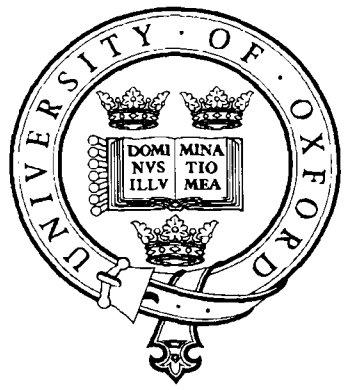
The electrokinetic bioremediation of hydrocarbons from the sub-surface

Thesis Submitted for the Degree of Doctor of Philosophy

Department of Engineering Science,
University of Oxford



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I hereby declare that this thesis has been entirely composed by myself and that no part of it has been accepted in any previous application for a degree at this or any other university. All sources of information have been specifically acknowledged by reference to the authors.

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Penny Catherine Carter

31 January 2007

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Hydrocarbon contamination of soil from accidental spills and negligence represents a major cause of sub-surface contamination. There are numerous physical, chemical and biological techniques available for contaminant remediation. However, many of these methods can be difficult to implement at sites where access to soils, or delivery of treatments, requires substantial excavation works and disruption (for example where contamination is located beneath or adjacent to building structures and underground services). An innovative technology that may provide a solution for the remediation of subsurface contamination without causing interim damage is that of electrokinetic remediation. Electrokinetic remediation is the application of a low voltage, direct current (of the order of mA/cm^2) between electrodes placed in a contaminated soil system. The production of an electric field causes a number of electrically-induced processes to occur that can promote soil remediation, principally: electromigration, electroosmosis, electrophoresis, and electrolysis.

This study examined the use of electrokinetics coupled with biodegradation to remediate kerosene and transformer oil from sub-surface soils. The principal investigations carried out within this study included: the use of electroosmosis as an *in situ* flushing technique; use of electrokinetics to deliver surfactants into the soil to promote mobilisation of contaminants within the sub-surface; and the use of electrokinetics to create optimum conditions for biodegradation by the delivery of nutrients and electron acceptors, the removal of toxic metabolites, and by controlling the pH and moisture content of the soil. As an integral part of the research, a field trial was designed and set up to determine whether electrokinetics could be used to promote the biodegradation of transformer oil from the subsurface. This was one of the first field trials in the UK for the electrokinetic remediation of hydrocarbon contaminants.

The results of the study support the use of electrokinetics as a field tool for the enhancement of remediation in the sub-surface. Laboratory-scale studies showed the potential to mobilise the more soluble hydrocarbons (such as toluene and xylene) in the soil, to deliver enhancing agents (*e.g.* surfactants and nutrients) and to remove toxic metabolites. The field-scale experiment demonstrated the concept of a relatively low cost treatment cell on-site and provided some evidence that biodegradation of the transformer oil was promoted. Consequently, it is suggested that electrokinetics, with the correct engineering controls in place, provides a valuable method of optimising the biodegradation of hydrocarbons in the sub-surface.

I will make rivers flow on barren heights,
and springs within the valleys.
I will turn the desert into pools of water,
and the parched ground into springs.

I will put in the desert
the cedar and the acacia, the myrtle and the olive.
I will set pines in the wasteland,
the fir and the cypress together,

so that people may see and know,
may consider and understand,
that the hand of the LORD has done this,
that the Holy One of Israel has created it.

Isaiah 41: 18-20

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Ø	Diameter
§	Section
ACL	Acceptable concentration limits
ANOVA	Analysis of variance
BLAST	Basic Local Alignment Search Tool
BSA	Bovine serum albumin
CEC	Cation exchange capacity
CFU	Colony forming units
CLARINET	Contaminated LAnd Rehabilitation Network for Environmental Technologies
CLEA	Contaminated Land Exposure Assessment
CMC	Critical micelle concentration
CoA	Coenzyme A
COD	Chemical Oxygen Demand
Cpm	Counts per minute
C^s	Aqueous solubility
C_s	Concentration of contaminant sorbed to the soil
Current density	Current per cross sectional area
C_w	Concentration of contaminant in aqueous phase
DC	Direct Current
DDL	Double Diffuse Layer
Defra	Department of environment, food and rural affairs
$d H_2O$	Deionised water
DNA	Deoxyribonucleic acid
DNAPL	Dense non-aqueous phase liquid
DOE	Department of Energy
DOM	Dissolved Organic Matter
d.p.	Decimal places
d/w	Dry weight
DXT	Contaminant mix containing dodecane, xylene and tetradecane
EA	Environment Agency
EBF	Electrokinetic Biofence
EK	Electrokinetic(s)
EM	Electromigration
EMS	Electrolyte management system
EO	Electroosmosis
EPA	Environmental Protection Act
EPSRC	Engineering & Physical Sciences Research Council
FIRS	Ferric Iron Remediation and Stabilisation
g	Gravity
GAC	Granular activated carbon
GC-FID	Gas Chromatograph-Flame Ionisation Detector
HA	Humic acid
HPLC	High Performance Liquid Chromatography
IC	Ion Chromatography
ICP-MS	Inductive Coupled Plasma – Mass Spectrometry
ISCO	*In Situ* chemical oxidation
K_d	Dissociation constant
K_H	Henry's Law Constant

K_{ow}	Octanol-water partition coefficient
LH-PCR	Length Heterogeneity-Polymerase Chain Reaction
LNAPL	Light Non-Aqueous Phase Liquid
mA	Milliamperes
Mains water	Supplied by Thames Water, Oxfordshire
mbgl	Metres below ground level
MNA	Monitored Natural Attenuation
MW	Molecular weight
NAPL	Non-Aqueous Phase Liquid
NCBI	National Center for Biotechnology Information
N/S	Sample not scheduled for analysis
ORC®	Oxygen release compounds
p	Probability of H_0 being true
PAH	Polycyclic aromatic hydrocarbon
PCA	Plate count agar
PCB	Polychlorinated biphenyl
PCR	Polymerase Chain Reaction
PDA	Potato dextrose agar
pH	$-\log[H^+]$
pH_{H20}	pH of soil determined in a 5:1, water to soil slurry
PRBs	Permeable reactive barriers
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride
rDNA	ribosomal DNA
rpm	Revolutions per minute
SE	Standard error = standard deviation 'divided by' √(number of samples)
Sec	Seconds
SDS	Sodium dodecyl sulphate
SGV	Soil Guideline Values
SOM	Soil organic matter
$Span^{85}$	Sorbitan trioleate
STL	Severn Trent Laboratories, Reading, UK
S_W	Water solubility
TCA	Tricarboxylic acid
TCE	Trichloroethylene
TE	*Tris*-EDTA
TPH	Total Petroleum Hydrocarbons
US EPA	United States Environmental Protection Agency
V	Volts
VOCs	Volatile organic contaminants
v/v	Volume/volume
WHC	Water holding capacity
w/w	Weight per weight
w/wt	Wet weight
Zeta - potential	The electric potential at the shear plane (interface between a moving liquid and the fixed liquid layer attached to the static soil)
ZPC	Zero point of charge

Chemical Abbreviations

Al^{3+}	Aluminium
$BaCl_2$	Barium chloride
BTEX	Benzene, toluene, ethylbenzene, and xylenes
C	Carbon
Ca^{2+}	Calcium
C_7H_8	Toluene
C_8H_{10}	xylene
$C_{12}H_{26}$	Dodecane
$C_{14}H_{30}$	Tetradecane
$C_{12}H_7Cl_3$	3-Chlorobiphenyl
$C_{24}H_{42}$,$C_6H_5(CH_2)_{12}CH_3$	Octadecylbenzene
3-CB	3-Chlorobiphenyl
3-CBA	3-Chlorobenzoate
Cl^-	Chloride
Cl_2	Chlorine
$ClC_6H_4CO_2H$	3-Chlorobenzoate
Cr	Chromium
CO_2	Carbon dioxide
^{14}C-toluene	Radiolabelled toluene
EDTA	Ethylenediaminetetraacetic acid
Fe^{3+}	Ferric iron
Fe^o	Zero valent iron
H^+	Hydrogen ion (proton)
He	Helium
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HDTMA	Hexadecyltrimethlammonium
HMN	Heptamethylnonane
HNO_3	Nitric acid
H_3PO_4	Phosphoric acid
H_2SO_4	Sulphuric acid
IrO_2	Iridium oxide
K^+	Potassium
KH_2PO_4	Potassium hydrogen phosphate
$KMnO_4$	Potassium permanganate
KNO_3	Potassium nitrate
M9	42.27 mM Na_2HPO_4, 22.06 mM KH_2PO_4, 8.62 mM NaCl, 18.6 mM NH_4Cl, 0.1 M $MgSO_4.7H_2O$, 0.01 M $CaCl_2.6H_2O$ (pH ~7.2)
Mg^{2+}	Magnesium
$MgNH_4PO_4$	Magnesium ammonium phosphate
Mn^{4+}	Manganese ion
MnO_4^-	Permanganate
m-xylene	*meta*-xylene (1,3-dimethylbenzene)
N_2	Nitrogen
Na^+	Sodium
NaCl	Sodium chloride
Na_2CO_3	Sodium carbonate
$NaHCO_3$	Sodium hydrogen carbonate

NaH_2PO_4	Sodium dihydrogen phosphate
$NaMnO_4$	Sodium permanganate
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
Na_2SO_4	Sodium sulphate
NH_3	Ammonia
NH_4^+	Ammonium ions
NH_4Cl	Ammonium chloride
NH_4NO_3	Ammonium nitrate
NH_4OH	Ammonium hydroxide
NO_3^-	Nitrate ion
NO_2^-	Nitrite ion
o-xylene	ortho-xylene (1,2-dimethylbenzene)
OH^-	Hydroxyl ions
P	Phosphorous
p-xylene	*para*-xylene (1,4-dimethylbenzene)
PO_4^{3-}	Phosphate
SO_4^{2-}	Sulphate
Ta_2O_5	Tantalum oxide
TNT	2,4,6-trinitroluene

1 Introduction

1.1 The requirement for alternative methods for the remediation of hydrocarbons

"There is a need for a remediation strategy that is easy, cost effective, less prone to accumulate toxic by-products and addresses both chemical and microbiological advantages and constraints" (Wildman and Alvarez, 2001).

Hydrocarbon contamination of soil from accidental spills and negligence represents a major cause of sub-surface contamination in the UK. Current legislation addressing the potential health and environmental impact of chemical contaminants dictates that all polluted soils causing significant 'risk' must be remediated (Butler and Petts, 2000). There are numerous physical, chemical and biological techniques available for the remediation of contaminated land. However, many of these methods can be difficult to implement at sites where contamination is located beneath or adjacent to building structures and underground services without carrying out substantial excavation that may cause interim damage (Figure 1.1). Furthermore, where sub-surface contamination occurs at industrial facilities, underground storage tanks within garage forecourts, or domestic heating supplies, there are frequently underground services and building structures that obstruct remedial works.



Figure 1.1 Photographs depicting the disruption caused by sub-surface remediation under buildings (Photo courtesy of RAW Consulting, 2006).

In some scenarios, listed buildings and public service properties (*e.g.* hospitals or water treatment plants) may be compromised in the process of remediating the site, in which case a novel non-invasive remediation technique could yield tangible financial savings. Remediation of hydrocarbons in residential areas is frequently expensive due to the short time-frames involved and the stringent consents that need to be achieved to comply with current legislation, especially with regard to health concerns. This investigation was designed to study the basic principles underpinning electrokinetics and bioremediation, an innovative, low-cost, non-disruptive approach capable of removing contaminants from below structures without the need for excavation or rebuilding. The utility of electrokinetics and bioremediation for the remediation of hydrocarbons from the sub-surface (electroremediation), with minimal site disruption, was investigated using laboratory based investigations and an *ex situ* field-trial.

1.2 Sources of hydrocarbon contamination

Leaks, spills, and disposal practices both past and present have led to considerable land contamination with hydrocarbons (Chilinger *et al.*, 1997). In the year 2000 it was estimated that approximately 360 000 hectares of land in the UK were deemed as contaminated (Bio-wise, 2000). There is a vast number of contributing contaminants including pesticides, fuels, and military waste (*e.g.* redundant munitions). Hydrocarbons are common environmental contaminants because of their wide use as fuels and lubricants. Hydrocarbons enter the environment principally as a result of faulty delivery pipes and boilers, delivery spills, tank failures and as a consequence of negligence. It is reported that with many households switching from using oil to natural gas or electricity, partially emptied fuel tanks are left buried in the soil where they eventually corrode, leaking their contents into the sub-surface (Biosoil, 2002). In the United States of America, 30 000 new releases of heating oil from underground storage tanks are reported each year (Eweis *et al.*, 1998).

1.2.1 Target hydrocarbons

Domestic heating oil (kerosene) and transformer oil were the target contaminants for this study. These are the most frequently encountered hydrocarbon contaminants in spill incidents attended by RAW Consulting, a representative UK-based environmental consultancy (RAW Consulting Ltd., Pers. Comm. 2002). Kerosene has a moderate volatility and low viscosity and therefore commonly occurs in the sub-surface, in the vadose (unsaturated) or saturated zone of the soil, persisting for months or even years

(Benazon *et al.*, 1995; Dror *et al.*, 2001). Kerosene is a complex mixture of hydrocarbons, the constituents of which can generally be placed into one of four generic groups: saturates (*e.g.* *n*-alkanes and cycloalkanes, C_6-C_{16}); aromatics (*e.g.* polycyclic aromatic hydrocarbons [PAHs] and BTEX [benzene, toluene, ethylbenzene, xylenes]); resins (*e.g.* amides); and asphaltenes (*e.g.* ketones) (Pollard *et al.*, 1994; Potter and Simmons, 1998). Transformer oil, an acid refined naphthenic product, consists mainly of cycloalkanes (C_{15}-C_{30}), *e.g.* cyclopentane, cyclohexane and alkyl derivatives. Polychlorinated biphenyls (PCBs) were also included in transformer oil as an insulating coolant and lubricant (Sierra *et al.*, 2003) until they were banned in the late 1970s. Kerosene and transformer oil contain functional and generally non-specified performance additives, added to improve properties such as electrical conductivity (Ellison *et al.*, 1999).

Contamination plumes in the soil resulting from domestic kerosene spills frequently contain concentrations of Total Petroleum Hydrocarbons (TPH) in the range between 1 000 mg kg^{-1} and 100 000 mg kg^{-1} (RAW Consulting Ltd., Pers. Comm. 2002). As petroleum hydrocarbons are complex mixtures, there are no specific legislative Soil Guideline Values (SGV) for TPH in soil systems. However, a recent guidance document produced by Land Quality Management (Nathanail *et al.*, 2006) defines the speciated TPH concentrations that should not be exceeded in the soils to prevent risk to human health. (refer to Appendix 8.1). As leaks generally occur under the ground or beneath building structures, contamination is not usually identified until a problem, such as structural damage or hydrocarbon odours becomes apparent. Where oil contacts with the soil large areas can become contaminated through lateral spread of the contamination influenced by the environmental conditions, including the physical characteristics of the soil and through the presence of water and an hydraulic gradient. The individual constituents of fuel oils have varied physico-chemical properties resulting in heterogeneous migration through a soil system (Dror *et al.*, 2001). The chemical composition of heating fuels and transformer oil in the environment will be dependent on its processing history and the extent of weathering it has been exposed to in the soil, as a result of chemical decomposition or physical disintegration (Nyer and Sladany, 1989).

1.2.1.1 Specific target contaminants for the study

The concentration of kerosene and transformer oil in soil samples is currently assessed by determining TPH fractions (a measure of the total hydrocarbons in a sample). However, these analytical methods are not effective for following the fate of individual constituents within hydrocarbon mixtures in environmental samples. In consequence, target

contaminants were selected for use in the laboratory investigations, including: n-dodecane and n-tetradecane (straight chain alkanes); xylene (*para* (*p*)-, *meta* (*m*)- and *orpho* (*o*)-isomers) and toluene (aromatic compounds); octadecylbenzene; and the PCB, 3-chlorobiphenyl (3-CB). Transformer oil was used for the field study (Chapter 5).

1.2.2 Toxicity of hydrocarbons

The presence of fuel oil hydrocarbons in the soil may produce hostile environments for biological systems and pose a significant health risk to humans and animals (IPCS, 1996; Mumtaz *et al.*, 1996; Ritchie *et al.*, 2001; She *et al.*, 2001; Samina and Adams, 2002; Wattiau, 2002). The toxicity of hydrocarbons, in particular to human health, is one of the main risks driving the remediation of hydrocarbon contaminated soils. With regard to human health, exposure to kerosene can result in dizziness, headaches, palpitations, and eye and skin irritations, with prolonged exposure leading potentially to renal failure, gastrointestinal symptoms and cancer (IPCS, 1996). PAHs, minor constituents of kerosene, are noted to have possible carcinogenic, mutagenic and genotoxic effects (Chen and Lio, 2006). Consequently, PAHs are considered priority pollutants by the European Community (Keith and Telliard, 1979), and hydrocarbons in general are included as List I contaminants under the EU Groundwater Directive (80/68/EEC) (as implemented by the Groundwater Regulations 1998) and as priority pollutants by the United States Environmental Protection Agency (US EPA) (Yerushalmi *et al.*, 2003). Health risks associated with transformer oil that is PCB-free are limited to eye and skin irritations. PCBs may be present in transformer oil produced prior to 1977, and these compounds are known to cause cancer in animals (*e.g.* rats) and are therefore likely to be carcinogenic to humans. There is also clinical evidence to suggest that PCBs can induce adverse reproductive, developmental and endocrine effects (Kimbrough, 1987; DeGranchamp and Barron, 2005).

1.2.3 Legislation

In the UK, following the implementation of Part IIA of the Environmental Protection Act (EPA) (introduced by section 57 of the Environment Act 1995 on April 1st 2000), and the development of the Contaminated Land Exposure Assessment (CLEA) model, local authorities are responsible for identifying contaminated land and overseeing its clean-up to comply with Acceptable Concentration Limits (ACL) (Kearney, 2002; Vik and Bardos, 2002). Under Part IIA, the statutory definition of contaminated land is "land which appears to the local authority in whose area it is situated to be in such a condition, by

reason of substances in, on or under the land, that (a) significant harm is being caused or there is a significant possibility of such harm being caused; or (b) pollution of controlled waters is being, or is likely to be, caused" (Defra, 2000). Where there is no sensitive receptor (*e.g.* residential area or neighbouring water course), the land would not meet the legal requirement for clean-up under this legislation. Contaminated land is the responsibility of the polluter unless an informed purchaser has accepted a transfer of liability, *e.g.* remediation of brownfield sites by property developers. In the instance that no owner is identified, the 'orphan site' becomes the responsibility of the local enforcing authority (Environment Agency [EA], 2002).

