Cd	Pb	Zn	Phenanthrene	Pyrene
S60D4	420 ± 21	436 ± 24	480 ± 31	412 ± 20	497 ± 17
S60D8	420 ± 21	436 ± 24	480 ± 31	412 ± 20	497 ± 17
S40D4	458 ± 21	494 ± 26	507 ± 27	396 ± 8	474 ± 14
S40D8	458 ± 21	494 ± 26	507 ± 27	396 ± 8	474 ± 14
K60D4	389 ± 14	470 ± 6	427 ± 23	348 ± 12	512 ± 18
K60D8	389 ± 14	470 ± 6	427 ± 23	348 ± 12	512 ± 18
K40D4	394 ± 14	403 ± 10	408 ± 16	351 ± 11	476 ± 9
K40D8	394 ± 14	403 ± 10	408 ± 16	351 ± 11	476 ± 9

The initial spiked concentration of heavy metals and PAHs are shown in Table 4.3. The average concentration of heavy metals initially spiked in natural soil and kaolin for the 8 electrokinetic treatments was 415 ± 32 mg/kg for Cd, 451 ± 40 mg/kg for Pb and 456 ± 46 mg/kg for Zn. The average concentration of PAHs initially spiked

in natural soil and kaolin for the 8 electrokinetic treatments was 377 ± 32 mg/kg for phenanthrene and 490 ± 18 mg/kg for pyrene.

4.1.2 Soil Water Content

Different electrokinetic tests were conducted and the final water content in natural soil and kaolin at different locations from the cathode is shown in Figures 4.1 and 4.2, respectively. All the tests in Figures 4.1 and 4.2 show a decreasing trend down the soil profile, with more water content at the cathode area and less water content at the anode area. This was likely due to the electroosmosis effect where the soil pore water moved from the anode towards the cathode under an electric field. However, the decreasing trend could also be likely due to pore water leaching out from the soil at the bottom of the cell and cathode circulating acid percolating into the soil at the top of the cell.

Figure 4.1 Water content of natural soil after electrokinetic treatments

Figure 4.2 Water content of kaolin after electrokinetic treatments

The initial water content in the soil for S60D4 and S60D8 was 62.1% (Table 4.2). The average final water content in the soil was 51.9% and 51.2% for S60D4 and S60D8, respectively (Figure 4.1). After 4 days of electrokinetic treatment, the final water content in the soil for S60D4 decreased by 10.2%. After 8 days of electrokinetic treatment, the final water content in the soil for S60D8 decreased by 10.9%. Both reductions were the highest compared to the other tests. This could be due to the pore water being removed from the top of the soil as electroosmosis efficiency likely increased with high water content. The reduction was also likely due to the leaching of the pore water from the bottom of the natural soil as the natural soil was more saturated than the other tests. This is evident in Table 4.4, which shows that the volume of leachate collected is more than the other tests.

The initial water content in the soil for S40D4 and S40D8 was 39.5% (Table 4.2). The average final water content in the soil was 41.3% and 40.1% for S40D4 and S40D8, respectively (Figure 4.1). The water content in the soil after 4 and 8 days of electrokinetic treatment remained relatively similar to the initial water content and this could be likely due to a smaller electroosmosis effect.

Table 4.4 Average volume of leachate collected daily for electrokinetic tests

Test	Average volume of leachate collected per day (ml)
S60D4	35
S60D8	33
S40D4	25
S40D8	29
K60D4	21
K60D8	26
K40D4	22
K40D8	27

The initial water content in the soil for K60D4 and K60D8 was 60.9% (Table 4.2). The average final water content in the soil was 61.4% for both K60D4 and K60D8 (Figure 4.2). The water content in the soil after 4 and 8 days of electrokinetic treatment remained relatively similar to the initial water content and this could be likely due to a smaller electroosmosis effect.

The initial water content in the soil for K40D4 and K40D8 was 41.2% (Table 4.2). The average final water content in the soil was 50.3% and 49.9% for K40D4 and K40D8, respectively (Figure 4.2). After 4 days of electrokinetic treatment, the final water content in the soil for K40D4 increased by 9.1%. After 8 days of electrokinetic treatment, the final water content in the soil for K40D8 increased by 8.7%. Both increases were the highest compared to the other tests. This could be likely due to the circulating acid percolating into the kaolin as it was less saturated than the other treatments.

The maximum difference in the final water content between tests with different treatment duration but with the same soil type and initial water content were as follows: $\pm 1.0\%$ between S60D4 and S60D8; $\pm 1.3\%$ between S40D4 and S40D8; $\pm 0.5\%$ between K60D4 and K60D8; and $\pm 0.5\%$ between K40D4 and K40D8 (Figures 4.1 and 4.2). The results showed that the doubling of the treatment duration from 4 to 8 days did not significantly affect the water content in the soil. This could

be probably because the electroosmosis flow in the soil tends to decrease over time due to the changing soil properties and chemical composition of pore water (Reddy and Ala 2005, Altin and Degirmenci 2005).

The effect of soil type was compared between S60D8 and K60D8, and also between S40D8 and K40D8 (Figures 4.1 and 4.2). After 8 days, the average final water content in the soil for K60D8 was about the same as the initial water content. For S60D8, the average final water content decreased by 10.9%. The average volume of leachate collected daily was 33 ml for S60D8 and 26 ml for K60D8 (Table 4.4). After 8 days, the average final water content in the soil for S40D8 was about the same as the initial water content. For K40D8, the average final water content increased by 8.7%. The average volume of leachate collected daily was 29 ml for S40D8 and 27 ml for K40D8 (Table 4.4).

The tests showed that for soils with higher initial water content, the reduction in water content after the electrokinetic treatment was higher for natural soil than kaolin. This could be due to more pore water leaching out from the natural soil as shown in the higher volume of leachate collected. The natural soil has a higher hydraulic conductivity and permeability than kaolin, which probably resulted in more pore water leaching out from the soil cell due to gravitational force and also increased the electroosmosis efficiency.

For soils with lower water content, the amount of pore water leaching out from natural soil and kaolin was about the same. The higher water content in kaolin as compared to natural soil after the electrokinetic treatment could be likely due to the circulating acid percolating into the cell.

4.1.3 Soil pH

Different electrokinetic tests were conducted and the final pH in natural soil and kaolin at different locations from the cathode is shown in Figures 4.3 and 4.4, respectively. All the tests showed a decreasing trend down the soil profile, with pH ranging from 3.52 to 4.74 at soil section 2 and pH ranging from 2.02 to 2.35 at soil

section 10. The soil was more acidic at the bottom and more basic at the top because the electrolysis of water produces OH^- ions at the cathode and H^+ ions at the anode, resulting in the migration of an acid front from the bottom anode up towards the cathode and a basic front from the top cathode down towards the anode. However, the basic front did not migrate further into the soil due to the circulating acid at the cathode area which helped to neutralise the OH^- ions. This resulted in the pH for soil sections 4, 6, 8 and 10 for all the tests to be less than the initial soil pH. For soil section 2, some of the tests have pH above the initial soil pH but the difference is not large with a maximum difference of 0.64.

Figure 4.3 pH of natural soil after electrokinetic treatments

Figure 4.4 pH of kaolin after electrokinetic treatments

The results for all the tests showed that as the treatment duration increased from 4 to 8 days, the pH in most of the soil sections decreased further by an average of 0.20. This indicated that the acid front kept the soil pH low and the circulating acid was effective in preventing the basic front from migrating down towards the anode. In addition, the results for all the tests showed that different soil types and initial soil water content did not affect the final soil pH much and the maximum difference was ± 0.54 and ± 0.27 , respectively.

4.1.4 Effluent pH

Figure 4.5 shows the pH of the effluent from the cathode area for the different tests with natural soil. Effluent collected after the first day showed higher pH at around 3.14 to 3.91. For the remaining days, effluent pH remained relatively constant at 2.33 to 2.80. Figure 4.6 shows the pH of the effluent from the cathode area for the different tests with kaolin. Effluent collected after the first day showed higher pH at around 2.90 to 3.43. For the remaining days, effluent pH remained relatively constant at 2.67 to 2.88.

Figure 4.5 pH of effluent during electrokinetic treatments for natural soil

Figure 4.6 pH of effluent during electrokinetic treatments for kaolin

For the tests with natural soil or kaolin, the pH of the effluent was higher initially because of the electrolysis of water at the cathode area to produce OH^- ions. This increased the initial pH of 2.00 of the circulating acid. However, the accumulation of metal precipitates on the cathode and reduced electroosmosis effect probably affected the current over time, resulting in less OH^- ions produced and stabilising

the effluent pH. In addition, the acid front generated at the anode could probably migrate to the cathode area and neutralised some of the OH^- ions there. The low pH of the effluent also showed that the circulating acid was sufficient to maintain low pH at the cathode area, thus preventing less precipitation of metal hydroxides.

4.1.5 Concentration of Heavy Metals in Soil

4.1.5.1 Concentration of Cadmium

The concentration profile of Cd in the natural soil and kaolin after electrokinetic treatments are shown in Figures 4.7 and 4.8, respectively. All the tests showed relatively similar trend. Soil at section 2 tends to have higher Cd concentration than the rest of the sections. The Cd concentration in soil sections 4, 6, 8 and 10 was lower than the initial Cd concentration in the soil. This showed that Cd in the soil had migrated upwards from the anode towards the cathode likely due to the electrokinetic process.

The initial Cd concentration for S60D4 and S60D8 was 420 ± 21 mg/kg (Table 4.3). The average Cd concentration in sections 6 to 10 decreased by 84% after 4 days and by 96% after 8 days. The Cd concentration in section 4 decreased by 36% after 4 days and by 87% after 8 days. The lower reduction in Cd concentration for section 4 after 4 days as compared to sections 6 to 10 could be likely due to the higher pH there (Figure 4.3), leading to possible formation of metal hydroxides and less migration. The initial Cd concentration for S40D4 and S40D8 was 458 ± 21 mg/kg (Table 4.3). The average Cd concentration in sections 4 to 10 decreased by 77% after 4 days and by 88% after 8 days.

Figure 4.7 Concentration of cadmium in natural soil after electrokinetic treatments

Figure 4.8 Concentration of cadmium in kaolin after electrokinetic treatments

The initial Cd concentration for K60D4 and K60D8 was 389 ± 14 mg/kg (Table 4.3). The average Cd concentration in sections 4 to 10 decreased by 75% after 4 days and by 92% after 8 days. The initial Cd concentration for K40D4 and K40D8 was $394 \pm$

14 mg/kg (Table 4.3). The average Cd concentration in sections 4 to 10 decreased by 66% after 4 days and by 90% after 8 days.

The above results indicated that an increase in the electrokinetic treatment duration from 4 to 8 days would increase the reduction in the Cd concentration for natural soil and kaolin. In addition, the Cd concentration in section 2 was lower after 8 days as compared to after 4 days, which indicated that Cd had migrated out of the soil into the circulating acid and into the effluent reservoir or deposited on the cathode.