1.3 Methods for the remediation of contaminated land

On entering the environment, a contaminant may be attenuated as a result of sorption, volatilisation, and through abiotic and biotic transformations (Bouwer, 1992). These processes alone are frequently insufficient to clean up land effectively in an acceptable time-frame. There are many techniques available for the remediation of contaminated land (both *in situ* and *ex situ*) including biological, chemical and physical methods (Vik and Bardos, 2002) (Table 1.1), with some techniques being a mixture (*e.g.* permeable reactive barriers [PRBs] and can be classified as both chemical and biological methods). The techniques described are not necessarily used in isolation; a combination of methods may be used to improve efficiency (Chilinger *et al.*, 1997). Effective remediation usually occurs in five steps, as follows: (1) characterisation of site geology, hydrology and contamination; (2) removal of any separate immiscible phase; (3) assessment of biotransformation and volatilisation; (4) system design, installation and operation; (5) monitoring of performance (*e.g.* Thomas and Ward, 1989). Planned land use, time available, the developer's knowledge, and available funds are key factors in determining the type of remediation programme undertaken (Vik and Bardos, 2002).

Remediation technologies can be implemented *in situ* or *ex situ*, either on-site or off-site. In the year 2000, the EA of England and Wales carried out a survey to gain an insight into the type of remediation programmes being performed in England and Wales from 1996 to 1999. The report concluded that out of 367 sites, civil engineering techniques (*e.g.* 'dig and dump') had been used on 345 (94%) of sites, *in situ* techniques (*e.g.* monitored natural attenuation [MNA]) on 59 (16%) and *ex situ* techniques (*e.g.* landfarming) on 18 (5%), with bioremediation accounting for only 11 sites (3%) overall (Rivett *et al.*, 2002; Vik and Bardos, 2002). Within Europe *ex situ* civil engineering technologies are most frequently

employed. The advantages of *ex situ* technologies tend to be related to speed of clean-up, a quick transfer of liability and the fact that 'remediation' companies are often most experienced in this area.

Table 1.1 Remedial technologies for contaminated land.

Remedial Treatment	Description and Aims of the Treatment
Containment Systems (Arctander and Bardos, 2002)	Barriers can be used to physically break the pathway in pollutant transport, thereby managing the risk, *e.g.* fencing, cover systems and cut-off walls.
Biological Processes (Pollard *et al.*, 1994; Nathanail *et al.*, 2001; Khan *et al.*, 2004)	The elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes, *e.g.* biopiling, bioreactors, bioslurries, windrows, MNA, bioaugmentation, composting, landfarming, bioventing, phytoremediation, biosparging and PRBs.
Chemical Processes (Arctander and Bardos, 2002)	The transformation, destruction or stabilisation of contaminants mediated by chemical reagents, *e.g.* chemical oxidation with the use of Fenton's reagent or potassium permanganate ($KMnO_4$), or PRBs.
Solidification/Stabilisation (Khan *et al.*, 2004)	Fixation of contaminants and/or physical encapsulation to reduce the availability and mobility of contaminated materials, *e.g.* liming, cement based stabilisation and solidification, use as road base materials and vitrification.
Physical Extraction (reviewed by Arctander and Bardos, 2002; Khan *et al.*, 2004)	Separation and/or concentration of contaminants by exploiting differences in physico-chemical properties of the contaminant and contaminated soil or groundwater, *e.g.* soil vapour extraction , air sparging, soil venting, 'pump and treat', 'dig and dump', soil washing, soil flushing and electrokinetics.
Thermal Processes (Arctander and Bardos, 2002)	Destruction or concentration of contaminants by heating the soil or groundwater, *e.g.* low temperature separation, thermal desorption, incineration and radio frequency heating.

On domestic sites, where correct insurance cover is in place, remediation programmes are funded by insurance companies whose main aim, through loss adjustors, is to solve the problem quickly and cost effectively. At present, the costs incurred for remediating contaminated land under buildings can be so high that on occasions it becomes cheaper to abandon the property. In an attempt to reduce clean-up costs, and therefore increase the

incentive for landowners to remediate land, there is substantial investment into research in this area, further driven by protective legislation and the ever growing requirement for more building developments. The use of natural systems to bioremediate/biodegrade or intercept contaminant plumes is of particular interest in a culture where sustainable 'environmentally friendly' technologies are increasingly desirable (Aldrich, 2005).

1.3.1 Physical remediation strategies

The excavation of contaminated soil for disposal in landfill ('dig and dump') or treatment by incineration are the most commonly used methods for the clean-up of hydrocarbons for reasons of simplicity and speed (Kong *et al.*, 1998; Nathanail, *et al.*, 2001). Where contamination is located beneath a building it is not possible to remediate the land via excavation without having to disturb and damage the buildings prior to remediation. Incineration and the disposal of contaminated soil in a landfill are not sustainable technologies; with regard to the landfilling of contaminated soils, the contamination is not treated but simply transferred to a secondary site. The use of landfills as a means of disposing of contaminated land is becoming more difficult following the introduction of the Landfill Tax on 1 October 1996 and the EU Landfill Directive (Council Directive 1999/31/EC) (HM Customs and Excise, 2002). The aim of the Landfill Directive was to reduce the negative impact of landfills on the neighbouring environment and dramatically reduce the amount of municipal waste going to landfill (Purchase, 2005). In the UK a significant decrease in the number of landfills accepting hazardous waste, from over 100 to around 12, has also had a significant impact on the ability to simply 'dig and dump' (Bethel, 2004). At present landfills charge approximately £15 per tonne for soils with a kerosene concentration of less than 25% w/w and approximately £150 when the kerosene concentration exceeds 25% w/w. Landfill tax exemptions have been implemented to encourage the reclamation of brownfield sites. Tax exemptions are given if contaminated land is remediated with the aim of '(a) facilitating development, conservation, the provision of a public park or other amenity, or the use of the land for agriculture or forestry; or (b) reducing or removing the potential of pollutants to cause harm' (HM Customs and Excise, 2003).

The incineration of hydrocarbon contaminated soils is not a favoured remediation strategy, both in terms of social and legislative acceptance. Incineration produces toxic combustion by-products, such as dioxins and furans, and the sterile ash formed may contain residual contaminants (Lee and Huffman, 1996). In addition, natural biodegradation of the end

product is limited by its sterility. Currently, 2.8 million tonnes (9%) of municipal waste and 100 000 tonnes (3%) of hazardous waste are incinerated in the UK each year (Environmental Services Association, 2006).

1.3.2 Bioremediation methods

The focus of this study is to develop a hybrid process combining both biological and physical technologies. As previously stated (Table 1.1) there are several bioremediation techniques available for the remediation of contaminated land that have been used with varying degrees of success. Bioremediation techniques performed *ex situ* are outlined below.

1.3.2.1 Landfarming

Landfarming has been used for the treatment of hydrocarbon contaminated soils, including kerosene spills (Ellison *et al.,* 1999). Contaminated soil is excavated, spread in a thin layer and then supplemented with nutrients, water, and oxygen by regular mixing. The technique has proven to be very useful but there are a number of disadvantages: a large amount of land is generally required and the efficiency of remediation is dependent on the properties of the soil (*e.g.* permeability affecting the efficiency of nutrient delivery), the climate, and the nature of the contamination (Bio-wise, 2000; Kearney, 2002; McCarthy *et al.,* 2004).

1.3.2.2 Engineered biopiling (windrows)

Engineered biopiling consists of mixing excavated contaminated soil with a controlled level of oxygen, water, nutrients and bulking agents. Contaminated land is excavated and then screened to remove large uncontaminated objects before being placed into lined pits or bays. A liner is used to avoid leachate seeping into the surrounding environment (Bio-wise, 2000; Nathanail *et al.*, 2001). Aeration is usually achieved by actively injecting air into the piles using a network of perforated pipes.

1.3.2.3 Composting

Composting is used for the treatment of hydrocarbon contaminated soil. In addition to the lined pits (or bays) of biopiles, a covering is applied to conserve heat and maintain optimum conditions for bioremediation. The contaminated soil is supplemented with bulking agents, such as animal manure, to enhance remediation via the addition of nutrients. One of the main risks associated with the technique is that through bulking, the

volume of contaminated soil is increased, so if remediation fails there is a larger quantity of contaminated soil to be disposed of (Bardos *et al.*, 2001).

1.3.2.4 Bioreactor treatment

Bioreactor treatment is used where contaminants present are resilient to the other technologies mentioned and where it is essential that they are remediated from the soil. The contaminated soil is mixed with water and treated in a bioreactor in which the environmental conditions are tightly controlled to maintain optimum degradative conditions. The use of bioreactors to remediate soil is a relatively expensive option, so its use is not widespread (Jou and Huang, 2003).

The bioremedial *ex situ* technologies described above would not provide an effective method for remediating contamination from the sub-surface under buildings (or around underground services), as they all require excavation of the soil. Bioreactors, as a complement to 'pump and treat', could be used in soils where permeability was sufficient to allow contaminants to be drawn from the sub-surface, but in the case of contaminants sorbed to the soil and in low permeability soils ($k_h < 10^{-7}$ cm s^{-1} [Vane and Zang, 1997]) the technology may not be effective. Methods that do not require intrusive excavation are described below (Sections [§] 1.3.2.5 – 1.3.2.9).

1.3.2.5 Bioventing and biosparging

Bioventing and biosparging involve promoting airflow through the contaminated area by supplying extra oxygen or compressed air, along with nutrients (if required) to promote the aerobic degradation of contaminants using microorganisms indigenous to the site. Bioventing occurs above the water table whereas biosparging takes place below. The technique is applicable to any contaminant that is susceptible to aerobic degradation (Nathanail *et al*, 2001; Khan *et al.*, 2004).

1.3.2.6 Permeable reactive barriers (PRBs)

PRBs are physical barriers installed in the pathway of a contamination plume to intercept and degrade the contaminants, preventing further migration (Kalin, 2004). The composition of the PRB is selected principally to immobilise the contaminant and then to promote degradation either chemically or biologically. For example, zero valent iron (Fe^o) can be used in a PRB to reduce chlorinated contaminants, or alternatively sand and gravel

with added nutrients and electron acceptors could be used in a PRB to promote the biodegradation of the contaminants.

1.3.2.7 Bioaugmentation

Bioaugmentation describes the addition of microorganisms that possess the metabolic capabilities of biodegrading target contaminants identified at a site. The aim of the technology is to enhance the biodegradation capacity at a site, particularly when analytical evidence indicates that the soil is depleted of appropriate degraders. Delivery of bacteria has been achieved by mechanically mixing developed cultures into the contaminated soils or by spraying liquid cultures onto the surface of the soil. However, evidence to suggest that effective bioaugmentation has been achieved in the field is limited, particularly as introduced bacteria appear to have a limited ability to establish and proliferate at a contaminated site and delivery methods tend to be inefficient (Thompson *et al.*, 2005).

1.3.2.8 Biostimulation

Biostimulation involves the addition of enhancing agents (*e.g.* nutrients and electron acceptors) to facilitate biodegradation *in situ* (*e.g.* Silva *et al.*, 2004). The technique relies on microorganisms indigenous to the site having the metabolic capabilities to degrade the target contaminants and also that the soil has a sufficient hydraulic conductivity to facilitate the delivery of the enhancing agents.

1.3.2.9 Monitored Natural Attenuation (MNA)

MNA includes monitoring the biodegradation and disappearance of contaminants at a site to ensure that natural remediation can occur, without supplements, within a defined time-frame. The technique is generally only used if there are no sensitive receptors in the area and if remediation does not have to meet a stringent time deadline. Monitoring and mathematical models are used to predict the fate of the contaminant(s) and to identify any transformation products that could pose further risks to the environment and to human health (Nathanail *et al.*, 2001; Khan *et al.*, 2004).

1.4 Bioremediation of hydrocarbons

Bioremediation is the process of using 'biological agents' (bacteria, fungi, higher plants, algae and cyanobacteria) to remove contaminants from the environment in order to remediate contaminated soils and water (Wackett and Hershberger, 2001). It is a multi-disciplinary area of study that can encompass microbiology, engineering, toxicology,

chemistry, hydrogeology, environmental science and botany (*e.g.* phytoremediation) (Alexander, 1964). The study of bioremediation, with the aim of remediating contaminated sites was developed in the early 1900s, due to the need to identify the fate of pesticides applied to farmland (Alexander, 1994) and marine hydrocarbon contamination (Zobell, 1946; McKee *et al.*, 1972; Litchfield and Clark, 1973). Biodegradation results in the biological transformation of a chemical compound to a product with a less complicated structure that may be less toxic. Where the organic parent compound is metabolised completely to inorganic products, carbon dioxide and water, biodegradation is known as mineralisation.

1.4.1 Bacterial adaptation to hydrocarbons in the environment

The initial concern regarding the use of microorganisms for biodegradation of contaminants was that there was a large division between the structures of natural and synthetic chemicals. The term 'xenobiotic', in the context of biodegradation, is used to describe chemicals that are not thought to occur naturally within the biosphere, *i.e.* chemicals that have been artificially synthesised and to which biological systems would not naturally be exposed (Wackett and Hershberger, 2001; Top and Springael, 2003). Hydrocarbons exist in the environment from both biogenic and anthropogenic sources and therefore historical bacterial exposure to these compounds is extensive. Consequently, very few compounds are truly xenobiotic. Biogenic sources of hydrocarbons are abundant within the biosphere, including plant and insect waxes (Chibnall *et al.*, 1934). Approximately 0.02% of solid materials produced by most plants is hydrocarbon in nature (Zobell, 1946).

Even in the rare instance that a compound is truly xenobiotic, bacteria are equipped with several mechanisms that enable their communities to adapt to the contaminant. Importantly, bacterial communities may respond in the following ways: (a) increasing the population size of those bacteria capable of tolerating the contaminant; (b) degrading the contaminant by the induction of appropriate genes; (c) developing mutations that result in resistance or degradative capabilities; and/or (d) through horizontal gene transfer to acquire additional genetic information (*e.g.* plasmids and transposons) from populations in the community exhibiting beneficial characteristics (Top and Springael, 2003). Substrate specificity is also important, with some structurally similar chemicals being fortuitously biotransformed. Other mechanisms that enable bacteria to be adaptable include chemotaxis (*e.g.* Wadhams and Armitage, 2004), biosurfactant production (*e.g.* Ron and

Rosenberg, 2002), and modification of their cell membrane surface properties (*e.g.* Wythe *et al.,* 1999) to allow the more efficient uptake of contaminants, or increased resistance.

Tolerance to hydrocarbons by microorganisms can be influenced by the exposure history of the soil; generally the longer the exposure the greater the tolerance exhibited (Allard and Neilson, 1997). Past studies have demonstrated that in heavily contaminated soils, microorganisms show higher metabolic potentials than in corresponding controls (Dobler *et al.,* 2000). The concentration of the organic contaminant will have an effect on the structure of the bacterial community in the soil. If the concentration of the contaminant is too high then it is likely to be inhibiting, but too low a concentration and alternative substrates will be used (Pollard *et* al., 1994). In circumstances (either aerobic or anaerobic) where complete mineralisation does not occur directly, the biotransformation products may be used by other microorganisms within the community, forming an efficient catabolic pathway (Alexander, 1994). It is often observed that bacterial communities at contaminated sites become less diverse with time, and the species most well suited to the particular contaminants thrive while others are diminished (*e.g.* Kozdrój and van Elsas, 2001).

1.4.2 Biodegradation of hydrocarbons by bacteria

That certain bacterial species are capable of degrading hydrocarbons, in both aerobic and anaerobic environments, is well established and reviewed extensively (*e.g.* Zobell, 1946; Atlas and Bartha, 1972; Lovley, 2001; Wattiau, 2002). It is estimated that there are between 10^5 and 10^6 hydrocarbon degrading bacteria in every gram of fertile top soil (Morgan and Watkinson, 1988), indicating that microorganisms indigenous to a contaminated site may provide an extensive metabolic capability to facilitate bioremediation. The bacterial group Pseudomonads (gram negative and generally aerobes) is a key example of hydrocarbon degraders frequently ubiquitous to soils, including the key genera *Pseudomonas, Burkholderia, Zymomonas,* and *Xanthomonas.* The group has been studied extensively in laboratory-based hydrocarbon degradation studies and it has been established that Pseudomonads are able to procure mobile genetic elements (*i.e.* plasmids and transposons), which has enhanced their hydrocarbon degrading capabilities. For the degradation of alkanes the most extensively studied pathway is encoded by the *Oct* plasmid, which can be found within *Pseudomonas putida* Gpo 1 (Shapiro, 1984; Hamme *et al.,* 2003); encoding the enzymes for the step-wise conversion of alkanes to Acetyl-CoA

(Figure 1.3). Gram positive bacteria, including the genera *Rhodococcus*, have also been extensively studied for their hydrocarbon-degrading abilities (Hamme *et al.*, 2003).

The susceptibility of hydrocarbons to biotransformation is dependent on their chemical structure, with the following sequence: alkanes > branched alkanes > branched alkenes > low molecular weight *n*-alkyl aromatics > monoaromatics > cyclic alkanes, polynuclear aromatics >>> asphaltenes (Pollard *et al.*, 1994). Therefore hydrocarbon mixtures (*e.g.* kerosene) should not be treated as a single contaminant (a pseudo-component) as the degradability of each of the constituents will vary considerably (Nocentini *et al.*, 2000).

The biotransformation of hydrocarbon contaminants is principally used by bacteria for growth and energy for cell maintenance (Bouwer, 1992). Hydrocarbons can be used as either primary or secondary substrates and maximum efficiency is achieved when the organic contaminant is used as a primary substrate. A primary substrate is one that a microorganism is able to utilise as its sole carbon source. A secondary substrate is biotransformed fortuitously; there are no energy benefits for the microorganism to transform the hydrocarbon but instead it relies on another carbon source for growth and other energy requirements. Secondary substrate metabolism of compounds may occur through substrates being structurally similar to the primary substrate or through low substrate specificity of the acting enzyme.

1.4.2.1 The use of electron acceptors for the degradation of hydrocarbons

For the biodegradation of carbon substrates, electron acceptors are required for oxidation to take place. In aerobic conditions, oxygen will act as the terminal electron acceptor for the biodegradation of organics, giving the highest energy yield in a completed reaction. Where oxygen is not available, in anaerobic and anoxic environments, other bioavailable ions may be used as alternative electron acceptors, for example nitrate (NO_3^-), sulphate (SO_4^{2-}) or ferric iron (Fe^{3+}), but not necessarily by the same organism (Zobell, 1946; Atlas, 1981). The electron acceptor used in a reaction, where there are a variety of acceptors present, depends on the redox potential. Table 1.2 illustrates some of the respiration reactions that can occur under the various reduction potentials. In addition to the acceptors given in the table, ferric iron and heavy metals may be used. Ferric iron would be used as an electron acceptor before SO_4^{2-} as it has a higher electron affinity (Maier *et al.*, 2000).

Table 1.2 Relationship between respiration and redox potential.

Type of Respiration	Reduction Reaction: electron acceptor ⟶ product	Reduction Potential (V)
Aerobic	$O_2 \rightarrow H_2O$	+ 0.81
Denitrification	$NO_3^- \rightarrow N_2$	+ 0.75
Manganese reduction	$Mn^{4+} \rightarrow Mn^{2+}$	+ 0.55
Nitrate reduction	$NO_3^- \rightarrow NH_4^+$	+ 0.36
Sulphate reduction	$SO_4^{2-} \rightarrow HS^-, H_2S$	– 0.22
Methanogenesis	$CO_2 \rightarrow CH_4$	–0.25

Adapted from Maier *et al.* (2000).

Hydrocarbon contamination from spills at domestic or industrial properties is often situated in the sub-surface under buildings. Oxygen is quickly consumed from the sub-surface and as gaseous exchange is generally inefficient in such environments, it frequently becomes a rate- limiting factor for aerobic degradation. Consequently, alternative electron acceptors facilitating biodegradation of hydrocarbons are of significance. Within this study, the use of NO_3^- as an alternative electron acceptor is of particular interest because of its aqueous solubility. Nitrate can be used as an electron acceptor in the oxidation of organic substances to obtain energy for growth using a membrane bound nitrate reductase. NO_3^- may be used in one of two pathways, either dissimilatory (respiratory denitrification and dissimilatory NO_3^- reduction) or assimilatory (Schlegel, 1992). The reduction of NO_3^- to N_2 in respiratory denitrification occurs via NO_2^-, nitrous oxide (NO), dinitrogen oxide (N_2O) and then finally to N_2, which is released into the atmosphere from the soil. The membrane-bound enzymes involved with the reduction of NO_3^- and NO_2^- are repressed by oxygen (NO_3^- and NO_2^- reductase, respectively). In the dissimilatory NO_3^- reduction pathway substrate derived electrons are transferred from the NO_3^- to NO_2^- (Schlegel, 1992). Conversely to denitrification the liberated NO_2^- may be further reduced to NH_4^+ and be used in the assimilatory NO_3^- reduction pathway or accumulate in the sub-surface as NO_2^-, but is not reduced to N_2.

Assimilatory nitrate reduction results in the formation of NH_4^+, which is then incorporated into amino acids and metabolites. The presence of NH_4^+ suppresses the assimilatory nitrate reduction pathways. *Alcaligenes*, *Bacillus*, *Pseudomonas*, and *Thiobacillus* bacterial species are all capable of utilising NO_3^-, reducing the NO_3^- to nitrite (NO_2^-) (Equation 1.1) (a), ammonia (b), or molecular nitrogen (denitrification) (c):

$$\text{Glucose} + 12\ NO_3^- \longrightarrow 6\ CO_2 + 12\ NO_2^- + 6\ H_2O \quad \text{(a)}$$

$$\text{Glucose} + 3\ NO_3^- + 3H^+ \longrightarrow 6\ CO_2 + 3\ NH_3^+ + H_2O \quad \text{(b)}$$

$$\text{Glucose} + 4.8\ NO_3^- + 4.8\ H^+ \longrightarrow 6\ CO_2 + 2.4\ N_2 + 8.4\ H_2O \quad \text{(c)}$$

Equation 1.1 Microbial nitrate reduction.

1.4.2.2 Aerobic biodegradation

The maximum free energy yield for microbial degradation occurs under aerobic conditions and therefore these are usually studied for stimulated bioremediation (Pollard *et al.*, 1994). Furthermore, aerobic biodegradation experiments are generally faster than anaerobic experiments and require less specialised laboratory equipment. Catechol and protocatechuate are the key intermediates in the degradation of aromatic compounds and act as ring fission substrates during aerobic metabolism (Figure 1.2). Part of the versatility of microorganisms in aerobic environments is a result of their ability to produce a wide range of oxygenases. Oxygenases are enzymes capable of incorporating molecular oxygen into the substrate molecule. There are two types of oxygenase: monooxygenases and dioxygenases. Monooxygenases, otherwise known as mixed function oxidases, are able to incorporate one atom of oxygen into the substrate while using an electron acceptor to reduce the other to water, whereas dioxygenases incorporate both atoms of oxygen from the oxygen molecule.

1.4.2.3 The aerobic biodegradation of kerosene constituents

Kerosene was the principal hydrocarbon mixture used in laboratory based investigations for this study. The biodegradative pathways of most kerosene constituents (detailed in Appendix 8.2) have been previously characterised. Of the aromatic compounds, benzene, toluene, and xylene are all degradable in the soil under optimum conditions. The principal pathways of degradation include dioxygenase attack on the aromatic ring and monoxygenase attack on the methyl substituents (Tsao *et al.*, 1998). Figure 1.2 details the aerobic degradative pathway of xylene to pyruvate and propanal, through catechol as the ring fission substrate.

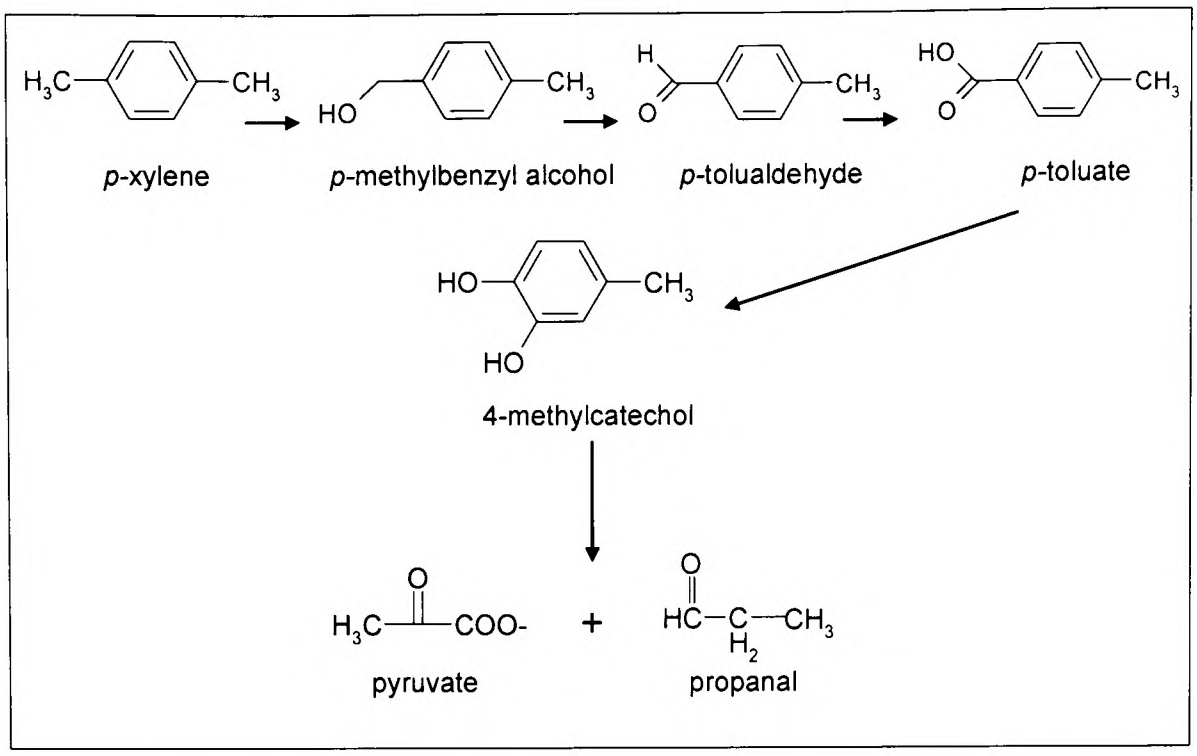


Figure 1.2 Biodegradation of *p*-xylene (UMBBD Databases, 2005).

The biodegradation of alkanes (cyclic and straight chain) involves oxidation of the chemical to the corresponding alcohol, aldehyde and fatty acid (Rosenberg and Ron, 1996). The fatty acids can then undergo β-oxidation to form acetate and propionate (Figure 1.3). In the case of even numbered carbon chains (*e.g.* dodecane), acetyl-Coenzyme A (CoA) will be the last residue before entering the tricarboxylic acid (TCA) cycle. Propionyl-CoA is the last residue for chains containing an odd number of carbons (*e.g.* undecane); the compound is then converted to succinyl-CoA, a 4-carbon intermediate in the TCA cycle.

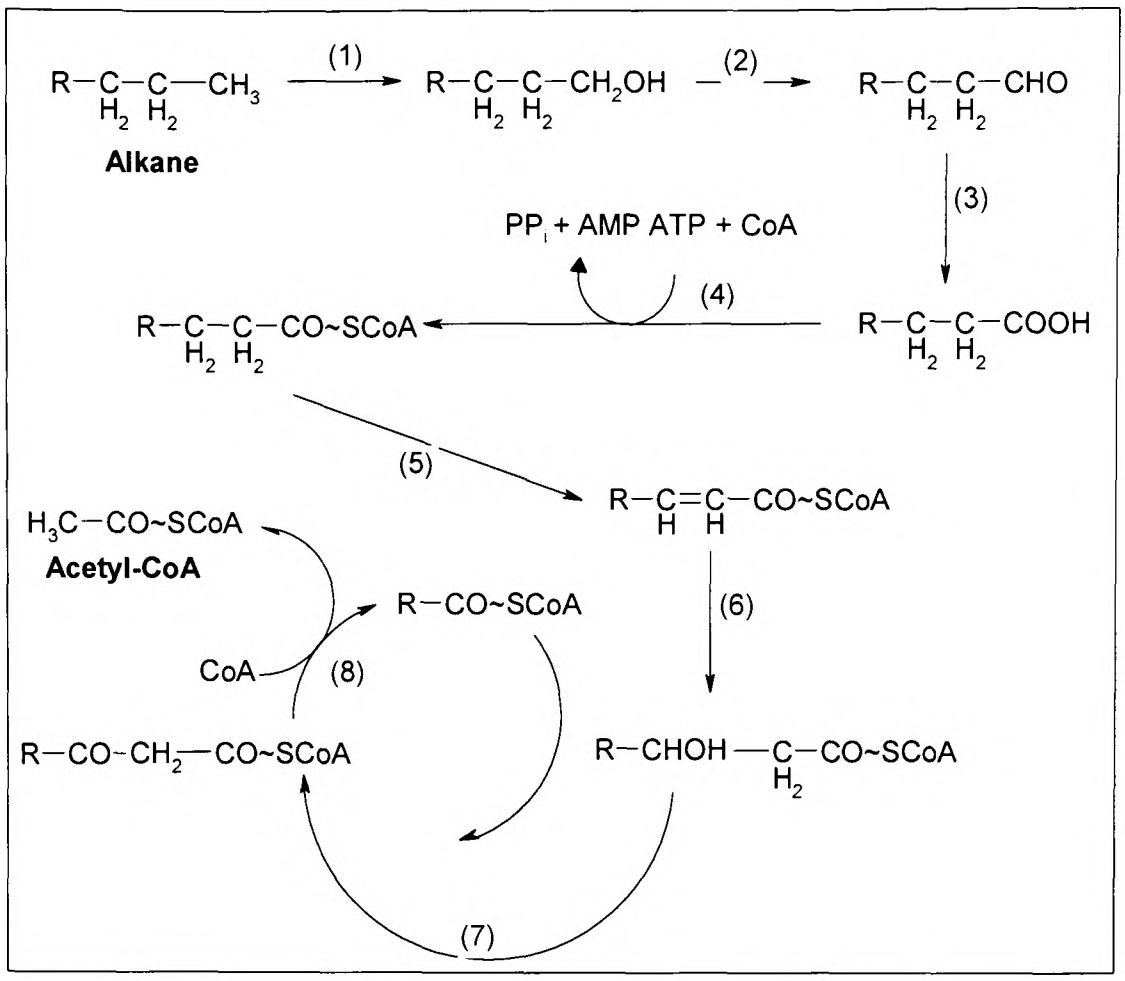


Figure 1.3 Biodegradation of alkanes via terminal oxidation by monooxygenase and β-oxidation (Schlegel, 1992). (1) Mono-oxygenase; (2) alcohol dehydrogenase; (3) aldehyde dehydrogenase; (4) acyl-CoA synthetase; (5) acyl-CoA dehydrogenase (6) 3-hydroxyacyl-CoA hydroxylase; (7) 3-hydroxyacyl-CoA dehydrogenase; (8) β-ketothiolase.

1.4.2.4 Anaerobic biodegradation

Anaerobic degradation was identified as a degradation mechanism for hydrocarbons, such as benzene, in the early 1900s (Tausson 1928, cited in Zobell, 1946). However, until the late 1980s there were far fewer studies investigating anaerobic degradation than aerobic degradation (Bouwer and McCarty, 1984; Karthikeyan and Bhandari, 2001; Boll *et al.*, 2002; Chang *et al.*, 2002; Boopathy, 2003). Anaerobic microbial populations tend to be less flexible in adapting to changes in substrate (Pollard *et al.*, 1994) and early evidence suggested that oxidation was key to degradation (Alexander, 1980; Rehm and Reiff, 1981). Nevertheless, recent studies have identified that anaerobes play a significant role in the transformation of contaminants (Lovley, 2001; Widdel and Rabus, 2001). Both laboratory and field-scale demonstrations have successfully shown efficient anaerobic remediation of chlorinated compounds (*e.g.* trichloroethylene [TCE]) via reductive dechlorination (Rabbi *et al.*, 2000; Magar *et al.*, 2001). Figure 1.4 illustrates the anaerobic degradation pathway of toluene.

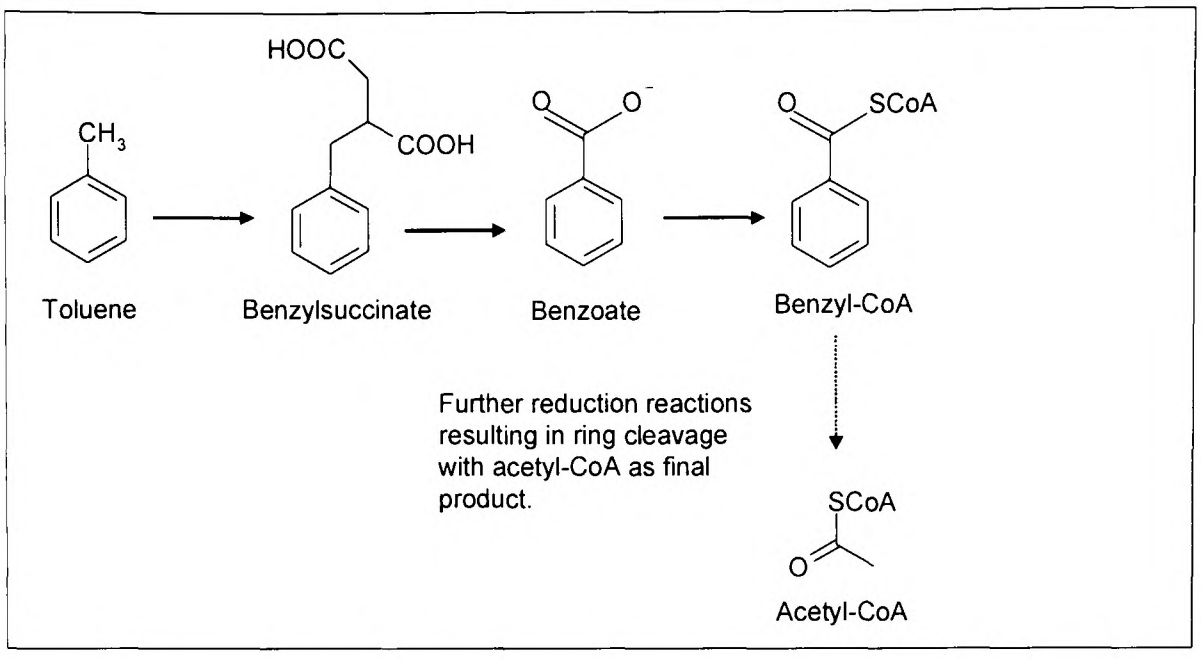


Figure 1.4 Anaerobic degradation of toluene and benzoate leading to ring cleavage (adapted from Boll *et al.*, 2002; UMBBD, 2005).

The anaerobic degradation of benzene, toluene, ethylbenzene, and xylene isomers (BTEX) has been described (Biegert *et al.*, 1996; Krumholz *et al.*, 1996; Heider and Fuchs, 1997; Boll *et al.*, 2002; UMBBD, 2002), with toluene appearing to be most readily degradable followed by xylene, ethylbenzene and benzene (Ball and Reinhard, 1996; Reinhard *et al.*, 1997).

1.4.3 Other potential hydrocarbon degraders

In addition to bacterial degradation, other enzymic and non-enzymic reactions occur. Non-enzymic reactions include photo-reactions and chemical transformations (Alexander, 1994). As this study focuses on the remediation of contaminants under buildings it is unlikely that photo-reactions will be significant. Certain species of fungi and other eukaryotic organisms are often capable of degrading hydrocarbons. Compared to bacteria, the degradation pathways of fungi tend to be less versatile and not as easy to control, but nevertheless their contribution can be significant (*e.g.* Wood, 1995; Rosenberg and Ron, 1996). Protozoa and earthworms may also have an influence on the remediation of contaminated soil. Protozoa are predators of bacteria, so an increase in protozoal numbers could have an effect on the size of bacterial populations (Wood, 1995) and earthworms may promote biodegradation, through increased aeration and the increased dispersion of microorganisms (Singer *et al.*, 2001). The use of plants to remediate soils (phytoremediation) contaminated with heavy metals and some organics has been investigated (*e.g.* Cunningham *et al.*, 1996) as a method to assimilate recalcitrant chemicals, by either storing or biotransforming them. The establishment of vegetation on contaminated sites has the potential to offer a lower maintenance approach to waste remediation and stabilisation, providing that time is not a constraint (Banks *et al.*, 2000).