The natural soil in S60D8 has higher initial soil water content than S40D8. The average reduction in Cd concentration for sections 6 to 10 was 96% for S60D8 and 88% for S40D8. The kaolin in K60D8 has higher initial soil water content than K40D8. The average reduction in Cd concentration for sections 6 to 10 was 90% for K60D8 and 88% for K40D8. The results showed that higher initial soil water content would help in the reduction of Cd concentration, especially for natural soil as compared to kaolin. This could be probably because higher water content increased the electroosmosis effect and removal of Cd. In addition, more pore water could likely result in a higher concentration of Cd being mobile in the water phase than adsorbed on the soil surface, thus improving the removal of Cd by electromigration. The natural soil has a higher hydraulic conductivity and permeability than kaolin, which probably increased the electroosmosis efficiency. The reduction in Cd concentration was thus higher in natural soil than in kaolin.

4.1.5.2 Concentration of Zinc

The concentration profile of Zn in the natural soil and kaolin after electrokinetic treatments are shown in Figures 4.9 and 4.10, respectively. All the tests showed relatively similar trend and were similar to the trend for the Cd concentration in Figures 4.7 and 4.8. Soil at section 2 tends to have higher Zn concentration than the rest of the sections. The Zn concentration in soil sections 4, 6, 8 and 10 was lower than the initial Zn concentration in the soil. This showed that Zn in the soil had migrated upwards from the anode towards the cathode likely due to the electrokinetic process.

Figure 4.9 Concentration of zinc in natural soil after electrokinetic treatments

Figure 4.10 Concentration of zinc in kaolin after electrokinetic treatments

The initial Zn concentration for S60D4 and S60D8 was 480 ± 31 mg/kg (Table 4.3). The average Zn concentration in sections 6 to 10 decreased by 74% after 4 days and by 86% after 8 days. The Zn concentration in section 4 decreased by 29% after 4 days and by 84% after 8 days. The lower reduction in Zn concentration for section 4

after 4 days as compared to sections 6 to 10 could be due to the higher pH there (Figure 4.3), leading to possible formation of metal hydroxides and less migration. The initial Zn concentration for S40D4 and S40D8 was 507 ± 27 mg/kg (Table 4.3). The average Zn concentration in sections 4 to 10 decreased by 72% after 4 days and by 82% after 8 days.

The initial Zn concentration for K60D4 and K60D8 was 427 ± 23 mg/kg (Table 4.3). The average Zn concentration in sections 4 to 10 decreased by 73% after 4 days and by 87% after 8 days. The initial Zn concentration for K40D4 and K40D8 was 408 ± 16 mg/kg (Table 4.3). The average Zn concentration in sections 4 to 10 decreased by 63% after 4 days and by 88% after 8 days.

The above results indicated that an increase in the electrokinetic treatment duration from 4 to 8 days would increase the reduction in the Zn concentration for natural soil and kaolin. In addition, the Zn concentration in section 2 was lower after 8 days as compared to after 4 days, which indicated that Zn had migrated out of the soil into the circulating acid and into the effluent reservoir or deposited on the cathode.

The natural soil in S60D8 has higher initial soil water content than S40D8. The average reduction in Zn concentration for sections 6 to 10 was 86% for S60D8 and 81% for S40D8. The kaolin in K60D8 has higher initial soil water content than K40D8. The average reduction in Zn concentration for sections 6 to 10 was 87% for K60D8 and 86% for K40D8. The results showed that higher initial soil water content would help in the reduction of Zn concentration, especially for natural soil as compared to kaolin. This could be probably because higher water content increased the electroosmosis effect and removal of Zn. In addition, more pore water could likely result in a higher concentration of Zn being mobile in the water phase than adsorbed on the soil surface, thus improving the removal of Zn by electromigration. The natural soil has a higher hydraulic conductivity and permeability than kaolin, which probably increased the electroosmosis efficiency. The reduction in Zn concentration was thus higher in natural soil than in kaolin.

4.1.5.3 Concentration of Lead

The concentration profile of Pb in the natural soil and kaolin after electrokinetic treatments are shown in Figures 4.11 and 4.12, respectively. All the tests showed different trend as compared to the trend for the Cd and Zn concentration. The tests in Figures 4.11 and 4.12 show higher Pb concentration in sections 8 and 10, which indicated that Pb is not too mobile at that region. This could be probably because Pb is known to have stronger adsorption on soil than Cd and Zn, and is usually difficult to remediate (Sah and Chen 1998; Vengris et al. 2001; Virkutyte et al. 2002). In addition, the low pH at sections 8 and 10 could affect the adsorption of Pb on soil. All the tests except for K40D4 showed that Pb concentration at section 2 tend to be higher than section 4. This could be likely due to the formation of lead hydroxides in the soil at section 2, causing less mobility and reduced migration of Pb into the effluent and on the cathode.

Figure 4.11 Concentration of lead in natural soil after electrokinetic treatments

Figure 4.12 Concentration of lead in kaolin after electrokinetic treatments

The initial Pb concentration for S60D4 and S60D8 was 436 ± 24 mg/kg (Table 4.3). The average Pb concentration in sections 2 to 10 decreased by 7% after 4 days and by 31% after 8 days. The initial Pb concentration for S40D4 and S40D8 was 494 ± 26 mg/kg (Table 4.3). The average Pb concentration in sections 2 to 10 decreased by 5% after 4 days and by 21% after 8 days.

The initial Pb concentration for K60D4 and K60D8 was 470 ± 6 mg/kg (Table 4.3). The average Pb concentration in sections 2 to 10 decreased by 13% after 4 days and by 34% after 8 days. The initial Pb concentration for K40D4 and K40D8 was 403 ± 10 mg/kg (Table 4.3). The average Pb concentration in sections 2 to 10 decreased by 9% after 4 days and by 26% after 8 days.

The above results indicated that an increase in the electrokinetic treatment duration from 4 to 8 days would increase the reduction in the Pb concentration for natural soil and kaolin. In addition, the Pb concentration in section 2 was lower after 8 days as compared to after 4 days, which indicated that Pb had migrated out of the soil into the circulating acid and into the effluent reservoir or deposited on the cathode.

The natural soil in S60D8 has higher initial soil water content than S40D8. The average reduction in Pb concentration for sections 2 to 10 was 31% for S60D8 and 21% for S40D8. The kaolin in K60D8 has higher initial soil water content than K40D8. The average reduction in Pb concentration for sections 2 to 10 was 34% for K60D8 and 26% for K40D8. The results showed that higher initial soil water content would help in the reduction of Pb concentration for natural soil and kaolin. This could be probably because higher water content increased the electroosmosis effect and removal of Pb. In addition, more pore water could likely result in a higher concentration of Pb being mobile in the water phase than adsorbed on the soil surface, thus improving the removal of Pb by electromigration.

4.1.6 Distribution of Heavy Metals

The total amount of heavy metals in the soil, effluent, leachate and on the cathode after electrokinetic treatments was compared to the total initial spiked amount. The mass balance for all the electrokinetic tests was $99.5 \pm 3.8\%$.

4.1.6.1 Distribution of Cadmium

The mass distribution of Cd in the soil, effluent, leachate and on the cathode after electrokinetic treatments is shown in Figures 4.13 and 4.14. All the treatments showed relatively similar trend. A higher quantity of Cd can be found in the soil and effluent than in the leachate and on the cathode. The amount of Cd that leached out from the soil into the leachate reservoir was less than 10% for all the treatments.

For S60D4 and S60D8, an increase in treatment duration from 4 to 8 days reduced the amount of Cd in the soil by 28%, increased the amount of Cd in the effluent and on the cathode by 27%, and increased the amount of Cd in the leachate by 1%. For S40D4 and S40D8, an increase in treatment duration from 4 to 8 days reduced the amount of Cd in the soil by 20%, increased the amount of Cd in the effluent and on the cathode by 19%, and increased the amount of Cd in the leachate by 1%. The results showed that an increase in the electrokinetic duration caused more Cd to migrate out of the natural soil to the effluent and cathode. The increase in

electrokinetic duration did not cause significant change (1%) in the amount of Cd leached out.

Figure 4.13 Mass distribution of cadmium after electrokinetic treatments with natural soil

Figure 4.14 Mass distribution of cadmium after electrokinetic treatments with kaolin

For K60D4 and K60D8, an increase in treatment duration from 4 to 8 days reduced the amount of Cd in the soil by 16%, increased the amount of Cd in the effluent and on the cathode by 13%, and increased the amount of Cd in the leachate by 3%. For K40D4 and K40D8, an increase in treatment duration from 4 to 8 days reduced the amount of Cd in the soil by 22%, increased the amount of Cd in the effluent and on the cathode by 18%, and increased the amount of Cd in the leachate by 4%. The results showed that an increase in the electrokinetic duration caused more Cd to migrate out of the kaolin to the effluent and cathode. The increase in electrokinetic duration did not cause significant change (3% to 4%) in the amount of Cd leached out.

4.1.6.2 Distribution of Zinc

The mass distribution of Zn in the soil, effluent, leachate and on the cathode after electrokinetic treatments is shown in Figures 4.15 and 4.16. All the treatments showed relatively similar trend. A higher quantity of Zn can be found in the soil and effluent than in the leachate and on the cathode. The amount of Zn that leached out from the soil into the leachate reservoir was less than 10% for all the treatments.

For S60D4 and S60D8, an increase in treatment duration from 4 to 8 days reduced the amount of Zn in the soil by 29%, increased the amount of Zn in the effluent and on the cathode by 27%, and increased the amount of Zn in the leachate by 2%. For S40D4 and S40D8, an increase in treatment duration from 4 to 8 days reduced the amount of Zn in the soil by 17%, increased the amount of Zn in the effluent and on the cathode by 11%, and increased the amount of Zn in the leachate by 7%. The results showed that an increase in the electrokinetic duration caused more Zn to migrate out of the natural soil to the effluent and cathode. The increase in electrokinetic duration did not cause significant change (2% to 7%) in the amount of Zn leached out.

Figure 4.15 Mass distribution of zinc after electrokinetic treatments with natural soil

Figure 4.16 Mass distribution of zinc after electrokinetic treatments with kaolin

For K60D4 and K60D8, an increase in treatment duration from 4 to 8 days reduced the amount of Zn in the soil by 18%, increased the amount of Zn in the effluent and on the cathode by 16%, and increased the amount of Zn in the leachate by 3%. For K40D4 and K40D8, an increase in treatment duration from 4 to 8 days reduced the amount of Zn in the soil by 23%, increased the amount of Zn in the effluent and on

the cathode by 20%, and increased the amount of Zn in the leachate by 3%. The results showed that an increase in the electrokinetic duration caused more Zn to migrate out of the kaolin to the effluent and cathode. The increase in electrokinetic duration did not cause significant change (3%) in the amount of Zn leached out.

4.1.6.3 Distribution of Lead

The mass distribution of Pb in the soil, effluent, leachate and on the cathode after electrokinetic treatments is shown in Figures 4.17 and 4.18. All the treatments showed relatively similar trends. A higher quantity of Pb can be found in the soil and effluent than in the leachate and on the cathode. The amount of Pb that leached out from the soil into the leachate reservoir was less than 4% for all the treatments.