1.5 The bioremediation of contaminated soil

1.5.1 Soil systems

Soil systems are extremely complex and have a significant influence over the efficiency of the bioremediation of contaminants. The aeration status, soil temperature, tilth (condition of the sub-surface) and water yield, retention and infiltration of water can all influence the retention of contaminants in the soil and ultimately affect the effectiveness of bioremediation (Pollard *et al.,* 1994). Soil consists of varying concentrations of organic matter, derived from dead and decaying matter and humic material (with non-polar moieties), which readily sorbs hydrocarbon contaminants.

Microbial activity may result in the formation of substances that are able to bind fine particles to form aggregates, with smaller aggregates having a larger surface area, and therefore a greater reactive surface. In soil systems two types of pores can exist. Pores between aggregates are referred to as inter-aggregate pores; pores that lie within the aggregate are known as intra-aggregate pores (Maier *et al.*, 2000). The intra-aggregate pores can be further divided into micropores and mesopores (Brady and Weil, 2004). The mesopores are involved in the capillary movement of solutions through soils and the micropores are involved with the entrapment of contaminants. The number and size of pores also influences the water holding capacity.

Clay soils have characteristically low permeability (for both water and air), reduced redox potential, high cation exchange capacity (CEC), and high plasticity, and tend to be slightly alkaline in nature (Virkutyte *et al.*, 2002). Even though both bacterial cells and clay aggregates tend to have negatively charged surfaces, attachment of bacterial cells to the clay surface can occur (Maier *et al.*, 2000). This is able to take place as the clay surface becomes neutralised by 'counterions' (*e.g.* K^+ and Na^+) creating an electrical double layer. Following attraction of the cells to the surface of the clay, other forces such as electrostatic and van der Waals forces are predominant in facilitating semi-permanent attachment to take place.

1.5.2 Association of contaminants with the soil

The fate of hydrocarbon contaminants in the environment is determined by the following factors (Mercer and Cohen, 1990; Pollard *et al.,* 1994): i) volume released; ii) area of infiltration; iii) duration of release; iv) bulk properties of the contaminant and soil media; and v) hydraulic properties of the sub-surface (*e.g.* hydraulic gradient and conductivity).

The soil will act as a chromatographic column for mixtures of hydrocarbons, separating individual constituents according to their physiochemical properties (IPCS, 1996). Contaminants have a tendency to migrate through the soil until the pressure, gravity and capillary forces are balanced (Dror *et al.*, 2001).

Sorption and desorption kinetics between the contaminant(s) and the soil will be dependent on the contaminant and the soil media, with hydrocarbons becoming associated with the soil matrix through absorption or adsorption. Adsorption is the binding of chemicals or particles to the soil surface through hydrogen bonds, van der Waals forces, or ion exchange (Alexander, 1994), whereas absorption refers to these species being held within the media, physically trapped within the pores of the soil. Sorption efficiency of organic contaminants (*i.e.* hydrocarbons) is determined by the percentage of organic carbon in the soil and the octanol-water partition coefficient of the contaminant (Rogers and Bunce, 2001). Recalcitrant hydrocarbon contaminants (*e.g.*, PAHs) commonly have low Henry's Law constants, high octanol-water coefficients and high soil organic-carbon water partition coefficients. These properties result in a preferential partitioning of contaminants from the water phase to the organic matrix (the higher the partition coefficient, the greater the hydrophobic nature of the compound). Humic molecules, the main organic chemical constituent of unpolluted soil, contain aromatic rings with carboxylic and phenolic groups, sugars and peptides (Cho *et al.*, 2002) providing binding sites and hydrophobic regions for PAHs to partition to, reducing bioavailability.

The hydrophobic nature of hydrocarbons gives them a high affinity to bind to the soil resulting in reduced bioavailability. Bioavailability describes the extent of interactions between bacteria and the target compound. Low bioavailability indicates that there is limited contact between the bacteria and target compound, leading to a reduction in degradation rate (Amellal *et al.*, 2001). Soil is a heterogeneous environment and contamination is likely to be unevenly distributed due to the presence of preferential binding sites. At low temperatures, hydrocarbon contaminants become increasingly viscous and less frequently enter the aqueous phase (Pollard *et al.*, 1994). Where hydrocarbon waste has remained in the soil for a long period of time, exposed to weathering, photolytic loss, evaporation, hydrolysis, and biotransformations, the contaminants tend to be resilient to further degradation (Pollard *et al.*, 1994). In general, the lighter constituents of kerosene (*e.g.* xylenes) will partition to the vapour phase, with the heavier components distributing to the soil (Ellison *et al.*, 1999) (Figure 1.5). Contamination that exists in the dissolved phase is able to freely migrate through the soil

system, so poses the greatest risk to humans, animals and structures, and could be susceptible to remediation via electroosmosis (refer to § 1.6.4.2).

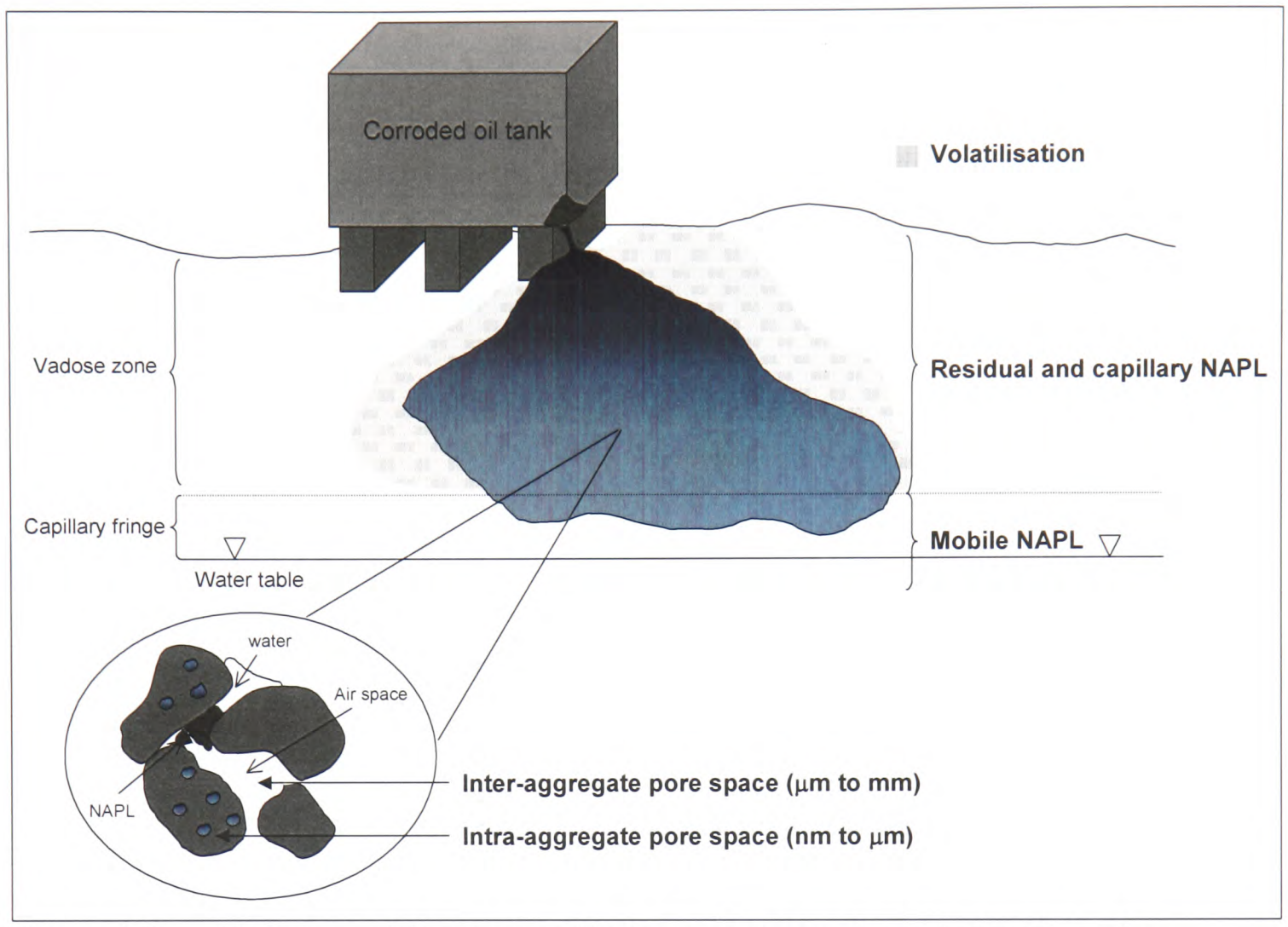


Figure 1.5 Migration and distribution of hydrocarbon contaminants in the sub-surface.

Contamination in the residual phase, either through physical entrapment or sorption, can act as a continual source of contamination. In remediation programmes it is important to consider contaminants in the residual phase. By removing free product (*e.g.* kerosene) and contamination from the vapour and dissolved phase, contaminants sorbed to the soil can then be released via mass transfer, referred to as a 'slow release mechanism'. In addition future use of the site, such as building, may release the contaminants physically trapped in the soil and pose a further risk to sensitive receptors at the site.

1.5.3 The use of biosurfactants to enhance bioavailability

Biosurfactants are produced by many bacterial and fungal genera (Déziel *et al.*, 1996; Miller and Zhang, 1997; Mulligan, 2005) and play an important role in enabling microorganisms to form physical interactions with the hydrocarbon contaminants and facilitating biodegradation (Figure 1.6). Surfactants are generally amphiphatic molecules, having a hydrophilic polar head and hydrophobic tail that lower the surface and interfacial tensions between NAPLs and the aqueous phase, promoting desorption of the hydrophobic contaminant from the soil (Christofi and Ivshini, 2002). In brief, biosurfactants are divided

into several groups: glycolipids (most commonly isolated biosurfactant), lipopeptides, lipopolysaccharides, phospholipids, and fatty acids/neural lipids (Miller and Zhang, 1997; Christofi and Ivshina, 2002). Generally, biosurfactants are either ionic or anionic (Bognolo, 1999). Both high molecular weight (*e.g.* amphipathic polymers) and low molecular weight compounds (*e.g.* lipopeptides) have been identified. These are produced, and used as surfactants/emulsifiers by bacteria (Rosenberg and Ron, 1996).

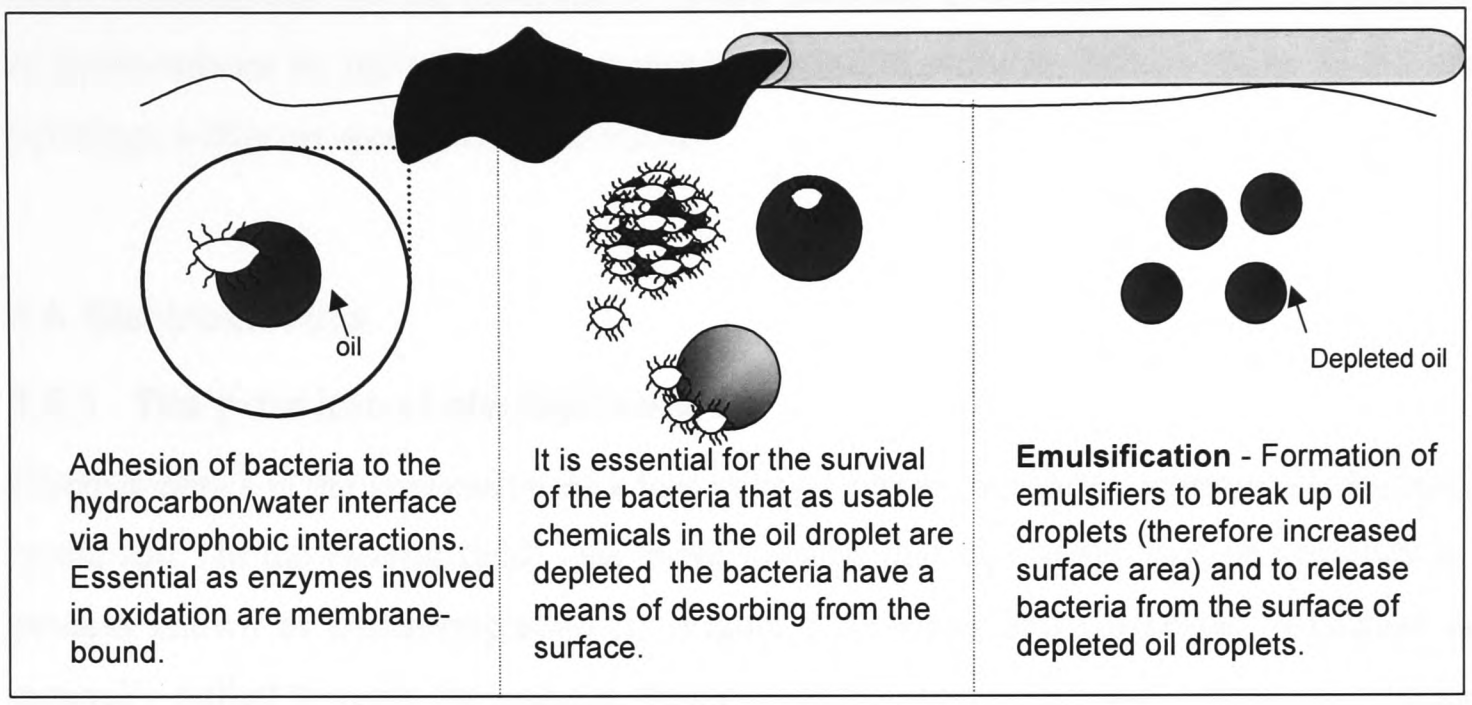


Figure 1.6 Physical interactions required for the degradation of hydrocarbons.

1.5.4 Potential limitations of bioremediation

There are a number of factors that can limit the effectiveness of bioremediation, including:

i. Sites are generally contaminated with a mixture of contaminants, but no organism is known to degrade or tolerate all organic wastes (Huesemann *et al.*, 2003).
ii. High concentrations of the contaminant may be inhibitory or toxic to the bacteria.
iii. Binding of the contaminant to the soil may occur, reducing its bioavailability (Hatzinger and Alexander, 1995; Allard and Neilson, 1997; Carmichael *et al.*, 1997), exacerbated by poor contaminant solubility and pollutant 'aging'.
iv. Limited nutrients and electron acceptors.
v. Build up of toxic transformation products (Pollard *et al.*, 1994; Lorton *et al.*, 2001; Wackett and Hershberger, 2001).

Although there are few synthetic compounds that can resist microbial digestion, environmental conditions can greatly hinder or enhance the progress of remediation (Thompson, 2001). It was identified in a CLARINET (Contaminated LAnd Rehabilitation

Network for Environmental Technologies) publication (Vik and Bardos, 2002) that available *in situ* and *ex situ* remediation technologies are difficult to use when there is complex infrastructure present in the vicinity of the contamination. For this reason there is a demand for a reliable *in situ* technology that is capable of remediating hydrocarbons by overcoming problems of bioavailability, nutrient delivery and the ability to optimise biodegradation without having direct access to the plume (McCarty, 1991). Electroremediation has the potential to create optimum conditions for the biodegradation of hydrocarbons by indigenous microorganisms and to mobilise hydrocarbons from under buildings within an acceptable time-frame.

1.6 Electrokinetics

1.6.1 The principle of electrokinetics

Electrokinetics is the application of a low voltage current between a number of electrodes in the soil. In the electric field: ions move towards the oppositely charged electrode in a process known as electromigration (i) (Figure 1.7); water and uncharged molecules are generally pulled towards the cathode through electroosmosis (ii); and, negatively charged bacterial cells and other particles can move to the anode through electrophoresis (iii). In addition, electrolysis at the anode and cathode of the circuit results in the production of hydrogen ions (H^+) at the anode and hydroxyl (OH^-) ions at the cathode. Their subsequent movement through the soil by electromigration can cause a pH gradient across the soil.

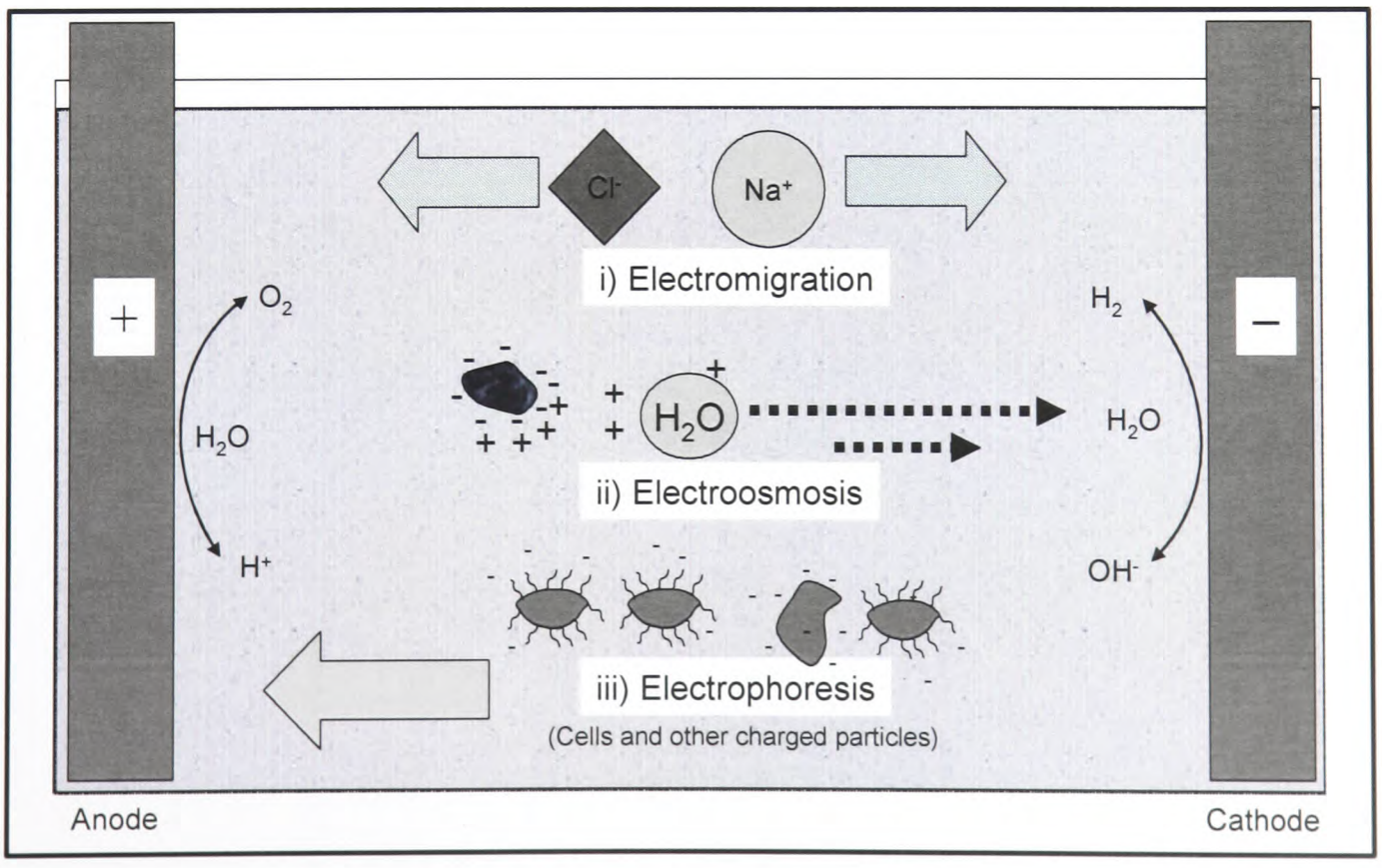


Figure 1.7 Schematic showing electromigration, electroosmosis and electrophoresis in a soil chamber in the presence of an electrical field.

1.6.2 The history of electrokinetics

Electrokinetics was demonstrated for the first time by F.F. Reuss in 1809 (Reuss, 1809; Elektorowicz and Boeva, 1996). Historically, electrokinetics has been used for dewatering clays, for the consolidation and stabilisation of fine-grained soils through dewatering (Casagrande, 1947; Alshawabkeh *et al.,* 2004), the insertion of metal grouts (Gray and Schlocker, 1969; Ozkan *et al.*, 1999), for geochemical exploration (Shmakin, 1985; Acar, 1992) and metal extraction (Will, 1995), and for controlling salt levels in agricultural land (Gibbs, 1966; Eid *et al.,* 2000). Recent studies have investigated the use of electrokinetics to remediate contaminated land and replace high energy consuming techniques for the dewatering and decontamination of sludges (Kim *et al.,* 2002; Yuan and Weng, 2002) and ball clays (a vital component of ceramics) (Sunderland, 1989b).

1.6.3 Electrokinetics and remediation of contaminants

The use of electrokinetics to attenuate pollution is of particular interest because of its application in low-permeability soils. Low-permeability soils are difficult to remediate using conventional remediation methods (Schnarr *et al.,* 1998; Reddy and Saichek, 2003) but could be susceptible to electroremediation. Heavy metals were the first contaminants to be targeted with electrokinetics (Hamnett, 1980; Segall *et* al., 1980). Following on from the founding work of Hamnett at the University of Manchester, many other contaminants have now been effectively targeted with electrokinetics (Table 1.3).

Table 1.3 Contaminants targeted with electrokinetics.

Contaminant	References
Arsenate, arsenic	Ribeiro *et al.*, 2000; Kim *et al.*, 2005
Atrazine	Ribeiro *et al.*, 2005
Benzene, toluene, *m*-xylene	Bruell *et al.*, 1992
Cadmium	Lageman *et al.*, 1989; Reddy and Parupudi, 1997; Huang *et al.*, 2003
Chromium (Cr) (VI)	Reddy and Parupudi, 1997; Pamukcu *et al.*, 2004
Copper	Lageman *et al.*, 1989; Lageman, 1993; Maini *et al.*, 2000a; Ribeiro *et al.*, 2000
Lead (II)	Lageman *et al.*, 1989; Lageman, 1993; Reed *et al.*, 1996; Yeung *et al.*, 1996; Coletta *et al.*, 1997
Mercury	Thöming *et al.*, 2000
Nickel	Reddy and Parupudi, 1997
Pentadecane	Kim *et al.*, 2005
Phenanthrene	Saichek and Reddy, 2003; Khodadoust *et al.*, 2004
Phenol (nitro-)	Ho *et al.*, 1995; Yang and Long, 1999
Radionuclides	Yu and Neretnieks, 1997b
TCE	Bruell *et al.*, 1992; Vesper *et al.*, 1995; Ho *et al.*, 1999a, 199b; Rabbi *et al.*, 2000
Zinc	Pamukca *et al.*, 1991; Lageman, 1993

The rate of mobilisation of the contaminants will be dependent on the hydraulic pressure gradient, the influence of electrokinetic transport and the level of contaminant diffusion (Chilinger *et al.*, 1997). If the contaminant can be successfully mobilised and focused at either the anode or cathode, then removal can be accomplished by several means. Abiotic methods of extraction include using an adsorptive material to soak up the contaminant(s), or the electrodeposition or precipitation methods to concentrate the contaminant(s) (Acar and Alshawabkeh, 1993; EGIS, 2002). Comparisons between the costs for 'clean-up' using conventional methods and those estimated to be incurred for electrokinetics give promising results. Electrokinetics can cost £10-80 per m^3 compared to £30-200 per m^3 for landfill and £100-400 per m^3 for incineration (Jackman *et al.*, 2001). As described by Acar (1992) there are a number of potential uses for electrokinetics when dealing with contaminant plumes, including: acting as barriers to impede migration; diversion and fencing schemes for migrating plumes; injection of nutrients; and *in situ* decontamination through species separation by migration, diffusion and electroosmosis.

1.6.4 Electrokinetic induced phenomena

1.6.4.1 Electromigration

The movement of both cations and anions towards their corresponding electrodes relies on there being sufficient fluid along their migration pathway and is independent of the charge density of the soil (Figure 1.7). Electromigration is the principal mechanism for removing cationic metal species from soil during electrokinetics (Maini *et al.*, 2000a). In addition, the phenomenon of electromigration could be exploited for the delivery of nutrients and electron acceptors to remediation zones for microbial use (Acar *et al.*, 1995; Lee and Lee, 2001). The injection and focusing of NO_3^- using electromigration in a soil system has been the focal point of recent studies (Segall and Bruell, 1992; Chew *et al.*, 1998; Eid *et al.*, 2000; Luo *et al.*, 2004), both in terms of the addition and removal of NO_3^- from a soil system at laboratory scale.

1.6.4.2 Electroosmosis

Electroosmosis (Figure 1.7) involves the movement of water, generally, from the anode towards the cathode of the circuit. As a result of a net negative charge of the soil/clay (due to isomorphous substitution and fracturing) there is an accumulation of cations, described as the double diffuse layer (DDL). The concentration of cations at the surface of the clay moving towards the cathode 'imparts momentum to the surrounding water molecules' resulting in the 'free' solution being dragged towards the cathode through friction forces (Figure 1.8) (Reuss, 1809; Renaud and Probstein, 1987; Acar, 1992; Chilinger et al., 1997).

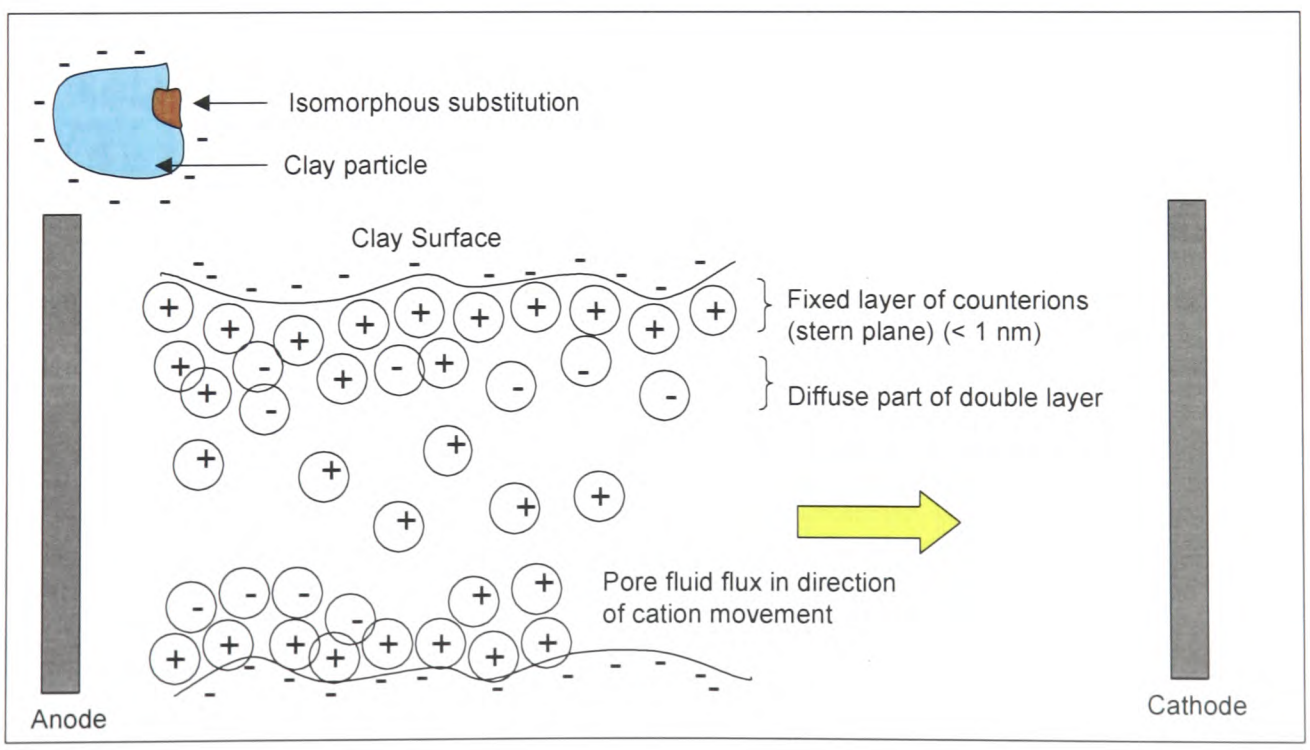


Figure 1.8 Schematic of pore fluid flux at the surface of clay and the double diffuse layer (DDL) of the soil. (Isomorphous substitution is the replacement of one atom by another without any change to the charge).

The electroosmotic phenomenon is described by the Helmholtz-Smoluchowski Theory that states that electroosmotic flow is proportional to the electric field applied (Equation 1.2) (Hemnet, 1980), based on the work of Helmholtz (1879), Pellat (1904) and Smoluchowski (1921) (discussed by Pamukcu, 1997).

$$\upsilon_{eo} = \frac{D\varepsilon_0\zeta}{\eta} \times E$$

υ_{eo} = electroosmotic velocity

ζ = zeta potential

η = viscosity of pore fluid

E_X = Applied voltage

D = dielectric constant

ε_o = permittivity of vacuum

Equation 1.2 The Helmholtz-Smoluchowski Theory.

The rate of electroosmosis (υ_{eo}) is influenced by the sub-surface conditions (Vane and Zang, 1997). For instance, the contaminants present, the pH and zeta potential (ζ) (a measure of the electrostatic potential across the double layer [Figure 1.8] resulting from an accumulation of ions around a static surface) can all affect the rate of electroosmosis (West and Stewart, 2000). The higher the pH, the more negative the ζ and thus the greater the electroosmotic velocity.

Generally, soils have a net negative charge but the specific surface area of the soil will affect the actual surface charge density. With increased surface area, the surface charge density increases, sand < silt < kaolinite < illite < montmorillonite. Hence, the electroosmotic rate is proportional to the specific surface area (Acar *et al.*, 1995). The density of the cationic layer around the surface of the clay and the dielectric properties of the pore fluid have a direct influence on the soil zeta potential and thus on the extent of pore fluid flux (Acar *et al.*, 1995). The presence of ions in the pore fluid has an effect on the size of the DDL; the more ions, the smaller the layer and thus the smaller the electroosmotic flow. Anions move toward the anode but the increased number of cations means that they have more influence on the movement of water, causing the net movement of fluid towards the cathode. Electroosmotic flow will cease if the soil surface potential approaches zero charge as a result of the ionic constituents of the pore fluid (Acar and Alshawabkeh, 1993). The total fluid flow (q) under hydraulic (Darcy's law) and electrosmotic gradients is regulated by the following parameters (Equation 1.3) (Alshawabkeh *et al.*, 2004):

$$q = -k_h \frac{\partial h}{\partial x} - k_e \frac{\partial \phi}{\partial x}$$

q = fluid flux per unit area due to hydraulic gradient ($L^3T^{-1}L^{-2}$)

k_h= hydraulic conductivity (LT^{-1})

h = hydraulic head (L)

k_e = coefficient of electroosmotic permeability ($L^2V^{-1}T^{-1}$)

x = longitudinal distance (L)

ϕ = electric potential (V)

Equation 1.3 Total fluid flow under hydraulic and electroosmotic gradients.

Reverse electroosmosis has been reported (*e.g.* Shapiro and Probstein, 1993; Yeung *et al.*, 1996). Movement of water from the cathode to the anode can occur where the zeta potential of the soil surface becomes positive. In this instance anion movement exceeds cation migration and the pore fluid is subsequently dragged towards the anode. There is general acceptance that the ζ value is influenced by the pH, with a decrease in pH, below the zero point of charge (ZPC), leading to ζ becoming more positive (West and Stewart, 2000). Thus, with an increase in H^+ concentration within the pore fluid, ζ becomes more positive, and electroosmosis from anode to cathode is slowed, or even reversed (Eykholt and Daniel, 1994; Shapiro and Probstein, 1993). Within this study, pH control at near neutral should prevent reverse electroosmosis occurring.

The flow rate of water through electroosmosis is dramatically decreased with an increase in hydraulic conductivity (Murdoch and Chen, 1997). If a soil has a higher hydraulic conductivity, and is therefore more permeable, the backward-acting hydraulic flux induced by electroosmosis can counteract electroosmotic flow. In this way electroosmosis only presents a valuable movement mechanism in less permeable soils, which do not exhibit such opposing hydraulic-fluxes. In some instances (with low hydraulic gradient) electroosmosis may be used to counteract the natural flow of water by creating a counter-current in the opposite direction to the groundwater gradient (Narasimhan and Ranjan, 2000), an important phenomenon if a sensitive receptor exists down an hydraulic gradient from the contaminant plume.

As kerosene and transformer oil (the target contaminants for this study) are largely composed of non-ionic hydrocarbons, movement of the contaminant through the system is dependent on the action of electroosmosis (Virkutyte *et al.*, 2002). In low permeability

soils electroosmosis can remove dissolved contaminants from the soil, independent of the soil pH, but is unable to move contaminants that are sorbed to the soil surface. Purging the system with water and surfactants may be suitable to increase the solubility of organic contaminants (Probstein and Hicks, 1993; Christof and Ivshina, 2002).

1.6.4.3 Electrophoresis

Electrophoresis (Figure 1.7) is the movement of suspended charged particles (generally less than 20 μm diameter [∅]) under the influence of an electric field (Sunderland, 1987a). The term particle includes all electrically charged entities such as clay colloids, clay particles, droplets and bacterial cells that are present in the pore fluid (Lageman *et al.*, 1989). Alshawabkeh *et al.* (2004) reported that the effect of electrophoresis is only significant where sufficient pore water exists and not in compacted soils, where pore fluid movement is restricted; some authors believe movement through electrophoresis in soils is completely insignificant and therefore can be ignored in soil systems (*e.g.* Reed *et al.*, 1996).

The outer cell membrane of bacterial cells contains ionisable polymers (*e.g.* peptidoglycan) (Beveridge *et al.*, 1997), the composition of which is dependent on growth conditions and genetics. At neutral and basic pH conditions the cells have an overall net negative charge (Wilson *et al.*, 2001). The greater the net negative charge the greater the electrostatic repulsion between the cells and soil surface (DeFlaun and Condee, 1997). The use of electrokinetics to inoculate soil in the laboratory with bacteria has been reported with some success (DeFlaun and Condee, 1997; Lee and Lee, 2001). However, soil type and structure and bacterial adhesion to the soil can render electrokinetics as ineffective at bioaugmentation.

1.6.4.4 Electrolysis

Electrolysis describes the splitting of water in the presence of an electric potential. Oxidation at the anode results in the formation of H^+ and the liberation of oxygen (O_2). At the cathode, reduction produces OH^- and hydrogen gas (H_2) (Equation 1.4).

$$2H_2O - 4e^- \longrightarrow O_2 + 4H^+ \qquad E_o = -1.23 \text{ V (a)}$$

$$2H_2O + 2e^- \longrightarrow H_2 + 2OH^- \qquad E_o = -0.83 \text{ V (b)}$$

Equation 1.4 (a) Electrolysis reaction at the anode; (b) electrolysis reaction at the cathode (Ho *et al.*, 1995; Reddy and Saichek, 2003).

The formation rate of both H^+ and OH^-, at their respective electrodes, depends on the presence of competing ions. At the anode, ions are competing to 'give up' electrons and if substances are present that are more oxidisable than water, then H^+ will be produced at a lower rate. Likewise, at the cathode, where ions are competing to gain electrons, OH^- production will be lowered if there is a more reducible substrate (Eid *et al.*, 2000). Importantly, the migration of these ions to their respective electrodes creates a pH gradient. As H^+ move approximately twice as fast as OH^- there is an advancing acid front through the soil, which increases the soil's ionic conductivity (Acar and Alshawabkeh 1993; Acar *et al.*, 1995). In certain instances the movement of the acid front is advantageous to the remediation of a site. For example, an acidic environment (pH < 3) is required for copper (Cu^{2+}) or zinc (Zn^{2+}) to be soluble (Maini *et al.*, 2000b) as cationic species are desorbed in acidic conditions. However, an advancing acidic front may need buffering to preserve neutrophilic bacteria, which have a pH optimum between pH 5.5 and 8.5 (DeFlaun and Condee, 1997). At the point where the H^+ and OH^- meet, closest to the cathode, a water front is created. In an unbuffered electrokinetic run, the pH values at the anode and cathode are likely to be 2 and 12, respectively (Acar, 1992).

As described (Equation 1.4), the evolution of H_2 and O_2 is a consequence of electrolysis of water at the electrodes. Tiehm *et al.* (2005) investigated the use of H_2 formed at the cathode as an electron donor and O_2 from the anode as electron acceptor in the biodegradation of chlorinated ethenes and metabolites (Tiehm *et al.*, 2005). Depending on the set-up of the electrokinetic system, the formation of O_2 and H_2 may be sufficient to cause an explosion (Alshawabkeh *et al.*, 2004), so considerations need to be made to ensure adequate venting of electrodes during electrokinetics.

1.6.4.5 Soil heating upon application of electrokinetics

In addition to the influence of the electric current on contaminant movement through the soil, the current may also have a heating effect on the soil. Electrical heating has been studied as a promising method for the remediation of volatile organic compounds (VOCs) (EGIS, 2002). The method uses an alternating current to heat the soil and induce volatilisation of contaminants so that they can be removed from the soil via an extraction method that can be implemented (*e.g.* vacuum extraction).