For S60D4 and S60D8, an increase in treatment duration from 4 to 8 days reduced the amount of Pb in the soil by 24%, increased the amount of Pb in the effluent and on the cathode by 22%, and increased the amount of Pb in the leachate by 2%. For S40D4 and S40D8, an increase in treatment duration from 4 to 8 days reduced the amount of Pb in the soil by 12%, increased the amount of Pb in the effluent and on the cathode by 10%, and increased the amount of Pb in the leachate by 2%. The results showed that an increase in the electrokinetic duration caused more Pb to migrate out of the natural soil to the effluent and cathode. The increase in electrokinetic duration did not cause significant change (2%) in the amount of Pb leached out.

Figure 4.17 Mass distribution of lead after electrokinetic treatments with natural soil

Figure 4.18 Mass distribution of lead after electrokinetic treatments with kaolin

For K60D4 and K60D8, an increase in treatment duration from 4 to 8 days reduced the amount of Pb in the soil by 16%, increased the amount of Pb in the effluent and on the cathode by 15%, and increased the amount of Pb in the leachate by 1%. For K40D4 and K40D8, an increase in treatment duration from 4 to 8 days reduced the amount of Pb in the soil by 14%, increased the amount of Pb in the effluent and on

the cathode by 12%, and increased the amount of Pb in the leachate by 1%. The results showed that an increase in the electrokinetic duration caused more Pb to migrate out of the kaolin to the effluent and cathode. The increase in electrokinetic duration did not cause significant change (1%) in the amount of Pb leached out.

4.1.7 Removal Efficiency of Heavy Metals

The removal efficiency of heavy metals from natural soil and kaolin after electrokinetic treatments is shown in Figures 4.19 and 4.20, respectively. The removal efficiency was calculated based on the amount of heavy metals present in the effluent and on the cathode, as compared to the initial amount of heavy metals. This showed the actual removal of heavy metals from the soil due to the electrokinetic effect without the effect of leaching of heavy metals from the bottom of the soil. All the treatments showed that the removal efficiency for Cd was the highest (45% to 72%), followed by Zn (38% to 62%) and Pb (9% to 37%). Previous studies have shown that the removal efficiency of Cd or Zn was generally better than Pb (Huang 2005; Kim et al. 2001; Kim et al. 2005; Vengris et al. 2001; Virkutyte et al. 2002; Wang et al. 2006).

Test S60D8 with electrokinetic treatment duration of 8 days and with natural soil (60% initial water content) showed the highest removal efficiency for all the heavy metals among the tests. The removal efficiency for S60D8 was $72 \pm 1\%$, $62 \pm 4\%$ and $37 \pm 0\%$ for Cd, Zn and Pb, respectively.

Figure 4.19 Removal efficiency of heavy metals from natural soil after electrokinetic treatments

Figure 4.20 Removal efficiency of heavy metals from kaolin after electrokinetic treatments

4.1.7.1 Effect of Treatment Duration

The removal efficiency of heavy metals after the electrokinetic treatments is shown in Table 4.5. For S60D4 and S60D8, an increase in treatment duration from 4 to 8

days increased the removal efficiency of Cd, Zn and Pb by 26%, 24% and 24% respectively. For S40D4 and S40D8, an increase in treatment duration from 4 to 8 days increased the removal efficiency of Cd, Zn and Pb by 15%, 12% and 10% respectively.

For K60D4 and K60D8, an increase in treatment duration from 4 to 8 days increased the removal efficiency of Cd, Zn and Pb by 10%, 16% and 13% respectively. For K40D4 and K40D8, an increase in treatment duration from 4 to 8 days increased the removal efficiency of Cd, Zn and Pb by 15%, 18% and 13% respectively.

Table 4.5 Removal efficiency of heavy metals after electrokinetic treatments with different treatment duration

Test	Removal efficiency (%)		
	Cd	Zn	Pb
S60D8	72 ± 1	62 ± 4	37 ± 0
S60D4	46 ± 3	38 ± 3	13 ± 4
S40D8	60 ± 6	51 ± 10	19 ± 5
S40D4	45 ± 4	39 ± 4	9 ± 3
K60D8	60 ± 6	57 ± 7	29 ± 2
K60D4	50 ± 8	42 ± 12	15 ± 5
K40D8	61 ± 3	59 ± 8	28 ± 3
K40D4	46 ± 5	41 ± 6	16 ± 4

The results showed that an increase in the electrokinetic duration improved the removal efficiency of heavy metals. This was supported by the low soil pH after the electrokinetic treatments and the low effluent pH during the treatment. The acid front generated at the anode kept the soil pH low while the circulating acid effluent was effective in preventing the basic front from migrating down towards the anode. This prevented precipitation of metal hydroxides near the cathode area. As the electrokinetic duration increased, the heavy metals migrated from the anode up towards the cathode and out of the soil into the circulating acid effluent or deposited on the cathode. This was shown by the reduction in heavy metal concentration in the

soil and the increase in mass distribution of heavy metals in the effluent and on the cathode.

4.1.7.2 Effect of Initial Soil Water Content

The removal efficiency of heavy metals after the electrokinetic treatments is shown in Table 4.6. For S40D8 and S60D8, an increase in initial soil water content from 40 to 60% increased the removal efficiency of Cd, Zn and Pb by 12%, 11% and 18% respectively. For S40D8 and S60D8, the average error bar was $\pm 4\%$, $\pm 5\%$ and $\pm 2\%$ for Cd, Zn and Pb, respectively. This probably indicates that the increase in removal efficiency is not likely due to measurement errors. For K40D8 and K60D8, an increase in initial soil water content from 40% to 60% did not significantly affect the removal efficiency and the difference was within $\pm 1\%$. For K40D8 and K60D8, the average error bar was $\pm 4\%$, $\pm 4\%$ and $\pm 2\%$ for Cd, Zn and Pb, respectively. There is no indication of an increase in removal efficiency.

Table 4.6 Removal efficiency of heavy metals after electrokinetic treatments with different initial soil water content

Test	Removal efficiency (%)		
	Cd	Zn	Pb
S60D8	72 ± 1	62 ± 4	37 ± 0
S40D8	60 ± 6	51 ± 10	19 ± 5
K60D8	60 ± 6	57 ± 7	29 ± 2
K40D8	61 ± 3	59 ± 8	28 ± 3

The results showed that natural soil with higher initial soil water content (higher saturation) improved the removal efficiency of heavy metals. This could be because higher water content probably increased the electroosmosis effect (Hamed 1990) and moved the heavy metals upwards. In addition, more pore water could likely result in a higher concentration of heavy metals being mobile in the water phase than adsorbed on the soil surface, thus improving the removal of heavy metals by electromigration. Kaolin has a lower hydraulic conductivity and permeability than natural soil, thus the enhanced effect of electroosmosis and electromigration due to

higher water content was probably insufficient to cause a significant increase in the removal efficiency.

4.1.7.3 Effect of Soil Type

The removal efficiency of heavy metals after the electrokinetic treatments is shown in Table 4.7. For S60D8 and K60D8, the removal efficiency of Cd, Zn and Pb increased by 12%, 4% and 8%, respectively when natural soil was used instead of kaolin. For S40D8 and K40D8, the removal efficiency of Cd, Zn and Pb increased by 1%, 8% and 10%, respectively when kaolin was used instead of natural soil.

Table 4.7 Removal efficiency of heavy metals after electrokinetic treatments with different soil type

Test	Removal efficiency (%)		
	Cd	Zn	Pb
S60D8	72 ± 1	62 ± 4	37 ± 0
K60D8	60 ± 6	57 ± 7	29 ± 2
S40D8	60 ± 6	51 ± 10	19 ± 5
K40D8	61 ± 3	59 ± 8	28 ± 3

The results showed that the removal efficiency of heavy metals was better for tests with natural soil than kaolin in a more saturated condition. The higher water content combined with higher permeability of natural soil improved the electrokinetic effect in natural soil as compared to kaolin. However, under less saturated conditions, kaolin seemed to show a better removal efficiency of heavy metals than natural soil. This could be probably due to the adsorption of heavy metals to natural soil under less saturated conditions, thus reducing the mobility of the heavy metals. In addition, less water content could likely result in reduced electroosmosis flow rate, uneven water distribution and development of negative pore pressures (Page and Page 2002), thus affecting the electrokinetic effect in natural soil and causing the difference between kaolin in removal efficiency.

4.1.8 Concentration of PAHs in Soil

4.1.8.1 Concentration of Phenanthrene

The concentration profile of phenanthrene in the natural soil and kaolin after electrokinetic treatments are shown in Figures 4.21 and 4.22, respectively. All the tests showed relatively similar trend, where the soil at sections 4 and 6 tend to have higher phenanthrene concentration than sections 8 and 10. This showed that phenanthrene in the soil at sections 8 and 10 had moved upwards from the anode towards the cathode likely due to the electrokinetic process.

Phenanthrene is electrically neutral and less likely to be removed by electromigration and electrophoresis. Electroosmosis was probably the main mechanism for the removal of phenanthrene from soil (Reddy and Saichek 2002; Reddy and Saichek 2003). Electroosmosis can cause the pore water in the soil to move upwards to the cathode and helped to transport the solubilised phenanthrene. In addition, phenanthrene being a light non-aqueous phase liquid tends to float on top of the pore water. The pore water thus provided an ‘uplifting effect’ and lifted the phenanthrene in the soil upwards (Wang et al. 2007). Besides electroosmosis, Wang et al. (2007) also described the effect of dielectrophoresis in the removal of phenanthrene but they suggested that it was not likely to be the main mechanism.

Figure 4.21 Concentration of phenanthrene in natural soil after electrokinetic treatments

Figure 4.22 Concentration of phenanthrene in kaolin after electrokinetic treatments

The initial phenanthrene concentration for S60D4 and S60D8 was 412 ± 20 mg/kg (Table 4.3). The average phenanthrene concentration in sections 2 to 10 decreased by 2% after 4 days and by 24% after 8 days. The initial phenanthrene concentration

for S40D4 and S40D8 was 396 ± 8 mg/kg (Table 4.3). The average phenanthrene concentration in sections 2 to 10 decreased by 3% after 4 days and by 15% after 8 days. The average error bar was $\pm 2\%$ for the tests and this probably indicate that the decrease in average phenanthrene concentration after 4 days could be likely due to measurement errors, whereas the decrease in average phenanthrene concentration after 8 days is not likely due to measurement errors.

The initial phenanthrene concentration for K60D4 and K60D8 was 348 ± 12 mg/kg (Table 4.3). The average phenanthrene concentration in sections 2 to 10 decreased by 16% after 4 days and by 26% after 8 days. The initial phenanthrene concentration for K40D4 and K40D8 was 351 ± 11 mg/kg (Table 4.3). The average phenanthrene concentration in sections 2 to 10 decreased by 15% after 4 days and by 16% after 8 days. The average error bar was $\pm 2\%$ for the tests and this probably indicate that the decrease in average phenanthrene concentration after 4 and 8 days is not likely due to measurement errors. The low reduction in phenanthrene concentration after 8 days (K40D8) as compared to after 4 days (K40D4) was due to the higher concentration of phenanthrene that was present in section 2.

The results indicated that an increase in the electrokinetic treatment duration from 4 to 8 days would increase the reduction in the phenanthrene concentration for natural soil and kaolin. In addition, the phenanthrene concentration in section 2 for most of the tests was lower after 8 days as compared to after 4 days, which indicated that phenanthrene had migrated out of the soil due to electroosmosis. The free phenanthrene removed from the soil tends to float on the circulating acid due to its low density and low solubility, and was unlikely to be transported to the effluent reservoir. Most of the free phenanthrene was then likely lost through volatilisation while some adhered to the cathode, cell and tubing surface.

The natural soil in S60D8 has higher initial soil water content than S40D8. The average reduction in phenanthrene concentration for sections 2 to 10 was 24% for S60D8 and 15% for S40D8. The kaolin in K60D8 has higher initial soil water content than K40D8. The average reduction in phenanthrene concentration for sections 2 to 10 was 26% for K60D8 and 16% for K40D8. The results showed that

higher initial soil water content would help in the reduction of phenanthrene concentration for natural soil and kaolin. This could be probably because higher water content increased the electroosmosis effect and removal of phenanthrene.

The use of natural soil or kaolin for the electrokinetic treatments (S60D8 and K60D8; S40D8 and K40D8) did not change the reduction in phenanthrene concentration significantly. The higher permeability of natural soil probably improved the electroosmosis effect in natural soil as compared to kaolin. This would help in the removal of phenanthrene from natural soil. However, phenanthrene was also probably adsorbed more strongly to natural soil than kaolin due to the higher organic content of natural soil. These two effects could be present in natural soil and resulted in phenanthrene not being removed effectively when compared to kaolin.

4.1.8.2 Concentration of Pyrene

The concentration profile of pyrene in the natural soil and kaolin after electrokinetic treatments are shown in Figures 4.23 and 4.24, respectively. The tests in Figure 4.23 showed different trends from the tests in Figure 4.24, where the soil at sections 2 and 4 tend to have higher pyrene concentration than sections 6, 8 and 10. This suggested that pyrene was not very mobile in the natural soil due to its lower solubility and higher adsorption capacity on soil as compared to phenanthrene. In addition, natural soil contained more organic content than kaolin and the organics present could probably adsorb more pyrene.

The initial pyrene concentration for S60D4 and S60D8 was 497 ± 17 mg/kg (Table 4.3). The average pyrene concentration in sections 2 to 10 decreased by 3% after 4 days and by 6% after 8 days. The initial pyrene concentration for S40D4 and S40D8 was 474 ± 14 mg/kg (Table 4.3). The average pyrene concentration in sections 2 to 10 decreased by 2% after 4 days and by 10% after 8 days. The average error bar was $\pm 2\%$ for the tests and this probably indicate that the decrease in average pyrene concentration after 4 days could be likely due to measurement errors, whereas the decrease in average pyrene concentration after 8 days is not likely due to measurement errors.

Figure 4.23 Concentration of pyrene in natural soil after electrokinetic treatments

Figure 4.24 Concentration of pyrene in kaolin after electrokinetic treatments

The initial pyrene concentration for K60D4 and K60D8 was 512 ± 18 mg/kg (Table 4.3). The average pyrene concentration in sections 2 to 10 decreased by 10% after 4 days and by 15% after 8 days. The initial pyrene concentration for K40D4 and K40D8 was 476 ± 9 mg/kg (Table 4.3). The average pyrene concentration in

sections 2 to 10 decreased by 5% after 4 days and by 16% after 8 days. The average error bar was $\pm 1\%$ for the tests and this probably indicate that the decrease in average pyrene concentration after 4 and 8 days is not likely due to measurement errors.

The results indicated that an increase in the electrokinetic treatment duration from 4 to 8 days would increase the reduction in the pyrene concentration for natural soil and kaolin. In addition, the pyrene concentration in section 2 for most of the tests was lower after 8 days as compared to after 4 days, which indicated that pyrene had migrated out of the soil likely due to electroosmosis. The free pyrene removed from the soil tends to float on the circulating acid due to its low density and low solubility, and was unlikely to be transported to the effluent reservoir. Most of the free pyrene was then probably lost through volatilisation while some adhered to the cathode, cell and tubing surface.

The natural soil in S60D8 has higher initial soil water content than S40D8, and the kaolin in K60D8 has higher initial soil water content than K40D8. However, the higher initial soil water content did not affect the reduction in pyrene concentration significantly. This could be because pyrene was adsorbed more strongly to soil and the enhanced electroosmosis effect due to higher initial water content was not able to remove pyrene effectively.

For S60D8 and K60D8, the reduction of pyrene concentration was increased by 9% when kaolin was used instead of natural soil. For S40D8 and K40D8, the reduction of pyrene concentration was increased by 6% when kaolin was used instead of natural soil.

The results showed that the reduction in the concentration of pyrene was better for tests with kaolin than natural soil. This could be probably because pyrene was not very mobile in the natural soil as it contained more organic content than kaolin and the organics present could probably adsorb more pyrene.

4.1.9 Removal Efficiency of PAHs

The removal efficiency of PAHs from natural soil and kaolin after electrokinetic treatments is shown in Figures 4.25 and 4.26, respectively. All the treatments showed that the removal efficiency for phenanthrene was the highest (10% to 29%), followed by pyrene (6% to 19%). Test K60D8 with electrokinetic treatment duration of 8 days and with kaolin (60% initial water content) showed the highest removal efficiency for all the PAHs among the tests. The removal efficiency for K60D8 was $29 \pm 1\%$ and $19 \pm 0\%$ for phenanthrene and pyrene, respectively.

The removal efficiency for PAHs was much lower compared to heavy metals. This was expected because PAHs were electrically neutral and less affected by the electrokinetic effect. Nevertheless, the tests showed that it was possible for the simultaneous removal of heavy metals and PAHs from soils using the electrokinetic process.

Figure 4.25 Removal efficiency of PAHs from natural soil after electrokinetic treatments

Figure 4.26 Removal efficiency of PAHs from kaolin after electrokinetic treatments

4.1.9.1 Effect of Treatment Duration

The removal efficiency of PAHs after the electrokinetic treatments is shown in Table 4.8. For S60D4 and S60D8, an increase in treatment duration from 4 to 8 days increased the removal efficiency of phenanthrene and pyrene by 14% and 8% respectively. For S40D4 and S40D8, an increase in treatment duration from 4 to 8 days increased the removal efficiency of phenanthrene and pyrene by 9% and 10% respectively.

For K60D4 and K60D8, an increase in treatment duration from 4 to 8 days increased the removal efficiency of phenanthrene and pyrene by 13% and 9% respectively. For K40D4 and K40D8, an increase in treatment duration from 4 to 8 days increased the removal efficiency of phenanthrene and pyrene by 3% and 7% respectively.

Table 4.8 Removal efficiency of PAHs after electrokinetic treatments with different treatment duration

Test	Removal efficiency (%)	
	Phenanthrene	Pyrene
S60D8	27 ± 2	14 ± 1
S60D4	13 ± 2	7 ± 2
S40D8	19 ± 0	17 ± 2
S40D4	10 ± 1	6 ± 2
K60D8	29 ± 1	19 ± 0
K60D4	16 ± 1	10 ± 0
K40D8	18 ± 0	15 ± 1
K40D4	15 ± 0	9 ± 0

The results showed that an increase in the electrokinetic duration improved the removal efficiency of PAHs. This was supported by the reduction in PAH concentration in the soil. Electroosmosis was probably the main mechanism for the removal of phenanthrene and pyrene from soil.

4.1.9.2 Effect of Initial Soil Water Content

The removal efficiency of PAHs after the electrokinetic treatments is shown in Table 4.9. For S40D8 and S60D8, an increase in initial soil water content from 40% to 60% increased the removal efficiency of phenanthrene by 8% and decreased the removal efficiency of pyrene by 2%. For K40D8 and K60D8, an increase in initial soil water content from 40% to 60% increased the removal efficiency of phenanthrene and pyrene by 11% and 4% respectively.

The results showed that natural soil and kaolin with higher initial soil water content (higher saturation) improved the removal efficiency of phenanthrene. This could be probably because higher water content increased the electroosmosis effect (Hamed 1990) and moved the phenanthrene upwards. On the other hand, natural soil and kaolin with higher initial soil water content (higher saturation) have varying effect on the removal efficiency of pyrene. This could be because pyrene was adsorbed

more strongly to natural soil and the enhanced electroosmosis effect due to higher initial water content was not able to remove pyrene effectively.

Table 4.9 Removal efficiency of PAHs after electrokinetic treatments with different initial soil water content

Test	Removal efficiency (%)	
	Phenanthrene	Pyrene
S60D8	27 ± 2	14 ± 1
S40D8	19 ± 0	17 ± 2
K60D8	29 ± 1	19 ± 0
K40D8	18 ± 0	15 ± 1

4.1.9.3 Effect of Soil Type

The removal efficiency of PAHs after the electrokinetic treatments is shown in Table 4.10. For S60D8 and K60D8, the removal efficiency of phenanthrene and pyrene increased by 2% and 5% respectively when kaolin was used instead of natural soil. For S40D8 and K40D8, the removal efficiency of phenanthrene and pyrene increased by 1% and 1% respectively when natural soil was used instead of kaolin.

The results showed that the removal efficiency of PAHs was not significantly affected (1% to 5% difference) by the type of soil used. The natural soil has a higher hydraulic conductivity and permeability than kaolin, which likely increased the electroosmosis efficiency. However, the improvement in electroosmosis was likely to be reduced by the stronger adsorption of PAHs on natural soil as it contained more organic content. Therefore, there was no significant change in the removal efficiency of PAHs when natural soil or kaolin was used for the electrokinetic treatments.