1.6.5 The use of electrokinetics to bioaugment contaminated soils

A number of studies have been undertaken to identify the possibility of using electrokinetics to bioaugment the sub-surface (*e.g.* Deflaun and Condee, 1997; Wick *et al.*, 2004; Kim *et al.*, 2005; Quishi *et al.*, 2005; Suni and Romantschuk, 2005). Deflaun and Condee (1997), showed that it was possible to distribute bacteria using electromigration, and to a certain degree electroosmosis. In the study of Wick *et al.* (2004) it appeared that electroosmosis played an important role in the movement of non-motile bacteria, with up to 90% transported by electroosmosis compared to 0-20% by electrophoresis. At circumneutral pH, bacteria are negatively charged and therefore have the potential to be moved via electromigration. If it can be proven that electrokinetics can be used to inject bacteria effectively into a soil system there is the potential to increase the contact efficiency between contaminants, nutrients and contaminant-degrading bacteria.

At the cell level, applying a current to bacteria can produce hydrophilic pores in the cell wall of bacteria, which could increase the transfer rate of nutrients into the cell (Weaver and Chizmadzhev, 1996). Luo *et al.* (2005) studied the effect of direct current on bacteria within an aqueous system, and found that when phenol degrading organisms are exposed to a current, cell hydrophobicity and the net negative charge of the cell surface are both increased at currents of 20 and 40 mA, respectively. Increasing the bacterial cell surface negativity could increase the ability to mobilise bacteria through electromigration towards the anode of the circuit, counteracting sorption of the bacterial cell to the soil matrix.

1.6.6 Commercial and field application of electrokinetics

There have been several commercial applications of electrokinetic remediation in the United States, with electrokinetics conducted for test and demonstration purposes, at pilot and full scale, reviewed by Van Deuren *et al.* (2002). Electrokinetics, Inc., and Geokinetics International, Inc. (Lageman, 1993; Lageman and Pool, 2001) have studied the field scale use of electrokinetics to remediate heavy metals from soil, and the first demonstrations in Europe took place in 1987. In addition, electrokinetics has been used at two sites near Cincinnati, Ohio, where graphite filled hydraulic fractures were investigated (Murdoch and Chen, 1997) within uncontaminated soils and at an electroplating site trial in Germany (Wieczorek *et al.*, 2005). Within Europe, electrokinetics has been used as a commercial remediation technology in Denmark, the Netherlands and France (Acar and Alshawabkeh, 1993; Vik and Bardos, 2002).

The 'Lasagna™' process is a form of electrokinetics first used in 1995 at the Paducah Gaseous Diffusion Plant, Kentucky, USA, for the remediation of TCE from clay using electroosmosis and zero valent iron (Terran, 1995). The term 'Lasagna' was adopted as the system consisted of horizontal layers of electrodes and treatment zones (Ho *et al.*, 1995, 2000a, 2000b; Roulier *et al.*, 2000) (Figure 1.9). A feature of the process is that the current is alternated at set intervals so that contaminants can be passed repeatedly through a bioremediation zone containing a consortium of methanotrophic TCE degrading bacteria; this also serves to limit the development of a pH gradient (McFurty, 1994; EGIS, 2002). Both horizontal and vertical configurations of the Lasagna process have now been studied. In trials, the catholyte (cathode electrolyte) has been recycled to the anode successfully, aiding buffering of the system. Other enhancements of the system involve the installation of microbial support zones (Ho *et al.*, 1995). A disadvantage to the Lasagna technique includes the entrapment of gases formed via electrolysis in the soil, which can disrupt the soil structure and presents a risk of explosion.

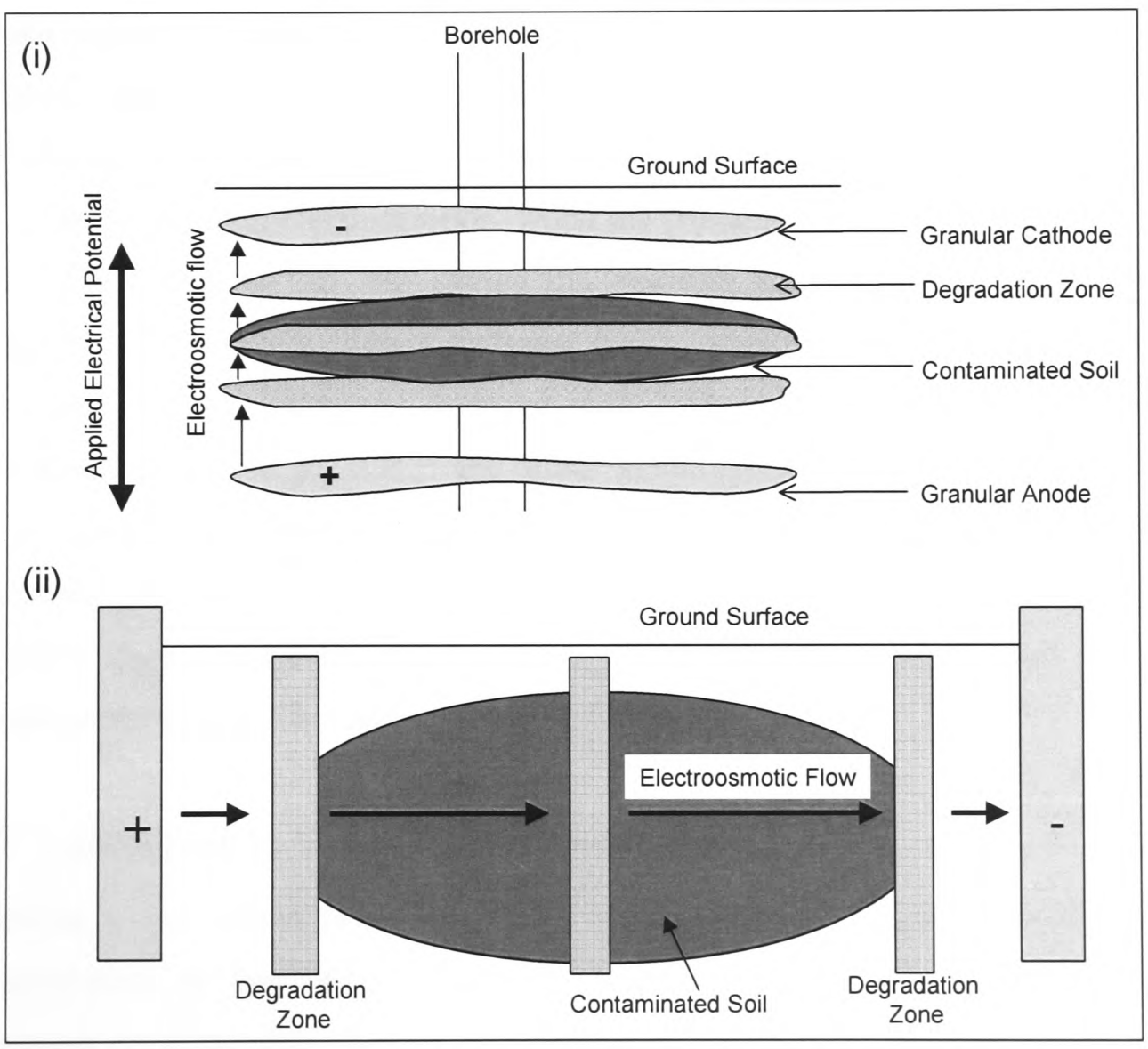


Figure 1.9 Schematic of the horizontal (i) and vertical (ii) configurations of the Lasagna™ process (Ho *et al.*, 1995; Roulier *et al.*, 2000). The electrodes are inserted into the ground using hydraulic fracturing.

Electro-klean™ is another *in situ* and *ex situ* electrokinetics technology, used for the first time in Louisiana, USA (Virkutyte *et al.*, 2002). The technique has successfully been used for the remediation of heavy metals, radionuclides, and specific VOCs. In addition to the standard electrokinetic set-up, enhancing fluids are added to the system (*e.g.* the addition of acids for the remediation of heavy metals). The technique of Electrosorb™ involves the use of cylindrical electrodes that are coated with a specially designed polymer, impregnated with pH regulating chemicals (Virkutyte *et al.*, 2002). An ion exchange system may be incorporated with the electrokinetics as a means of filtering out contaminants. Although the technique appears to be very effective, the ion exchange materials are expensive.

Research carried out in the UK has included that of Sunderland (1987a,b,c), Cundy and Hopkinson (2004), and Electrokinetic Limited (Electrokinetic Ltd., 2005). Cundy and Hopkinson, (2004) demonstrated the use of sacrificial iron electrodes to produce a pH gradient across the soil and force the precipitation of an iron-rich barrier that restricts leachate movement, especially leachate containing heavy metals; the process is referred to as Ferric Iron Remediation and Stabilisation (FIRS) (requested Patent number W020040287170). They have also carried out work (unpublished) on the movement of hydrocarbons, through electroosmosis, when the contaminants are present in the aqueous phase. Electrokinetic Ltd. has carried out research into the use of electrokinetics to manage the water content of sport turfs and for the dewatering of sludges (Lamont-Black, 2002), and they are currently involved with DTI funded research to commercialise electrokinetics for sewage, sludge and waste minimisation through dewatering (CIWEM, 2006). Sunderland (1987a,b,c) reviewed a selection of technologies that have utilised electroosmosis and electrophoresis to dewater soils and sludges and for chemical deposition (*e.g.* concentration of rubber latex) and also investigated the use of electrokinetics to remediate heavy metals at field-scale.

1.6.7 Limitations to the use of electrokinetics

Following a site investigation to determine the extent of the contamination and site characteristics, an appraisal of available remedial options would aid in making an assessment to determine whether electrokinetics could offer an effective solution for remediation. Parameters affecting the success of electrokinetics include: strength of the electric field; extent of contamination; soil type; and the type of microorganisms present at the site.

1.6.7.1 The presence of buried objects

Lageman (1993) showed that the presence of metal objects or isolating materials in contaminated soils could impede electrokinetics, through diversion of the current. It was concluded that metal items >100 mm can have a significant effect on the electrical current by providing preferential flow paths. In electrokinetic field trials performed by Geokinetics Inc. (Lageman, 1993), the presence of metal tins and other objects greatly increased the power expenditure for the remediation of arsenic. Other objects such as wooden beams, plastic sheeting and concrete blocks were also considered to be a significant problem. The present study focuses on the remediation of contaminants from the sub-surface beneath buildings. It is therefore necessary to consider the complications that will arise as a result of any cables, pipes or other underground services that may be present.

1.6.7.2 A reduction in the efficiency of electrokinetics

Over time, deterioration of the electrokinetic apparatus may occur and precipitation of metals, occurring at the cathode, may impede the electrokinetic process (Cauwenberghe, 1997). The movement and precipitation of calcium at the cathode, as bicarbonates and/or hydroxides, is a common problem (Acar *et al.*, 1995; Maini *et al.*, 2000b). Energy requirements to power the circuit are dependent on the electrical potential difference across the electrodes (Acar, 1992). Where precipitation has occurred, an area of low conductivity may arise increasing the electrical potential and thus increasing running time and costs. Another consideration is the moisture content of the soil. Van Deuren *et al.*, (2002) report that the effectiveness of electrokinetics is greatly reduced for wastes with moisture content of less than 10% because of restricted movement through electroosmosis and electrophoresis.

1.7 Aims and objectives

The overall aim of this study was to investigate and develop electrokinetics as a process for remediating hydrocarbon contamination from logistically difficult sites. The study was devised to assess: the use of electrokinetics to mobilise the target hydrocarbon compounds within the sub-surface; bioremediation, as a process, to remediate the target hydrocarbon compounds; and finally the development of a technology that combines electrokinetics and bioremediation to remediate hydrocarbons.

In order to achieve the overall aim the following objectives were developed:

1. To investigate the feasibility of mobilising hydrocarbons from the sub-surface using electroosmosis induced by the application of electrokinetics to soil, and the measurement of parameters in the soil that may be affected by the applied electric current.

2. To determine whether surfactants can be delivered into the soil using electrokinetics to increase the mobilisation of hydrocarbons that have limited solubility in water and to investigate the efficiency of surfactant delivery using electrokinetics.

3. To assess the potential of using electrokinetics as a tool for the delivery of enhancing agents (*e.g.* nutrients, electron acceptors and chemical oxidants) to facilitate degradation of hydrocarbons in the sub-surface.

4. To undertake preliminary feasibility studies to investigate the potential use of electrokinetics to enhance biodegradation of the target hydrocarbons.

5. To undertake a controlled *ex situ* field-scale pilot trial to further investigate the potential to use electrokinetics to remediate hydrocarbons from the sub-surface. The field-scale trial was to build on the results from the bench-scale experiments undertaken to achieve objectives 1 to 4.

The main purpose of the Industrial CASE Studentship was to investigate the principles and potential of electrokinetics to be used for the remediation of hydrocarbons from the sub-surface. At both laboratory- and field-scale, work has been carried out to determine the feasibility of the technology and throughout the study there has been a keen focus on investigating the subject in a way that minimises the gap between research and application in the field, making the transition between bench-scale and field-scale experiments achievable.

2 The use of electrokinetics to mobilise hydrocarbons

2.1 Introduction

The application of a low-voltage direct current in a soil system can induce a number of physicochemical processes including electroosmosis, electromigration, electrophoresis and electrolysis (Section (§) 1.6). The main focus of the work reported in this chapter was to ascertain whether electroosmosis could be used as an *in situ* flushing technique for the mobilisation of the non-charged hydrocarbon contaminants, specifically kerosene and transformer oil, from the sub-surface. Once accessible, contaminants could then be treated *ex situ* by physical or biological treatment processes without the requirement to excavate sub-surface soils. Following the accepted use of electroosmosis in the construction industry for dewatering soils, Segall *et al.* (1980) suggested the use of electroosmosis as a flushing technique for the mobilisation of pesticide or organic contaminants from the soil.

The main prerequisite for *in situ* flushing to be effective is that the contaminant(s) are present in the aqueous phase, either as a result of solubility or with the aid of carrier agents (*e.g.* surfactants or through association with dissolved organic matter [DOM]). To an extent, water solubility (C^s) and octanol-water partition coefficients (K_{ow}) can be used to predict the fate of a contaminant in the sub-surface (Page *et al.*, 2000; Saichek & Reddy, 2005), and could be used to determine if electroosmosis would be appropriate to use. However, where contaminants exist as a mixture, predictions may have limited relevance. With regard to water solubility, there are marked differences between the physicochemical properties of the target contaminants. For example, *o*-xylene has a solubility in water of 175 mg l^{-1} whereas tetradecane has a limited solubility of 0.0022 mg l^{-1} (Howard & Meylan, 1996).

Owing to the hydrophobic nature and generally low solubility of the target contaminants used in this study, the association of contaminants with dissolved organic matter (DOM) is likely to be of importance. Principally, the contaminants may become tightly bound to the organic fraction of the soil limiting the effectiveness of electroosmosis induced *in situ* flushing. Conversely, it has been reported that the partitioning of hydrophobic contaminants on humic materials (such as humic acid and dextran) can result in increased

movement in the aqueous phase where they are associated with DOM. (Ding & Wu, 1995; Kördel *et al.,* 1997). Once in the aqueous phase the contaminants would be likely to be susceptible to *in situ* flushing through electroosmosis.

For electroosmosis to be the driving force for the removal of contaminants, the hydraulic conductivity of the soil should be less than 10^{-5} mm s^{-1} (Bruell *et al.,* 1992). If the opposing hydraulic gradient is greater than 10^{-5} mm s^{-1} the movement of contaminants towards the cathode of the circuit would be negated by water movement in the opposite direction. It is reported (Casagrande, 1983) that electroosmotic conductivity is generally between 1 to 10 x 10^{-9} $m^2(V \cdot s)^{-1}$ for a variety of soil types.

To date, a number of studies have been undertaken to investigate the use of electroosmosis as a process to remediate contaminated soils. The electroosmotic movement of phenol and acetic acid was achieved in laboratory-scale experiments (Shapiro & Probstein, 1993), and *para (p)*-nitrophenol (Ho *et al.,* 1995), TCE, benzene, toluene and m-xylene (BTX) (Bruell *et al.,* 1992; Segall & Bruell, 1992) have been investigated. Ho *et al.* (1995) showed the potential to reduce *p*-nitrophenol concentrations in the soil by mobilising the contaminant by electroosmosis into treatment zones (Lasagna process [§1.6.6]). Bruell *et al.* (1992) compared conventional contaminant-transport equations to contaminant breakthrough graphs using glass column experiments, set up to analyse the electroosmotic movement of BTX and TCE. This provides some supporting evidence that the electroosmotic removal of contaminants with high solubility and low distribution coefficients is possible. Renaud & Probstein, (1987) identified that it may be feasible to use electroosmosis to control groundwater flow in contaminated soils and landfill sites, although their conclusions were based predominantly on theoretical models.

In contrast to the work carried out by previous authors (*e.g.* Bruell *et al.,* 1992) the experimental set up within this study was aimed at identifying the possibility of mobilising hydrocarbon contaminant mixtures using electroosmosis, while maintaining the pH and moisture content throughout the soil. Reproducible 14 day experiments have been undertaken using electrokinetic soil chambers spiked with a mixture of contaminants. Many electrokinetics studies cited in the literature have shorter run times of 4 days (*e.g.* Reddy & Parupudi, 1997). Adsorption and desorption studies were also performed to ascertain the likely behaviour of the contaminant mixture in a soil system.

2.2 Chapter objectives

The objectives of the investigations carried out in this chapter were to determine whether electroosmosis could be used to mobilise hydrocarbon contaminants from the soil (§ 1.7, Objective 1) (Figure 2.1). The target contaminants chosen for investigation included dodecane, xylene (*para*, *meta*, *ortho*), tetradecane, toluene, 3-chlorobiphenyl (3-CB), and octadecylbenzene (§ 1.2.1.1). Experiments were first performed to investigate the desorption and sorption characteristics of the target contaminants and to identify whether they partitioned to the aqueous phase after being spiked into the soil. The second set of experiments were designed and carried out to determine if contaminants in the aqueous phase could be mobilised through a soil system under the influence of an electric field. Prior to the investigative work it was also necessary to optimise the electrokinetics apparatus to be used and also develop methods to achieve more reproducible results.