Table 4.10 Removal efficiency of PAHs after electrokinetic treatments with different soil type

Test	Removal efficiency (%)	
	Phenanthrene	Pyrene
S60D8	27 ± 2	14 ± 1
K60D8	29 ± 1	19 ± 0
S40D8	19 ± 0	17 ± 2
K40D8	18 ± 0	15 ± 1

4.1.10 Models for Removal Efficiency

Multiple regression analysis was performed on the data shown in Table 4.11 using Microsoft Excel. Based on the analysis, models for calculating the removal efficiency of heavy metals and PAHs (dependent variables) from the treatment duration and initial soil water content (independent variables) were obtained. The R^2 value obtained was 0.87, 0.91, 0.79, 0.85 and 0.88 for Cd, Zn, Pb, phenanthrene and pyrene, respectively. The R^2 values were close to 1, which indicated that the multiple regression models were accurate. The models obtained from the multiple regression analysis were as follows:

Removal efficiency of Cd (%)

$$= 19.434 + 0.213 \times \text{Initial soil water content (\%)} + 4.136 \times \text{Treatment duration (days)}$$

$$R^2 \text{ value} = 0.87$$

Removal efficiency of Zn (%)

$$= 16.583 + 0.116 \times \text{Initial soil water content (\%)} + 4.356 \times \text{Treatment duration (days)}$$

$$R^2 \text{ value} = 0.91$$

Removal efficiency of Pb (%)

$$= -15.292 + 0.270 \times \text{Initial soil water content (\%)} + 3.732 \times \text{Treatment duration (days)}$$

$$R^2 \text{ value} = 0.79$$

Removal efficiency of Phenanthrene (%)

$$= -11.193 + 0.297 \times \text{Initial soil water content (\%)} + 2.474 \times \text{Treatment duration (days)}$$

$$R^2 \text{ value} = 0.85$$

Removal efficiency of Pyrene (%)

$$= -2.760 + 0.044 \times \text{Initial soil water content (\%)} + 2.124 \times \text{Treatment duration (days)}$$

$$R^2 \text{ value} = 0.88$$

Table 4.11 Data used for the multiple regression analysis

Test	Initial soil water content (%)	Treatment duration (days)	Removal efficiency (%)				
			Cd	Zn	Pb	Phenanthrene	Pyrene
S60D4	60	4	46.1	37.7	12.8	13.3	6.9
S60D8	60	8	72.2	61.7	36.6	27.2	14.4
S40D4	40	4	44.7	38.7	8.9	9.7	6.4
S40D8	40	8	59.8	51.0	18.8	19.0	16.7
K60D4	60	4	50.0	41.9	15.2	16.1	10.0
K60D8	60	8	59.9	57.4	28.5	29.2	19.1
K40D4	40	4	45.8	40.9	15.6	15.1	8.5
K40D8	40	8	60.9	58.7	28.3	18.3	15.4

4.1.11 Current Change during Electrokinetic Treatments

The change in current during the electrokinetic treatments with natural soil and kaolin are shown in Figures 4.27 and 4.28, respectively. All the tests reached a peak after 1 to 5 hours and started to decline. The current stabilised after 24 hours and showed an average current of 20.2 ± 4.8 mA and 19.8 ± 3.1 mA for electrokinetic treatments with natural soil and kaolin, respectively. The high initial current was consistent with previous studies (Reddy and Ala 2005; Reddy and Chinthamreddy 2004; Sah and Chen 1998).

Figure 4.27 Change of current during electrokinetic treatments with natural soil

Figure 4.28 Change of current during electrokinetic treatments with kaolin

The high initial current values for the tests corresponded to the movement of mobile ions in the soil. When the current was switched on, electroosmosis probably caused the movement of pore water which increased soluble metal ions. In addition, metals ions probably migrated to the electrodes due to electromigration. The electrolysis of water also caused the movement of H^+ and OH^- ions. The increase in mobile ions

likely resulted in the high initial current. However, the current declined over time likely due to reduced electroosmosis and electromigration of metal ions. Some metal ions also precipitated as hydroxides in the soil due to the OH^- ions or accumulated as precipitation at the cathode.

The change in current during the electrokinetic treatments that used natural soil and kaolin with 60% initial soil water content and 8 days of treatment duration are shown in Figure 4.29. Both the tests reached a peak after 1 hour and started to decline. The current stabilised after 24 hours and showed an average current of 22.7 ± 4.1 mA and 21.6 ± 2.4 mA for electrokinetic treatments with natural soil and kaolin, respectively. Test S60D8 showed the highest removal efficiency of heavy metals and was higher than that of K60D8. This was supported by the higher average current for S60D8 as compared to K60D8, which indicated higher mobility of metal ions.

Figure 4.29 Change of current during electrokinetic treatments with natural soil and kaolin

4.1.12 Energy Consumption and Cost for Electrokinetic Treatments

The energy consumption and cost involved for the electrokinetic treatments are shown in Table 4.12. Test S60D8 with electrokinetic treatment duration of 8 days and with natural soil (60% initial water content) showed the highest energy consumption. This was consistent with the removal efficiency for heavy metals as S60D8 showed the highest heavy metal removal efficiency among the tests. Higher metal removal could indicate more movement of metal ions and thus increased the current during the electrokinetic treatment. The local electricity tariff rate (in April 2007) was about S\$0.1888 / kWh. The average unit energy consumption per day was 6.4 ± 0.6 kWh/m³ for all the tests.

Table 4.12 Energy consumption and cost for electrokinetic treatments

Test	Soil type	Test duration (days)	Water content (%)	Unit energy (kWh/m ³)	Unit cost (S\$/m ³)
S60D4	Natural soil	4	60	25.3	4.8
S60D8	Natural soil	8	60	57.8	10.9
S40D4	Natural soil	4	40	29.1	5.5
S40D8	Natural soil	8	40	45.5	8.6
K60D4	Kaolin	4	60	25.9	4.9
K60D8	Kaolin	8	60	54.3	10.3
K40D4	Kaolin	4	40	23.4	4.4
K40D8	Kaolin	8	40	46.7	8.8

For S60D4 and S60D8, an increase in the treatment duration from 4 to 8 days increased the average removal efficiency of heavy metals and the unit cost by 2.0 and 2.3 times, respectively. For K60D4 and K60D8, an increase in treatment duration from 4 to 8 days increased the average removal efficiency of heavy metals and the unit cost by 1.5 and 2.1 times, respectively. The results indicated that it

might be worthwhile to increase the treatment duration at a higher cost to achieve higher removal efficiencies.

For S60D8 and S40D8, an increase in the initial soil water content from 40% to 60% increased the average removal efficiency of heavy metals and the unit cost by 1.5 and 1.3 times, respectively. For K60D8 and K40D8, an increase in the initial soil water content from 40% to 60% increased the average removal efficiency of heavy metals and the unit cost by 1.0 and 1.2 times, respectively. The results indicated that it might be worthwhile to increase the initial soil water content at a higher cost to achieve higher removal efficiencies for natural soil but not for kaolin.

According to Virkutyte et al. (2002), the costs for selected soil remediation technologies were US\$ 65-123 /yd³ (S\$ 129-245 /m³) for soil heating/vapour extraction technology, and US\$ 130-200 /m³ (S\$ 198-305 /m³) for chemical oxidation (with potassium permanganate or hydrogen peroxide). The costs for the electrokinetic treatments in this study were lower and ranged from S\$ 4.4-10.9 /m³. There could be potential for the electrokinetic technology to be more cost-effective than conventional technologies for treating contaminated soil.

4.2 Bioremediation Study

The results are presented and discussed in the following sections for the fungi inoculum, and degradation of phenanthrene and pyrene.

4.2.1 Fungi Inoculum

The actual parameters for the different bioremediation tests are shown in Table 4.13. For test C0, no liquid inoculum was added to the soil. For tests C10, C30, C50 and C70, autoclaved liquid BSM without fungi was added to the soil as inoculum. The five control tests were used to determine the abiotic loss of PAHs. The results of the five control tests were combined and reported as an average result labelled as test “C” in Figures 4.30 to 4.33.

Table 4.13 Actual parameters for the bioremediation tests

Test	Type of fungi	Fungi inoculum concentration (mg/l)	Fungi inoculum to soil concentration (% v/w)	Initial concentration of PAHs in soil (mg/kg)	
				Phenanthrene	Pyrene
C0	None	0	0	261	486
C10	None	0	10 (BSM)	261	496
C30	None	0	30 (BSM)	261	491
C50	None	0	50 (BSM)	261	482
C70	None	0	70 (BSM)	261	487
F10	Cultivated	727	10	286	481
F30	Cultivated	727	30	265	494
F50	Cultivated	727	50	243	483
F70	Cultivated	727	70	245	507
P10	Pure	397	10	161	496
P30	Pure	397	30	215	492
P50	Pure	397	50	205	489
P70	Pure	397	70	240	496

4.2.2 Degradation of Phenanthrene in Soil

The degradation of phenanthrene for the different tests with the cultivated fungi and pure fungi is shown in Figures 4.30 and 4.31, respectively. The control test C showed that the abiotic loss of phenanthrene after 56 days was $27.2 \pm 1.7\%$. The loss of phenanthrene in the control was lower than the other tests throughout the bioremediation treatment. This was expected as no fungi inoculum was added in the control to stimulate the biodegradation. The abiotic loss of phenanthrene may be likely due to volatilisation since phenanthrene has high vapour pressure properties.

Figure 4.30 Degradation of phenanthrene for different tests with the cultivated fungi

Figure 4.31 Degradation of phenanthrene for different tests with the pure fungi

4.2.2.1 Effect of Treatment Duration

All the tests with cultivated and pure fungi (Figures 4.30 and 4.31) showed increasing degradation over time, although the change in degradation decreased over

time. This could be probably because the production of enzymes responsible for the biodegradation of phenanthrene increased within the first few weeks and then decreased. Another explanation for the lower degradation over time was that probably the relatively high levels of phenanthrene were available initially for biodegradation by the fungi but the remaining lower levels of phenanthrene over time might not be easily available for degradation. Since phenanthrene is a hydrophobic compound with low solubility in water, it was more likely to bind with organic matter or sediment, and thus the remaining phenanthrene was not available to the fungi for degradation.

4.2.2.2 Effect of Fungi Type and Fungi Inoculum to Soil Concentration

The final degradation of phenanthrene after 56 days of bioremediation treatment with the cultivated fungi was 52.5%, 55.6%, 65.3% and 75.3% for F10, F30, F50 and F70, respectively (Figure 4.30). The final degradation of phenanthrene after 56 days of bioremediation treatment with the pure fungi was 42.2%, 51.6%, 57.3% and 68.9% for P10, P30, P50 and P70, respectively (Figure 4.31).

The results showed that an increase in the fungi inoculum to soil concentration increased the degradation of phenanthrene. This was expected as more fungi increased the degradation rate. In addition, the results showed that tests with cultivated fungi have higher phenanthrene degradation than tests with pure fungi. This could be because of the higher concentration of fungi inoculum for the cultivated fungi tests as compared to the pure fungi tests (about 1.8 times higher). The cultivated fungi tend to grow faster than the pure fungi as shown in the incubation stages on agar plates and in liquid BSM. This resulted in a higher concentration of cultivated fungi used for the tests.

4.2.3 Degradation of Pyrene in Soil

The degradation of pyrene for the different tests with the cultivated fungi and pure fungi is shown in Figures 4.32 and 4.33, respectively. The control test C showed that the abiotic loss of pyrene after 56 days was $7.5 \pm 2.9\%$. The loss of pyrene in the control was lower than the other tests throughout the bioremediation treatment. This was expected as no fungi inoculum was added in the control to stimulate the biodegradation. The abiotic loss of pyrene may be likely due to volatilisation since pyrene has high vapour pressure properties. In addition, the loss of pyrene in the control was much less than the loss of phenanthrene. This was expected as phenanthrene is more volatile and has lower soil adsorption capacity than pyrene.

Figure 4.32 Degradation of pyrene for different tests with the cultivated fungi

Figure 4.33 Degradation of pyrene for different tests with the pure fungi

4.2.3.1 Effect of Treatment Duration

Most of the tests with cultivated and pure fungi (Figures 4.32 and 4.33) showed increasing degradation over time, although the change in degradation decreased over time. This could be probably because the production of enzymes responsible for the biodegradation of pyrene increased within the first few weeks and then decreased. Another explanation for the lower degradation over time was that probably relatively high levels of pyrene were available initially for biodegradation by the fungi but the remaining lower levels of pyrene over time might not be easily available for degradation. Since pyrene is a hydrophobic compound with low solubility in water, it was more likely to bind with organic matter or sediment, and thus the remaining pyrene was not available to the fungi for degradation.

4.2.3.2 Effect of Fungi Type and Fungi Inoculum to Soil Concentration

The final degradation of pyrene after 56 days of bioremediation treatment with the cultivated fungi was 17.5%, 18.9%, 25.2% and 25.4% for F10, F30, F50 and F70,

respectively (Figure 4.32). The final degradation of pyrene after 56 days of bioremediation treatment with the pure fungi was 17.1%, 17.4%, 18.5% and 21.0% for P10, P30, P50 and P70, respectively (Figure 4.33).

The results showed that an increase in the fungi inoculum to soil concentration slightly increased the degradation of pyrene. This slight increase was expected as pyrene is harder to degrade even though more fungi are added. In addition, the results showed that tests with cultivated fungi have higher pyrene degradation than tests with pure fungi. This could be because of the higher concentration of fungi inoculum for the cultivated fungi tests as compared to the pure fungi tests (about 1.8 times higher). The cultivated fungi tend to grow faster than the pure fungi as shown in the incubation stages on agar plates and in liquid BSM. This resulted in a higher concentration of cultivated fungi used for the tests.

4.2.4 Degradation of PAHs

The degradation results for all the tests showed that it was possible to degrade PAHs using the *Pleurotus ostreatus* fungi, regardless of whether it is the pure strain or a cultivated strain from commercial mushroom. This agreed with previous studies that reported on the possible biodegradation of PAHs by white rot fungi (Marquez-Rocha, F. J. et al 2000; Novotny et al. 1999; Pointing 2001; Pozdnyakova et al. 2006; Pozdnyakova et al. 2006a).

In addition, the degradation results showed that phenanthrene degradation after 56 days was higher than pyrene degradation by 2.5 to 3.3 times. This was consistent with a review that reported that it was harder to degrade PAHs with higher molecular weight and more benzene rings (Juhasz and Naidu 2000; Pointing 2001). Phenanthrene is a 3-ring PAHs while pyrene is a 4-ring PAHs.

4.3 Integrated Study

The electrokinetic test (S60D8 described earlier in Section 4.1) was first conducted by using the natural soil with 60% initial water content and electrokinetic treatment

for 8 days. After 8 days, the removal of Cd, Zn, Pb, phenanthrene and pyrene from the soil was 72%, 62%, 37%, 27% and 14%, respectively (Figures 4.19 and 4.25). The natural soil after the electrokinetic test S60D8 was then used for the bioremediation test. The soil was mixed so that the pH and the concentration of PAHs were consistent throughout the soil. The parameters for the integrated test are shown in Table 4.14. For test C0, no liquid inoculum was added to the soil. For test C70, autoclaved liquid BSM without fungi was added to the soil as inoculum. The 2 control tests were used to determine the abiotic loss of PAHs. The results of the 2 control tests were combined and reported as an average result labelled as test “C” in Figure 4.34.

Fungi cultivated from commercial mushroom was used in this integrated test since the bioremediation study in Section 4.2 showed that tests with cultivated fungi achieved better PAH degradation than tests with pure fungi. The fungi inoculum to soil concentration was also chosen as 70% since the bioremediation study showed that using that concentration in the tests resulted in the highest degradation for PAHs.

Table 4.14 Parameters for the integrated tests

Test	Type of fungi	Fungi inoculum concentration (mg/l)	Fungi inoculum to soil concentration (% v/w)	Initial concentration of PAHs in soil (mg/kg)	
				Phenanthrene	Pyrene
C0	None	0	0	365	426
C70	None	0	70 (BSM)	344	379
F	Cultivated	727	70	354	399

4.3.1 Degradation of Phenanthrene and Pyrene

The degradation of phenanthrene and pyrene for the integrated test with the cultivated fungi is shown in Figure 4.34. The control test C showed that the abiotic loss of phenanthrene and pyrene after 56 days was $19.4 \pm 2.9\%$ and $6.8 \pm 0.2\%$,

respectively. The loss of phenanthrene and pyrene in the control was lower than the test with cultivated fungi throughout the bioremediation treatment. This was expected as no fungi inoculum was added in the control to stimulate the biodegradation.

The final degradation of phenanthrene and pyrene after 56 days of bioremediation treatment with the cultivated fungi was 68.4% and 19.3%, respectively. The test showed increasing degradation over time, although the change in degradation decreased over time, especially for pyrene. This could be probably because the production of enzymes responsible for the biodegradation of PAHs increased within the first few weeks and then decreased (Baldrian et al. 2000; Eichlerova et al. 2000). Another explanation for the lower degradation over time was that probably relatively high levels of PAHs were available initially for biodegradation by the fungi but the remaining lower levels of PAHs over time might not be easily available for degradation. Since PAHs are hydrophobic compounds with low solubility in water, it was more likely to bind with organic matter or sediment, and thus the remaining PAHs were not available to the fungi for degradation (Eggen and Majcherczyk 1998; Novotny et al. 1999)

Figure 4.34 Degradation of phenanthrene and pyrene for the integrated test

The degradation results for the test also showed that phenanthrene degradation after 56 days was higher than pyrene degradation by 3.5 times. This was consistent with a review that reported that it was harder to degrade PAHs with higher molecular weight and more benzene rings (Juhasz and Naidu 2000; Pointing 2001) since phenanthrene is a 3-ring PAHs while pyrene is a 4-ring PAHs.

The electrokinetic remediation process was able to remove heavy metals and PAHs simultaneously although the removal of PAHs was limited. The bioremediation process using white rot fungi can be used to further degrade the remaining PAHs and acted as a polishing step. This integrated study showed that it was possible for the further removal of PAHs from soil using bioremediation after the electrokinetic remediation.

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

In this research, the tests conducted showed that electrokinetic remediation using the UESR technology was able to remove most of the heavy metals and some amount of PAHs from the soil. Further bioremediation tests conducted also showed that the white rot fungi, *Pleurotus ostreatus*, was capable of degrading PAHs. The bioremediation process can be used as an additional step to improve the degradation of the PAHs remaining after the electrokinetic remediation.

5.1 Conclusions for the Electrokinetic Study

Based on the results obtained, the following conclusions can be drawn:

- a) The removal efficiency of Cd was the highest, followed by Zn and Pb. The removal efficiency of Pb tends to be lower because Pb is known to have stronger adsorption on soil than Cd and Zn, and is usually difficult to remediate.
- b) An increase in the electrokinetic duration improved the removal efficiency of heavy metals. As the electrokinetic duration increased, more heavy metals migrated up towards the cathode and out of the soil into the circulating acid effluent or deposited on the cathode.
- c) Natural soil with higher initial soil water content improved the removal efficiency of heavy metals. This could be because higher water content increased the electroosmosis and electromigration effect. The removal efficiency of heavy metals was also better for natural soil than kaolin in a more saturated condition. The higher water content combined with higher permeability of natural soil improved the electrokinetic effect in natural soil.

- d) The removal efficiency for PAHs was much lower compared to heavy metals. This was expected because PAHs were electrically neutral and was less affected by the electrokinetic effect. Electroosmosis was probably the main mechanism for the removal of PAHs from soil.
- e) The removal efficiency for phenanthrene was higher than pyrene. This might be because pyrene was not very mobile due to its lower solubility and higher adsorption capacity on soil as compared to phenanthrene.
- f) An increase in the electrokinetic duration improved the removal efficiency of PAHs. This was supported by the reduction in PAHs concentration in the soil.
- g) Natural soil and kaolin with higher initial soil water content improved the removal efficiency of phenanthrene. This could be because higher water content probably increased the electroosmosis effect. The removal efficiency of PAHs was not significantly affected by the type of soil used. The natural soil had a higher hydraulic conductivity and permeability than kaolin, which probably increased the electroosmosis efficiency. However, the improvement in electroosmosis was likely to be reduced by the stronger adsorption of PAHs on natural soil as it contained more organic content.
- h) The costs for selected soil remediation technologies were S\$ 129-245 /m³ for soil heating/vapour extraction technology, and S\$ 198-305 /m³ for chemical oxidation (with potassium permanganate or hydrogen peroxide). The costs for the electrokinetic treatments in this study were lower and ranged from S\$ 4.4-10.9 /m³. There could be potential for the electrokinetic technology to be more cost-effective than conventional technologies for treating contaminated soil.

5.2 Conclusions for the Bioremediation Study

Based on the results obtained, the following conclusions can be drawn:

- a) It was possible to degrade PAHs using the *Pleurotus ostreatus* fungi, regardless of whether it was the pure strain or a cultivated strain from commercial mushroom.
- b) Tests with cultivated fungi showed higher phenanthrene and pyrene degradation than tests with pure fungi. This could be because of the higher concentration of fungi inoculum for the cultivated fungi tests as compared to the pure fungi tests, or due to *Pleurotus ostreatus* strain differences.
- c) The loss of phenanthrene and pyrene in the control was lower than the other tests throughout the bioremediation treatment. This was expected as no fungi inoculum was added in the control to stimulate the biodegradation. In addition, the loss of pyrene was much less than phenanthrene. This was expected as phenanthrene was more volatile and had lower soil adsorption capacity than pyrene.
- d) All the tests with cultivated and pure fungi showed increasing degradation of phenanthrene and pyrene over time although that increase became smaller over time. This could be because the production of enzymes responsible for the biodegradation of PAHs probably decreased over time or the remaining lower levels of PAHs were probably not available to the fungi for degradation.
- e) An increase in the fungi inoculum to soil concentration increased the degradation of phenanthrene. This was expected as more fungi increased the degradation rate. However, an increase in the fungi inoculum to soil concentration only slightly increased the degradation of pyrene. This slight increase was expected as pyrene was harder to degrade even though more fungi were added. It was harder to degrade PAHs with higher molecular weight and more benzene rings.

5.3 Conclusions for the Integrated Study

Based on the results obtained, the following conclusions can be drawn:

- a) After the electrokinetic treatment, most of the heavy metals were removed but a substantial amount of PAHs still remained in the soil. The bioremediation process using white rot fungi can be used to further degrade the remaining PAHs and acted as a polishing step. It was possible for the further removal of PAHs from soil using bioremediation after the electrokinetic remediation.
- b) The loss of phenanthrene and pyrene in the control was lower than the test with cultivated fungi throughout the bioremediation treatment. This was expected as no fungi inoculum was added in the control to stimulate the biodegradation.
- c) The degradation of phenanthrene was higher than pyrene after 56 days. Pyrene was harder to degrade as it had higher molecular weight and more benzene rings. In addition, pyrene was not very mobile in soil likely due to its lower solubility and higher adsorption capacity on soil as compared to phenanthrene.
- d) The test showed increasing degradation over time although that increase became smaller over time, especially for pyrene. This could be because the production of enzymes responsible for the biodegradation of PAHs probably decreased over time or the remaining lower levels of PAHs were probably not available to the fungi for degradation.