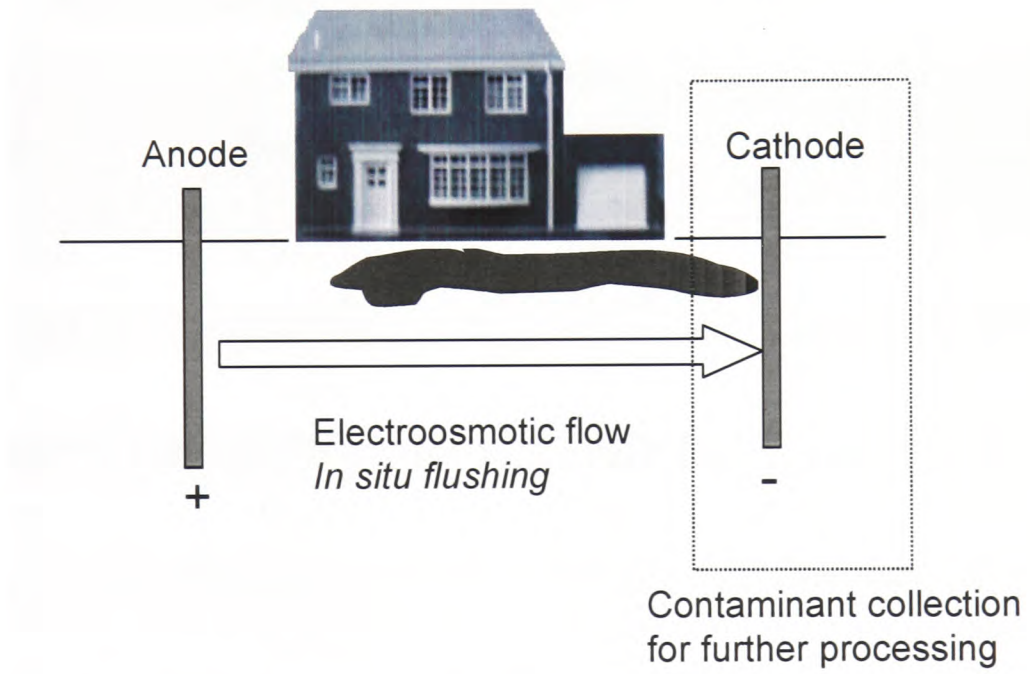


Figure 2.1 **Objective 1**. The schematic depicts the use of electrokinetics as an *in situ* flushing technique for the remediation hydrocarbon contaminants from the sub-surface.

2.3 Materials and methods

Unless otherwise stated, all chemicals were purchased from Sigma Aldrich, UK. Mains water, described in several of the experiments, was supplied by Thames Water, Oxfordshire. Where methods are described in this chapter and are referred to in proceeding chapters 'general method' is included in the title (*e.g.* § 2.3.1).

2.3.1 Target contaminants (general method)

The principal target contaminants for the study were combined in a mixture containing 47% dodecane, 37% xylene and 16% tetradecane (DXT mix). In several experiments *e.g.* mobilisation of the target hydrocarbons under an hydraulic gradient (§ 2.3.3.2), the DXT

mix was used as the representative hydrocarbon contaminant mixture. The concentration of contaminants chosen in the experiments was based on the concentrations detailed for a typical sub-surface plume (TPHCWGS, 1998), a concentration that is likely to have saturated the soil. The emphasis on the presence of each of the xylene isomers at contaminated sites varies considerably throughout the literature (*e.g.* TPHCWGS, 1998; Franzmann *et al.*, 2002) so a laboratory standard solution of xylene was used, consisting of *p*- (21.4%), *m*- (62.7%), and *o*- (15.9%) isomers. Unless explicitly stated otherwise, whenever 'xylene' appears in the text it will refer to this mixture of its structural isomers. Octadecylbenzene and 3-CB were studied in several of the electrokinetics experiments because of their relevance to the field study (Chapter 5). Toluene was also investigated as an example of a water-soluble aromatic hydrocarbon. The physical characteristics of the target contaminants are summarised in Table 2.1.

Table 2.1 Physical characteristics of the target contaminants.

Compound	Formula	Molecular Weight (MW) (g mol^{-1})	Solubility (C^s) (mg l^{-1})	Vapour pressure (mm Hg @ 25°C)	Log K_{ow}	Density g ml^{-1}
3-Chlorobenzoate	$ClC_6H_4CO_2H$	156.6	450	0.00233	2.68	1.227
3-Chlorobiphenyl	$C_{12}H_7Cl_3$	257.5	1.3	0.00735	5.62	1.370
Dodecane	$C_{12}H_{26}$	170.0	0.0037	0.12	7.24	0.749
Octadecylbenzene*	$C_{24}H_{42}$,$C_6H_5(CH_2)_{12}CH_3$	330.59	/	4.95	/	0.857
Tetradecane	$C_{14}H_{30}$	198.0	0.0022	0.0286	7.20	0.763
Toluene	C_7H_8	92.1	530	22	2.69	0.867
p- xylene	C_8H_{10}	106.2	156	8.6	3.15	0.861
m-xylene	C_8H_{10}	106.2	161	8.3	3.20	0.897
o-xylene	C_8H_{10}	106.2	175	5.2	2.77	0.868

For chemical structures refer to Appendix 8.3.
References: Prager, (1995); *European Chemicals Bureau, (2000).
K_{OW}: Octanol-water partition coefficient

2.3.2 Soil preparation (general method)

Two uncontaminated soils were used in the study, Reading (supplied by the Department of Soil Science, University of Reading) and Wytham (supplied from the Oxford University field station at Wytham). Both soils were obtained from a depth of between 200 and 500 mm. Prior to use, soils were air-dried and hand sieved through a 2 mm aperture. Drying of the soil facilitated the homogenous packing of the electrokinetic soil chambers and soil columns. Owing to the high clay content of Wytham soil, preparation and

sampling was labour intensive and inefficient, consequently in some experiments only Reading soil was used as a model soil.

1. Reading soil is a sandy loam with (w/w) 73% sand, 12% silt and 14% clay (Bending *et al.*, 2004), pH_{H2O} (pH of soil determined in a 5:1, water to soil slurry) 7.52 ± 0.06. The water holding capacity (WHC) of the soil was calculated as 17.0 ± 1.4 % w/w (data point is the mean ± standard error (SE); n = 3) using standard methods (BS1377: 1990). The water holding capacity was determined by adding 10 g dry weight (d/w) of soil to a pre-weighed wet filter paper cone and placed in a beaker of water for 12 h. The soil-filled cone was then allowed to drain in a humid environment for a further 12 h before weighing to assess the change in weight due to water uptake; the procedure was repeated in triplicate.

2. Wytham soil is an Evesham Series heavy silty clay with (w/w) 25% sand, 22% silt and 53% clay and ~ 8.5% organic content (Lilley *et al.*, 1996; Lear *et al.*, 2004), pH_{H2O} 7.56 ± 0.03. WHC of the soil was calculated as 66.3 ± 2.3 % (data point is the mean ± SE; n = 3) using standards methods, as described above.

Table 2.2 Concentration of selected inorganic ions in the model soils (the method of analysis is described in § 2.3.6.4).

	Concentration of inorganic ions (mg kg^{-1})			
	NO_3^-	SO_4^{2-}	Cl^-	PO_4^{3-}
Reading soil	66.3 ± 2.2	37.3 ± 2.1	28.1 ± 1.4	4.5 ± 4.4
Wytham soil	115.4 ± 16.6	10.0 ± 5.1	20.5 ± 3.2	0.0 ± 0.0

2.3.2.1 Sterilisation of the soil

Soil was sterilised by autoclaving (121°C, 15 psi, for 15 min) three times in a 48 h period. Inoculations of the soil onto plate count agar (PCA) were used to verify the sterility of the soil in triplicate using a dilution series.

2.3.3 Feasibility studies for the electroosmotic mobilisation of the target contaminants

A series of experiments were undertaken to identify the feasibility of using electroosmosis to flush the target contaminants through the soil after being 'spiked' into the soil. In the principal electrokinetic chamber experiments (described in § 2.3.7.1) the approximate concentration of the DXT contaminant mix was 11 000, 9 100 and 4 000 mg kg^{-1},

respectively. Consequently, this concentration of DXT contaminant mix was used in the feasibility studies. Toluene was investigated at a concentration of ~9 000 mg kg^{-1}.

2.3.3.1 Partitioning of the target hydrocarbons from the soil to the aqueous phase

Soil, 1 g (d/w) (data normalised to 3 decimal places [d.p.]), weighed into 7-ml glass vials was sterilised (§ 2.3.2.1) and then directly spiked with either a mix containing 15 µl dodecane, 10 µl xylene and 5 µl tetradecane or was spiked with 10 µl toluene. 2.5 ml filtered (< 0.2 µm) mains water was added to the spiked soil before the vials were sealed with PTFE-lined (polytetrafluoroethylene) lids. Samples (in triplicate) were placed on a laboratory rotor wheel and shaken for < 0.05, 6, 12, 18, 24 or 48 h at ambient temperature (~20°C) before being destructively sampled.

The aqueous phase was separated from the soil by centrifugation at 2060 x *g* (*g* = acceleration due to gravity, ≈ 9.8 ms^{-2}) using a Beckman J2-HC centrifuge with a JS4.3 rotor (Buckinghamshire, UK). The supernatant was transferred and weighed into clean 7-ml glass vials, extracted with hexane on a rotor wheel (designed and constructed in-house) for 24 h, and then dried over anhydrous sodium sulphate (Na_2SO_4), before analysis using gas chromatography – flame ionisation detection (GC-FID) (§ 2.3.6.3). To calculate a mass balance between the contaminants present in the aqueous phase and those present in the soil phase after incubation for 6 h, and to assess extraction efficiency, the supernatant and soil were separated and extracted using hexane and analysed using GC-FID (§ 2.3.6.3).

2.3.3.2 Mobilisation of the target hydrocarbons through the soil under an hydraulic gradient

The model soils were homogenously spiked with the dodecane, xylene and tetradecane mix at concentrations of 11 000, 9 100 and 4 000 mg kg^{-1}, respectively. Soil columns (in triplicate) were prepared by compacting 16.00 g (d/w) spiked model soil into 25-ml glass syringes (Fischer Scientific, Loughborough, UK). The spiked soil was added to the glass syringes in 20 mm intervals, rewetted with filtered (< 0.2 µm) mains water (3.8 ml in total for 16.00 g of the model soil; one pore volume of the soil column) and then compressed using PTFE tubing before the next layer of soil was added, up to the 10 ml graduation of the syringe. At time point zero, 3.8 ml of filtered mains water were added and the breakthrough solution (eluent) was collected. This was repeated every 24 h for 14 days. Between each addition of mains water the syringe tops were covered with a thin Teflon

sheet to prevent drying of the soil and reduce the loss of contaminants through volatilisation. The eluent was extracted with hexane for 24 h on the rotor shaker, dried over anhydrous Na_2SO_4 and analysed using GC-FID (§ 2.3.6.3). Additional columns were prepared that were treated in the same way but destructively sampled on either day 3, 7 or 14 to calculate a mass balance between the concentration of contaminants in both the aqueous and soil phase.

2.3.3.3 Partitioning of the target hydrocarbons from the aqueous to the soil phase

Feasibility studies were carried out to investigate the likely outcome of the target hydrocarbons being mobilised from the saturated 'spiked' soil region into 'clean' soil. Vial studies were used to identify uptake of the contaminants from the aqueous phase into the soil phase. Soil, 1 g d/w (data normalised to 3 d.p.), was weighed into 7-ml glass vials and sterilised (§ 2.3.2.1). The vials were then spiked with either a mix of 15 µl dodecane, 10 µl xylene and 5 µl tetradecane (DXT mix) or 10 µl toluene suspended in 2.5 ml filtered (< 0.2 µm) mains water, and the vials were sealed with PTFE-lined lids. Samples (in triplicate) were placed on a laboratory rotor wheel for < 0.05, 6, 12, 18, 24 or 48 h at ambient temperature (~20°C), before being destructively sampled (the entire soil sample was extracted).

To separate the aqueous phase from the soil, the samples were centrifuged at 2060 x *g*. The supernatant was transferred and weighed into clean 7-ml glass vials, extracted with hexane on a rotor wheel for 24 h, and then dried over anhydrous Na_2SO_4, before analysis using GC-FID (§ 2.3.6.3). At time point 6 h, after the supernatant from the samples was removed, the soil was also extracted using hexane and analysed using GC-FID in order to calculate a mass balance and assess the efficiency of the extraction technique and any loss of spiked contaminant from the system.

To investigate whether there were any differences in partitioning of the hydrocarbon contaminants in non-sterilised soil and autoclaved soil because of alterations in sorption capacity of sterilised soil, a second experiment was carried out using identical methodology except that the soil was not autoclaved prior to its use in the experiments. To calculate a mass balance both the aqueous and soil phases were extracted separately for each time point.

2.3.4 Determination of electroosmotic flow

In the literature it is frequently reported that electroosmosis is measured by monitoring a change in the volume of electrolytes (*e.g.* Probstein & Hicks, 1993). Since the experiments in this study were performed in a laboratory extraction fume hood, evaporation meant that the electroosmotic flow rate could not be calculated by simply measuring a change in electrode reservoir volume. An alternative method (detailed by Wick *et al.*, 2004), that used a solution of phenolphthalein (0.5% in 50% aqueous ethanol) as a marker compound in the aqueous phase, was investigated. However, extracting the dye from the pore space of clay-rich soil proved problematic and therefore was not used further.

Apparatus was therefore developed to determine the electroosmotic flow rate of a given soil sample with greater accuracy. The apparatus was designed based on equipment similar to that described by Reuss (1809). A glass U-tube (Figure 2.2) was constructed in which a column of soil, separated with a permeable membrane, was placed between two electrodes, stainless steel cathode and carbon anode (carbon). 60 V (~10 mA) was applied between the two electrodes using an Isotech IPS 601A power pack (RS Components, Leicester, UK, 0-1 A) over a soil surface area of 1.8 cm^2, total area of soil was 18 cm^3 (23 g d/w Wytham soil and 26 g d/w Reading soil) for 24 h. The electroosmotic head was measured using a graduated pipette to replace displaced water in the anode side of the apparatus to the starting volume, which was marked on the two sides of the U-tube.

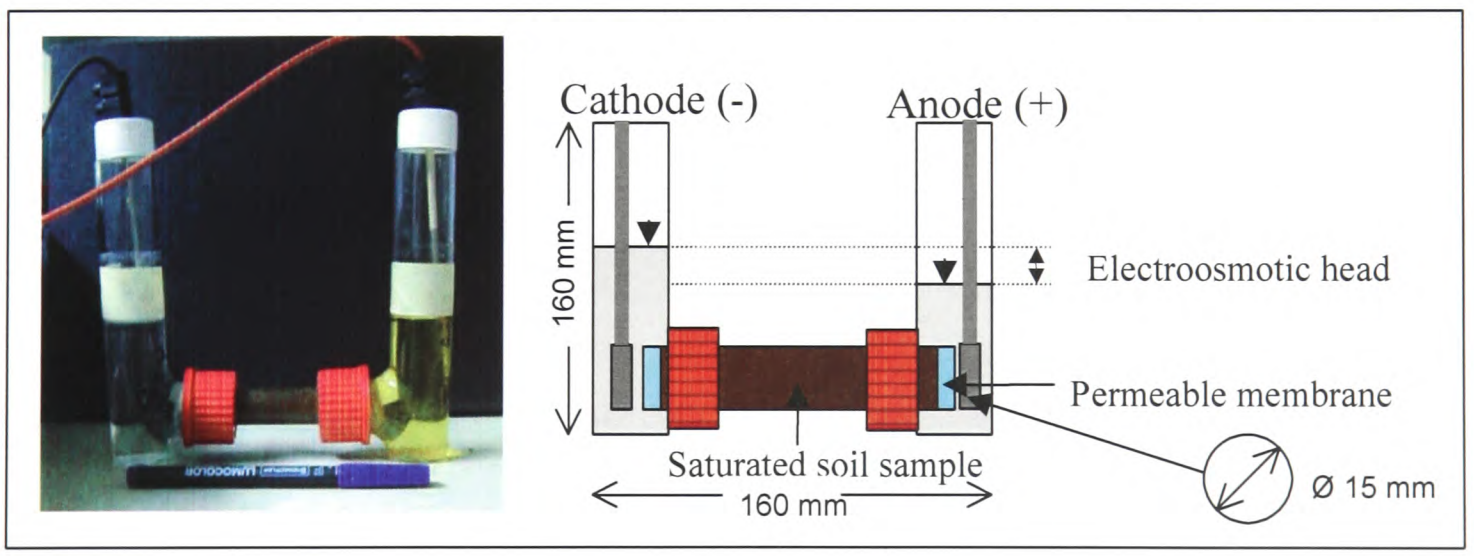


Figure 2.2 Photograph and schematic of the equipment used to determine the electroosmotic flow rate in a soil sample. The yellow discolouration of the electrolyte is likely to be chromium, a corrosion product of the stainless steel cathode that has migrated to the anolyte (Sunderland, 1987a).

2.3.5 Electrokinetics equipment (general method)

2.3.5.1 Chamber setup

The electrokinetics equipment, adapted from Jackman *et al.* (2001) consisted of a plastic trough (internal dimensions 255 mm x 85 mm x 64 mm) with two terminal electrodes (Figure 2.3). Electrode chambers were housed in PVC (polyvinyl chloride) with a porous Daramic front (Daramic, Industrial profile 707, supplied by Grace GmbH, Sélestat, Germany). Daramic is a colloidal silica loaded polythene with pores of approximately 0.1 μm (used as a membrane separator in batteries). The silica is hydrophilic, which facilitates the required electrical conductivity.

A constant voltage (60 V) was supplied using an Isotech IPS601A power pack, passing a direct current between a stainless steel cathode and an inert carbon anode; non-inert metallic electrodes may break down through electrolysis introducing corrosive products into the soil (Figure 2.2). In pilot studies the anode chamber was filled with approximately 2 mm carbon graphite chips (derived from petroleum coke). However, it was observed that the graphite chips rapidly degraded when an electric field was applied, with the smaller fragments blocking the peristaltic tubing, used to circulate the electrolyte, so their use was discontinued. Instead, solid carbon strips were used as the anode in the form of three strips of carbon held horizontally by a vertical strip (see Appendix 8.4). On application of a constant voltage of 60 V a current of approximately 10 mA was observed across the soil chamber. The electrolyte was mains water, which contained sufficient ions for conductivity and was economically feasible for use in the field-scale study (Chapter 5).