5.4 Recommendations

Some recommendations for future studies are summarised as follows:

- a) Real contaminated soil contains other types of pollutants besides those studied in this research. These other contaminants may affect the electrokinetic process and some heavy metals may migrate in the opposite direction towards the anode. In

addition, aged contaminated soil may have different properties compared to spiked soil, especially the adsorption of heavy metals on soil. Further studies using real contaminated soil are needed to investigate the effect on the electrokinetic process.

- b) The addition of a purging or flushing fluid at the anode helps to increase the electroosmosis flow and the transportation of contaminants as the fluid travels from the anode towards the cathode. However, the UESR process used a vertical migration system and no flushing fluid was used. Further studies are needed to explore the possibility of adding flushing fluid to the anode and the type of fluid needed to enhance the removal of heavy metals or organic contaminants.
- c) Studies have been conducted to determine which non-ligninolytic and ligninolytic type enzymes in white rot fungi help to break down PAHs. However, some studies disagreed on the types of enzymes responsible for the degradation mechanism. Further studies can be conducted to determine the enzymes produced by the white rot fungi and investigate whether it corresponds to the degradation rate.
- d) White rot fungi can degrade organic contaminants but with different degradation efficiencies. Further studies can be conducted on other types of PAHs or organic compounds to determine whether the fungi is effective in the degradation.

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APPENDIX A EXPERIMENTAL SET-UP

Figure A.1 Experimental set-up for electrokinetic study

Figure A.2 Cathode with circulating acid

Figure A.3 Commercial mushroom

Figure A.4 Cultivated fungi from mushroom

Figure A.5 Close-up of cultivated fungi

Figure A.6 Pure fungi

Figure A.7 Close-up of pure fungi

APPENDIX B RESULTS FOR ELECTROKINETIC STUDY

Table B.1 Water content of natural soil and kaolin after electrokinetic treatments

	Water content of natural soil (%)				Water content of kaolin (%)			
Distance to cathode (cm)	S60D4	S60D8	S40D4	S40D8	K60D4	K60D8	K40D4	K40D8
2	55.5	55.5	44.4	42.5	64.8	65.4	53.6	53.0
4	54.7	55.0	41.8	40.6	62.3	62.6	50.4	49.9
6	52.4	51.4	41.1	40.0	61.3	61.0	49.8	49.3
8	49.6	48.2	40.1	39.0	59.5	59.4	49.1	48.9
10	47.3	45.9	39.0	38.4	59.1	58.6	48.8	48.6

Table B.2 pH of natural soil and kaolin after electrokinetic treatments

	pH of natural soil				pH of kaolin			
Distance to cathode (cm)	S60D4	S60D8	S40D4	S40D8	K60D4	K60D8	K40D4	K40D8
2	4.56	3.82	4.74	4.04	4.61	4.58	4.33	4.32
4	3.45	2.88	3.07	2.89	2.89	2.77	2.82	2.77
6	2.76	2.56	2.70	2.59	2.55	2.43	2.50	2.50
8	2.57	2.35	2.46	2.37	2.39	2.21	2.30	2.22
10	2.35	2.05	2.15	2.13	2.23	2.02	2.21	2.05

Table B.3 pH of effluent during electrokinetic treatments for natural soil and kaolin

Day	S60D4	S60D8	S40D4	S40D8	K60D4	K60D8	K40D4	K40D8
1	3.52	3.91	3.84	3.14	3.17	3.43	2.9	2.98
2	2.65	2.76	2.93	2.71	2.73	2.88	2.81	2.78
3	2.35	2.47	2.61	2.33	2.79	2.81	2.78	2.71
4	2.42	2.53	2.58	2.44	2.81	2.72	2.71	2.67
5		2.78		2.62		2.85		2.75
6		2.72		2.69		2.8		2.71
7		2.8		2.68		2.78		2.79
8		2.69		2.68		2.68		2.77

Table B.4 Concentration of cadmium in natural soil and kaolin after electrokinetic treatments

	Cd concentration in natural soil (mg/kg)				Cd concentration in kaolin (mg/kg)			
Distance to cathode (cm)	S60D4	S60D8	S40D4	S40D8	K60D4	K60D8	K40D4	K40D8
2	681.0	288.5	854.9	511.0	608.1	482.0	346.7	284.0
4	269.1	55.9	99.2	49.8	82.6	15.8	92.0	16.2
6	60.0	15.6	108.4	48.9	86.7	27.4	146.1	28.6
8	66.9	18.3	112.7	55.5	113.4	42.4	137.6	39.8
10	79.2	21.0	109.3	62.2	111.2	42.1	153.0	75.4

Table B.5 Concentration of zinc in natural soil and kaolin after electrokinetic treatments

	Zn concentration in natural soil (mg/kg)				Zn concentration in kaolin (mg/kg)			
Distance to cathode (cm)	S60D4	S60D8	S40D4	S40D8	K60D4	K60D8	K40D4	K40D8
2	791.9	349.7	1040.4	765.6	779.8	517.8	396.3	339.6
4	340.7	78.2	135.0	89.7	116.3	43.8	124.4	28.6
6	123.4	65.5	140.4	93.5	99.4	39.7	156.6	39.2
8	140.5	67.2	146.2	91.2	126.5	56.0	165.3	49.8
10	111.8	68.6	141.5	96.9	127.1	76.7	163.2	85.4

Table B.6 Concentration of lead in natural soil and kaolin after electrokinetic treatments

	Pb concentration in natural soil (mg/kg)				Pb concentration in kaolin (mg/kg)			
Distance to cathode (cm)	S60D4	S60D8	S40D4	S40D8	K60D4	K60D8	K40D4	K40D8
2	423.3	268.6	464.2	364.8	348.0	258.2	269.5	215.1
4	364.7	175.2	412.4	301.9	315.6	149.3	387.6	181.0
6	384.8	284.7	486.8	398.5	451.8	335.1	383.6	378.5
8	427.5	384.3	492.6	437.0	459.5	408.8	399.3	349.7
10	433.8	403.5	496.8	445.5	459.0	407.8	384.3	370.9

Table B.7 Mass distribution of cadmium after electrokinetic treatments using natural soil and kaolin

	Cd content (%)							
	S60D4	S60D8	S40D4	S40D8	K60D4	K60D8	K40D4	K40D8
In Soil	46.5	18.6	53.6	33.5	46.9	30.8	46.7	24.4
In Effluent	35.2	55.0	37.0	50.7	33.9	49.8	39.2	53.9
In Leachate	8.6	9.5	1.3	2.4	4.9	8.2	5.4	9.8
Deposited on Cathode	9.8	16.9	8.1	13.3	14.4	11.3	8.7	11.9

Table B.8 Mass distribution of zinc after electrokinetic treatments using natural soil and kaolin

	Zn Content (%)							
	S60D4	S60D8	S40D4	S40D8	K60D4	K60D8	K40D4	K40D8
In Soil	54.8	26.2	59.3	41.9	52.0	33.7	51.0	28.2
In Effluent	30.0	48.7	31.4	40.3	28.9	45.9	34.2	50.8
In Leachate	7.9	9.6	2.4	9.1	7.4	10.0	6.7	9.7
Deposited on Cathode	7.3	15.5	6.8	8.6	11.7	10.4	8.1	11.3

Table B.9 Mass distribution of lead after electrokinetic treatments using natural soil and kaolin

	Pb Content (%)							
	S60D4	S60D8	S40D4	S40D8	K60D4	K60D8	K40D4	K40D8
In Soil	85.8	62.2	90.5	78.3	84.5	68.7	84.7	71.2
In Effluent	8.5	22.5	6.1	13.5	9.1	20.4	10.6	18.9
In Leachate	1.4	3.2	0.4	2.4	0.4	1.1	0.4	1.2
Deposited on Cathode	4.3	12.2	2.9	5.7	6.1	9.8	4.4	8.8

Table B.10 Concentration of phenanthrene in natural soil and kaolin after electrokinetic treatments

	Phenanthrene concentration (mg/kg)							
Distance to cathode (cm)	S60D4	S60D8	S40D4	S40D8	K60D4	K60D8	K40D4	K40D8
2	418.1	302.4	383.1	318.1	279.1	255.8	279.2	309.7
4	423.7	332.3	399.6	345.6	306.5	276.8	320.0	303.5
6	407.6	332.4	392.4	349.9	309.7	279.1	318.9	301.9
8	377.0	305.8	370.5	339.8	292.4	246.3	295.9	284.3
10	384.5	290.0	369.1	334.6	276.4	236.1	283.1	278.5

Table B.11 Concentration of pyrene in natural soil and kaolin after electrokinetic treatments

	Pyrene Concentration (mg/kg)							
Distance to cathode (cm)	S60D4	S60D8	S40D4	S40D8	K60D4	K60D8	K40D4	K40D8
2	482.4	498.0	511.8	468.5	468.4	408.2	423.4	360.7
4	527.0	505.9	476.8	455.5	469.7	439.7	460.4	413.5
6	481.4	455.4	450.2	423.0	506.6	476.7	483.1	450.4
8	471.0	454.3	435.2	416.1	439.1	429.0	453.9	401.5
10	454.5	427.2	449.5	364.3	431.4	417.7	442.5	386.3

Table B.12 Removal efficiency of heavy metals and PAHs from natural soil and kaolin after electrokinetic treatments

Test	Removal efficiency (%)				
	Cd	Zn	Pb	Phenanthrene	Pyrene
S60D4	46.1	37.7	12.8	13.3	6.9
S60D8	72.2	61.7	36.6	27.2	14.4
S40D4	44.7	38.7	8.9	9.7	6.4
S40D8	59.8	51.0	18.8	19.0	16.7
K60D4	50.0	41.9	15.2	16.1	10.0
K60D8	59.9	57.4	28.5	29.2	19.1
K40D4	45.8	40.9	15.6	15.1	8.5
K40D8	60.9	58.7	28.3	18.3	15.4

Table B.13 Multiple regression analysis for Cd

Regression Statistics								
Multiple R	0.931239002							
R Square	0.867206079							
Adjusted R Square	0.81408851							
Standard Error	4.228729094							
Observations	8							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	2	583.8937806	291.9468903	16.32616292	0.006426071			
Residual	5	89.41074876	17.88214975					
Total	7	673.3045294						
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	19.43391675	8.845021491	2.197158793	0.079377441	-3.302934826	42.17076832	-3.302934826	42.17076832
Initial soil water content (%)	0.213213443	0.149508151	1.426099122	0.213172322	-0.171109494	0.59753638	-0.171109494	0.59753638
Treatment duration (days)	4.136445496	0.747540755	5.533404661	0.002643826	2.214830811	6.05806018	2.214830811	6.05806018

Table B.14 Multiple regression analysis for Zn

Regression Statistics								
Multiple R	0.953829384							
R Square	0.909790494							
Adjusted R Square	0.873706692							
Standard Error	3.500345918							
Observations	8							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	2	617.8471198	308.9235599	25.2132657	0.002444166			
Residual	5	61.26210773	12.25242155					
Total	7	679.1092275						
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	16.58320991	7.321498771	2.265002075	0.072893537	-2.237301833	35.40372166	-2.237301833	35.40372166
Initial soil water content (%)	0.115916855	0.123755917	0.936657077	0.391934877	-0.202207856	0.434041567	-0.202207856	0.434041567
Treatment duration (days)	4.355663504	0.618779584	7.039119613	0.000893525	2.765039946	5.946287062	2.765039946	5.946287062

Table B.15 Multiple regression analysis for Pb

Regression Statistics								
Multiple R	0.891320168							
R Square	0.794451643							
Adjusted R Square	0.7122323							
Standard Error	5.106935289							
Observations	8							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	2	504.0158231	252.0079116	9.662588077	0.019155127			
Residual	5	130.4039402	26.08078804					
Total	7	634.4197633						
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-15.29202154	10.68192154	-1.431579654	0.211682664	-42.750775	12.16673192	-42.750775	12.16673192
Initial soil water content (%)	0.270038745	0.180557429	1.49558369	0.195006511	-0.194098901	0.734176392	-0.194098901	0.734176392
Treatment duration (days)	3.731952756	0.902787143	4.133812475	0.009051337	1.411264524	6.052640987	1.411264524	6.052640987

Table B.16 Multiple regression analysis for Phenanthrene

Regression Statistics								
Multiple R	0.922323369							
R Square	0.850680397							
Adjusted R Square	0.790952556							
Standard Error	3.058435206							
Observations	8							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	2	266.4515019	133.225751	14.24261086	0.00861573			
Residual	5	46.77012954	9.354025907					
Total	7	313.2216314						
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-11.19288779	6.397176201	-1.749660699	0.14058381	-27.63735272	5.25157715	-27.63735272	5.25157715
Initial soil water content (%)	0.29703086	0.108132014	2.746928034	0.040457569	0.01906867	0.57499305	0.01906867	0.57499305
Treatment duration (days)	2.474050554	0.540660068	4.575981653	0.005969274	1.084239603	3.863861504	1.084239603	3.863861504

Table B.17 Multiple regression analysis for Pyrene

Regression Statistics								
Multiple R	0.940437533							
R Square	0.884422754							
Adjusted R Square	0.838191855							
Standard Error	1.952313736							
Observations	8							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	2	145.8333285	72.91666424	19.13055513	0.004541307			
Residual	5	19.05764463	3.811528925					
Total	7	164.8909731						
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-2.760415709	4.083557156	-0.67598312	0.529049481	-13.25753356	7.736702138	-13.25753356	7.736702138
Initial soil water content (%)	0.043499665	0.069024714	0.630204206	0.556236203	-0.133934011	0.220933341	-0.133934011	0.220933341
Treatment duration (days)	2.12367276	0.345123571	6.153369233	0.001648475	1.236504379	3.010841141	1.236504379	3.010841141

Table B.18 Change of current during electrokinetic treatments using natural soil

Time (h)	Current (mA)			
	S60D4	S60D8	S40D4	S40D8
0	32.0	33.6	36.8	34.7
1	39.6	43.0	43.4	42.3
2	38.4	39.6	37.0	36.0
3	34.2	35.1	32.2	32.5
4	31.1	32.6	28.9	29.7
5	29.8	30.5	28.1	27.2
6	29.1	29.9	27.1	27.4
8	25.6	26.9	25.4	23.5
17	18.4	19.1	20.1	15.0
18	18.7	18.4	19.3	14.3
19	18.8	20.9	18.5	15.6
20	17.3	19.3	18.2	14.4
21	16.4	18.5	17.4	13.8
22	16.0	18.0	18.1	13.6
23	15.8	17.9	17.5	13.7
24	15.6	17.7	17.7	13.3
26	18.7	18.8	20.4	19.3
43	11.3	12.2	12.9	10.3
44	13.5	14.6	15.3	13.2
45	12.2	13.6	16.1	12.9
48	10.7	12.3	15.3	11.8
51	23.3	26.7	30.5	22.0
52	20.9	23.2	28.9	21.0
53	20.4	23.2	29.2	21.0
54	20.4	23.2	29.1	21.5
55	20.5	23.6	29.5	21.6
65	21.9	26.8	26.0	19.1
66	23.0	26.1	27.3	19.6
67	23.1	25.1	26.3	18.9
68	22.6	26.1	26.2	18.4
70	27.8	33.1	26.2	21.3
72	20.0	24.5	23.4	17.0
74	21.5	25.0	26.4	19.9
91	16.8	19.3	17.8	14.8
92	16.5	18.9	18.1	14.4

93	16.2	18.6	18.0	14.4
94	16.0	18.3	17.8	14.4
96	16.2	18.2	17.7	14.4
98		26.5		17.7
99		25.4		16.8
102		26.2		17.6
113		26.8		18.6
119		19.1		13.7
120		19.0		13.7
121		24.7		18.5
140		22.3		13.9
142		20.8		13.6
143		24.1		16.0
144		25.1		17.0
149		24.9		17.2
163		23.4		16.1
164		23.4		15.9
166		23.0		16.0
167		23.6		16.4
168		24.4		16.2
170		25.4		19.2
171		26.2		17.6
173		26.0		18.1
177		22.9		18.7
186		22.7		18.0
188		23.2		19.9
189		22.7		19.4
191		22.1		17.4
192		22.5		19.6

Table B.19 Change of current during electrokinetic treatments using kaolin

Time (h)	Current (mA)			
	K60D4	K60D8	K40D4	K40D8
0	38.4	39.0	13.1	14.3
1	42.0	39.7	17.2	16.3
2	33.4	34.5	23.1	19.9
3	31.8	32.6	24.9	21.5
4	28.7	29.5	24.8	22.1
5	25.9	27.8	24.2	22.9
7	22.1	25.5	23.3	22.6
8	26.2	27.2	22.0	21.7
18	17.6	18.9	19.0	19.4
19	20.9	20.2	19.7	19.4
21	17.2	18.5	19.1	17.7
22	17.2	18.3	19.0	17.7
24	16.1	17.8	18.6	16.9
26	22.1	24.4	24.9	22.9
27	18.9	20.9	21.0	20.0
44	17.5	20.7	18.5	17.0
45	28.4	25.3	18.8	18.8
46	23.1	23.6	18.9	18.0
47	19.4	20.6	17.0	16.6
48	19.2	20.1	16.4	15.2
50	20.4	21.7	19.0	16.7
51	19.3	19.8	17.2	15.4
52	17.5	18.7	16.6	14.6
54	18.0	18.3	16.5	14.4
57	17.0	17.8	17.2	14.4
66	17.0	17.7	16.1	13.5
67	19.0	17.4	15.8	13.5
68	19.0	17.3	15.7	13.7
69	18.4	17.3	15.6	13.8
71	18.1	17.6	15.7	13.8
72	17.8	17.5	15.6	14.1
73	25.6	22.5	19.5	17.9
75	22.1	19.8	17.8	15.7
92	19.1	20.6	17.1	16.1
94	24.2	21.2	17.4	17.1

96	24.8	20.4	17.5	16.8
98		19.2		16.5
99		23.0		18.6
118		25.1		18.4
119		25.5		19.7
120		25.5		19.7
121		23.7		19.9
122		22.5		19.1
123		21.1		18.7
142		23.2		18.7
144		24.2		20.4
145		25.7		22.8
164		23.8		21.5
165		24.0		21.7
166		23.4		21.7
167		23.2		21.2
168		23.0		21.7
170		22.5		22.9
171		22.1		22.5
172		21.1		21.7
173		22.1		22.0
178		22.4		23.0
186		22.0		21.8
187		22.5		22.2
188		22.4		22.2
189		22.1		21.8
190		22.3		21.6
191		22.1		22.4
192		22.5		21.6

APPENDIX C RESULTS FOR BIOREMEDIATION STUDY

Table C.1 Degradation of phenanthrene for different tests using the cultivated fungi and pure fungi

	Degradation of phenanthrene (%)							
Day	C0	C10	C30	C50	C70	C		
7	2.6	1.6	4.1	0.7	4.0	2.6		
14	1.7	2.8	6.6	1.6	2.0	2.9		
21	10.0	10.6	10.3	12.6	13.3	11.4		
35	15.9	16.9	17.2	15.6	17.1	16.5		
56	27.4	25.8	26.9	26.0	29.9	27.2		
Day	F10	F30	F50	F70	P10	P30	P50	P70
7	13.4	17.9	15.0	26.4	11.4	14.7	11.1	25.3
14	31.8	32.2	37.8	42.7	18.9	16.4	26.1	39.1
21	40.4	38.5	49.6	59.0	25.8	32.8	33.1	53.7
35	49.3	52.1	59.7	67.9	38.9	40.0	52.5	64.8
56	52.5	55.6	65.2	75.3	42.2	51.6	57.3	68.9

Table C.2 Degradation of pyrene for different tests using the cultivated fungi and pure fungi

	Degradation of pyrene (%)							
Day	C0	C10	C30	C50	C70	C		
7	1.1	1.5	1.8	1.5	1.0	1.4		
14	4.7	4.2	3.1	1.9	1.8	3.1		
21	7.2	3.4	5.1	2.7	3.1	4.3		
35	9.6	5.2	6.5	4.7	3.5	5.9		
56	11.5	7.4	8.3	6.6	3.6	7.5		
Day	F10	F30	F50	F70	P10	P30	P50	P70
7	9.2	9.4	8.5	9.1	3.8	3.6	4.8	5.6
14	12.7	14.1	13.4	14.6	7.3	6.6	11.6	11.0
21	16.8	16.7	17.4	18.3	10.8	11.5	13.8	14.7
35	16.5	17.6	22.5	22.1	15.1	15.5	16.5	19.9
56	17.5	18.9	25.2	25.4	17.1	17.4	18.5	21.0

APPENDIX D RESULTS FOR INTEGRATED STUDY

Table D.1 Degradation of phenanthrene and pyrene for the integrated test

Day	Degradation of phenanthrene (%)				Degradation of pyrene (%)			
	C0	C70	C	F	C0	C70	C	F
7	2.0	1.7	1.9	22.6	2.0	1.8	1.9	9.9
14	4.1	3.8	3.9	40.0	4.3	4.8	4.5	16.5
21	8.3	7.0	7.7	54.3	5.3	5.5	5.4	16.8
35	13.7	12.5	13.1	62.3	6.6	6.4	6.5	17.6
56	21.4	17.3	19.4	68.4	6.9	6.7	6.8	19.3