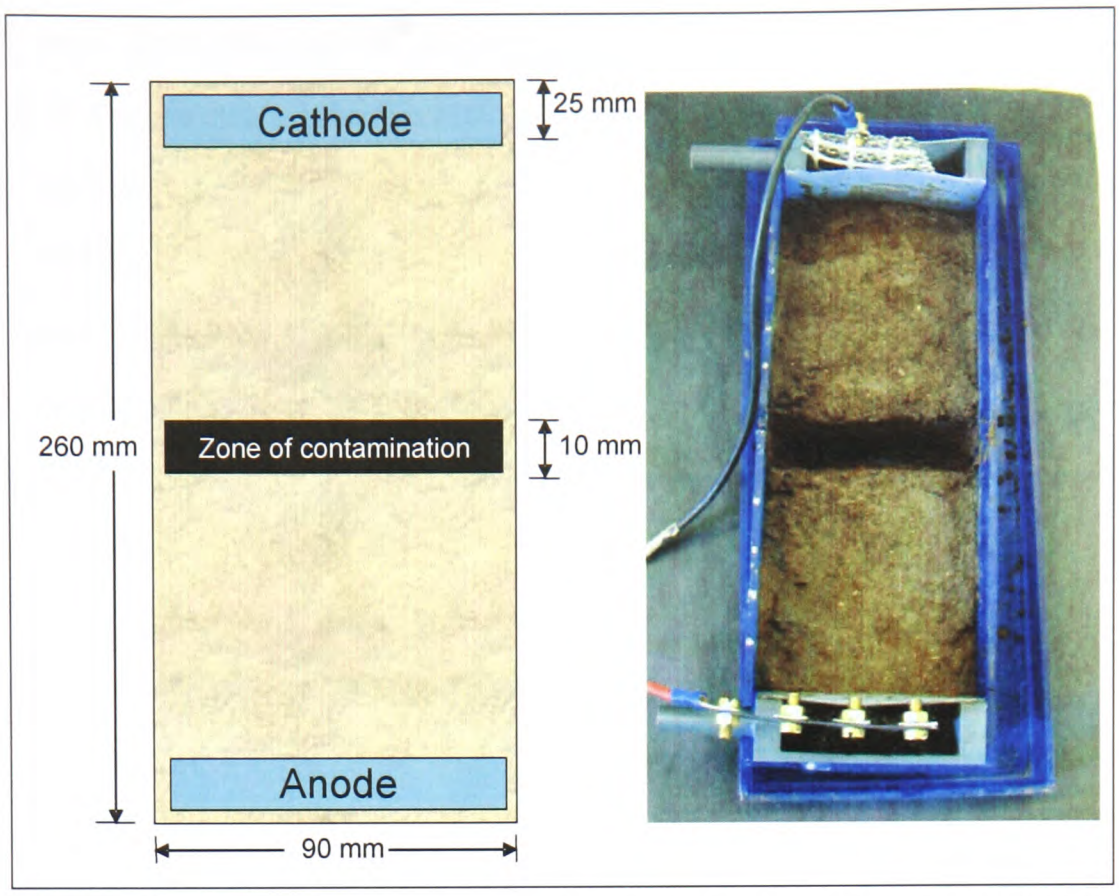


Figure 2.3 Photograph and schematic showing zone of spiking contaminants into the electrokinetics soil chamber. The chamber consisted of a plastic trough (internal dimensions 255 mm x 85 mm x 64 mm [length x width x depth]) with two terminal electrodes.

2.3.5.2 Spiking and packing of soil into electrokinetics chamber

Soil, 1 100 g d/w, was packed into the chamber in 1 cm layers, which were rewetted to 70% of the soil's WHC (§ 2.3.2), with mains water, before subsequent layers were compacted on top. Unsaturated soil was used in the experiments to ensure oxygen could penetrate the soil system allowing aerobic biodegradation to take place.

A 1 cm wide central portion was removed from the chamber (~100 g) and homogenously spiked in a glass beaker with the target contaminant(s) before being repacked into the soil chamber (Figure 2.3). Care was taken to avoid cross contamination of the soil chamber by covering the remaining two sections with tin foil while repacking was carried out. To reduce possible interferences from the PVC chamber (*e.g.* adsorption of the contaminant) the trough was lined with a 0.25 mm thick PTFE sheet (Adtech Polymer Engineering, Gloucester, UK). In addition, a PTFE strip was also placed across the upper surface of the spiked zone to limit volatilisation and disturbance of the region during temperature and voltage readings.

2.3.5.3 pH control of the electrolyte

The electrolyte of each electrode chamber was circulated into separate reservoirs using an overflow weir, which was actively pumped into a glass reservoir using peristaltic pumps with Norprene tubing (06404-16) (Masterflex Console Drive, Cole-Palmer Instruments,

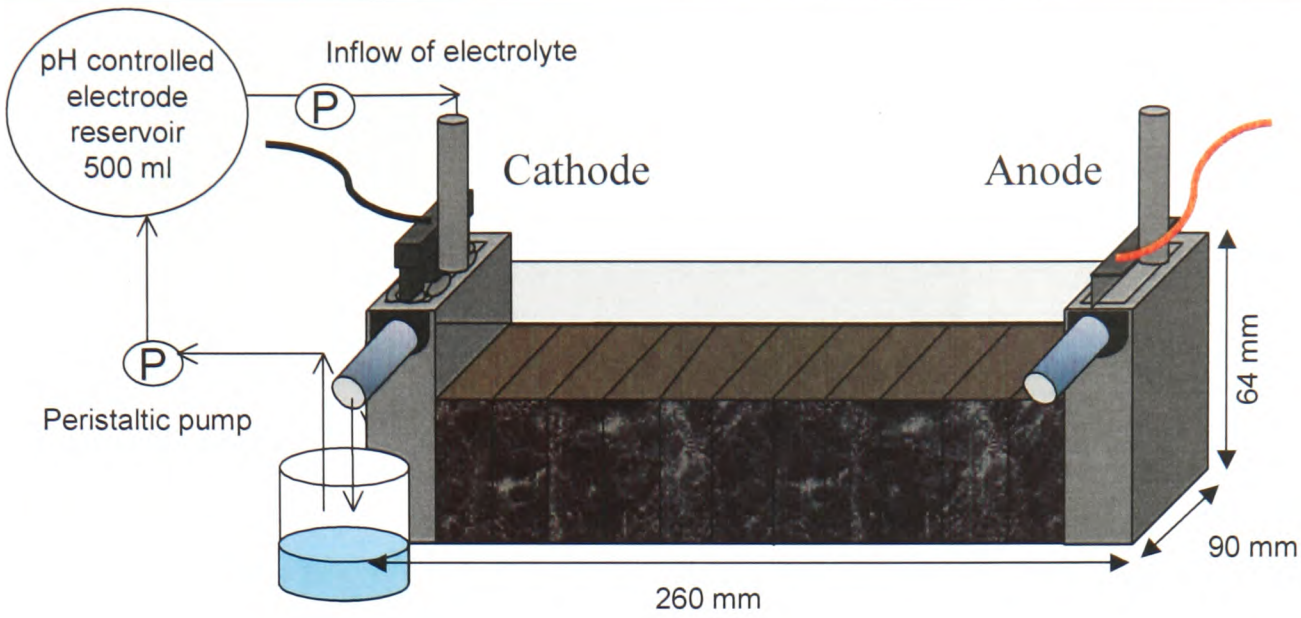
London, UK) at a flow rate of 48 ml min^{-1}. The pH was adjusted in the collection reservoirs to 6 at the cathode and 7 at the anode with 0.1 M sulphuric acid (H_2SO_4) and 0.1 M sodium hydroxide (NaOH), respectively (Figure 2.4). Hydrochloric acid (HCl) was not used as a buffer solution because of the possibility of producing chlorine gas (Cl_2) through oxidation-reduction reactions at the electrodes. The pH was adjusted automatically using a Gelplas pH probe (BDH, Ltd., Poole, UK) connected to a pH monitor (3305, Jenway Ltd., Felsted, UK). Daramic, used in the front of the electrode chamber, is sensitive to alkali and above pH 8 the hydrophilic silica is liable to leach out.

2.3.5.4 Monitoring of voltage and temperature in the soil chamber

Ten voltage readings at a soil depth of 10 mm, evenly spaced along the length of the electrokinetics soil chamber, were measured at 24 h intervals using a handheld Wavetek Meterman (Fisher Scientific, Loughborough, UK). The temperature was measured at the anode end, cathode end and at the centre of the chamber using a digital thermometer (Fisher Scientific, Loughborough, UK) also at 24 h intervals.

2.3.5.5 Sampling of the soil chambers after termination of the experiment

At the end of an experimental run, the electrokinetics soil chamber was disconnected from the peristaltic pumps and power supply. The soil was then divided into eleven equally sized cross sections (~19 mm width) of approximately 100 g (Figure 2.4). Each cross section was placed into a separate glass beaker and homogenised using a stainless steel spatula, before being weighed and divided for moisture content, pH, anion and hydrocarbon analysis. In all instances soil samples were immediately stored at –80°C if they could not be analysed immediately. Destructively sampling at the end of the experiment reduced the possibility of cross contamination and limited the amount of disruption to the structure of the soil within the chamber during the experiments. The electrolyte solution was also analysed for the target contaminants. In triplicate 1 ml of electrolyte was extracted with hexane and analysed using the general method (§ 2.3.6.3 GC-FID).



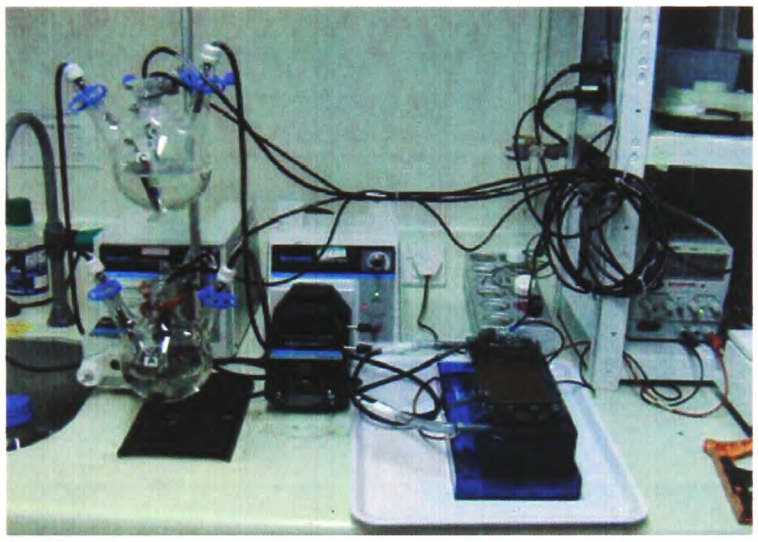


Figure 2.4 Schematic and photograph of the electrokinetics apparatus. Both the anode and the cathode electrodes have the same reservoir arrangement as depicted for the cathode chamber in the illustration. The soil chamber was divided into eleven equal slices for analysis, as illustrated.

2.3.5.6 Sampling of the soil chamber before termination of the experiment

Where it was necessary to sample the soil chamber prior to the completion of the experiment, soil was sampled using metal tubing, (9.5 mm Ø, Oxford Instruments, UK) as a soil corer. The soil chamber was divided into sections (corresponding to the number of time-points for the experiment) across the width of the chamber and for each time point 11 soil cores were collected along the length of the soil chamber (Figure 2.5). Soil cores of approximately 5 g were extracted, mixed in a foil weighing boat (Cole-Palmer, UK) to homogenise and then analysed for anion and hydrocarbon content.

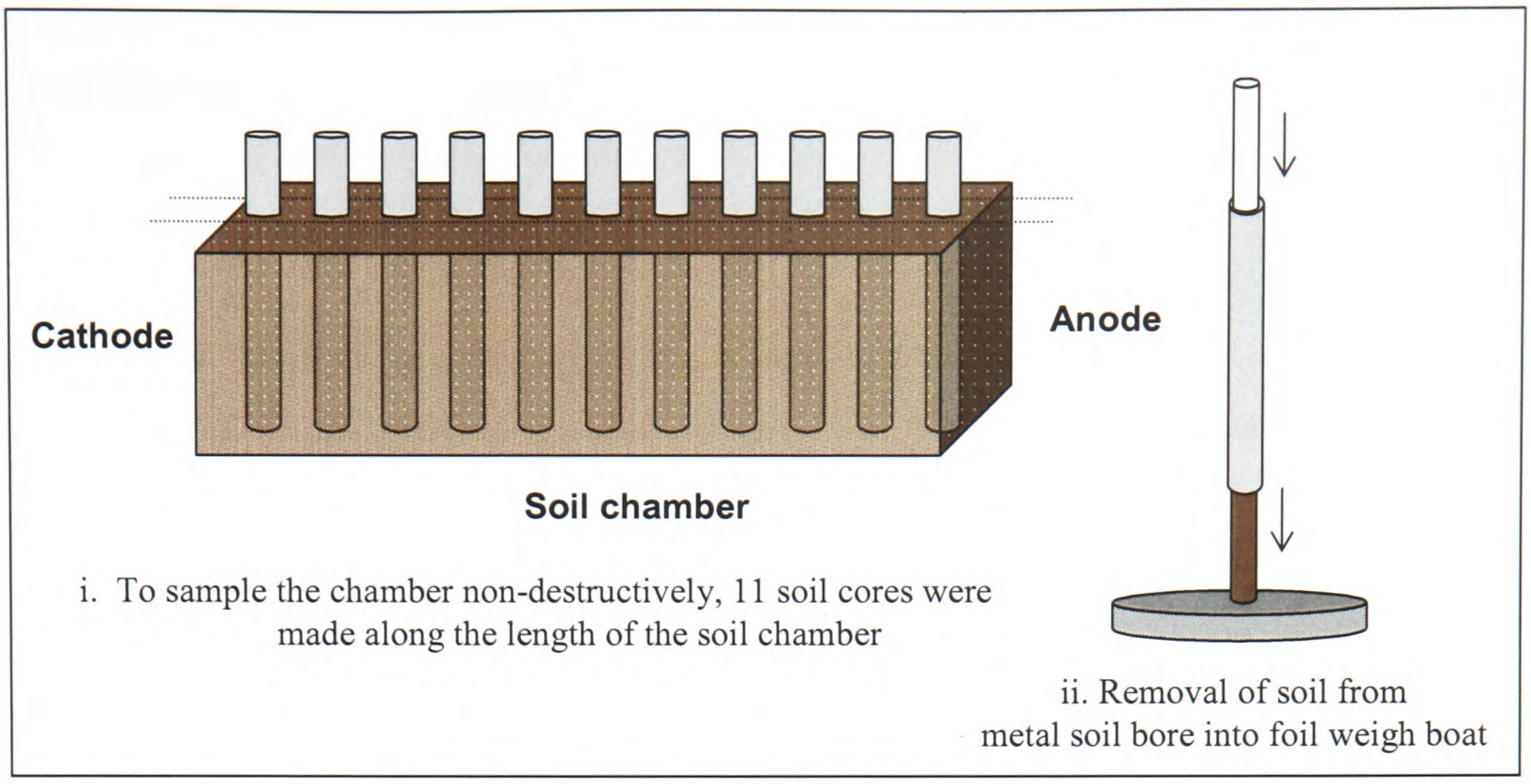


Figure 2.5 Schematic of the electrokinetics apparatus to illustrate sampling prior to termination of the experiment. Soil was sampled along the length of the soil chamber using metal tubing. Approximately 5 g of soil was extracted in this way and homogenised in a foil weigh boat.

2.3.6 Analytical methods (general methods)

2.3.6.1 Moisture content of soils

The moisture content of soil was determined by drying pre-weighed soil at 105°C for 24 h in foil weighing boats and assessing the difference in weight.

2.3.6.2 Determination of the soil pH

The pH_{H2O} throughout the chamber was measured using standard methods. 1 g of soil was suspended in 10 ml of mains water by vortexing on a bench-top vortex (10 s at 3 000 rpm) and then the pH was analysed using a pH Meter (3305 Jenway, Felsted, UK). The standard error using the described method and apparatus was calculated as ± 0.03.

2.3.6.3 GC-FID – analysis of hydrocarbons extracted from soil

Hydrocarbons were extracted from the soil using hexane and 3% sodium dodecyl sulphate (SDS), which was rotated for 24 h on an upright rotor. Heptamethylnonane (HMN) was used as an internal standard within the hexane, at a concentration of 100 $\mu l\ l^{-1}$. HMN was chosen because of its close retention time to dodecane compared with other internal standards tested (*e.g.* biphenyl). Analysis of hydrocarbons was performed using GC-FID. The carrier gas was helium (He), column DB5-ms (Agilent, Cheshire, UK) (30 m long; internal diameter 250 μm), and temperature programme as follows: 65°C for 1 min, rising

by 10°C min^{-1} until 105°C, holding for 45 s, then rising 30°C min^{-1} until 220°C, holding for 1 min. 1 µl of the extracted sample was injected onto the column. Splitless injection at 50 ml min^{-1} occurred at 0.5 min.

2.3.6.4 Ion chromatography – analysis of anions from soil

Soil samples of 1 g (samples weighed and normalised to 3 d.p.), were placed into 2-ml plastic microcentrifuge tubes, and 1.5 ml of deionised water (dH_2O) was added. The soil slurries were then mixed using a bench-top whirlimixer (Fisher Scientific, Loughborough, UK) for 30 s before being centrifuged using a Labnet Microcentrifuge (Fisher Scientific, Loughborough, UK) for 5 min at 7200 x *g*. The supernatant was then decanted into clean microcentrifuge tubes and centrifuged in the same way before being analysed by Ion Chromatography (IC). IC was carried out using a Metrohm 733 IC separation Centre and a Metrohm Anion Dual 2 column (6.1006.100) (Buckinghamshire, UK), with a mobile phase consisting of 1.3 mM Na_2CO_3, 2.0 mM $NaHCO_3$, and 5% (v/v) acetone, degassed for 10 min with He prior to use. The flow rate was 0.8 ml min^{-1}. Samples were analysed for chloride (Cl^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}) and nitrate (NO_3^-).

2.3.6.5 Analysis of metal distribution through the electrokinetics soil chamber

Soil collected from the electrokinetics soil chamber after 14 days of applying 60 V (~10 mA) was analysed for the concentration (mg kg^{-1}) of calcium (Ca^{2+}), potassium (K^+) and iron ($Fe^{2+/3+}$). The analysis was carried out using inductively-coupled plasma mass spectrometry (ICP-MS) by I_2 Middlesex, UK (private laboratory).

2.3.7 Movement of hydrocarbons through electroosmosis

2.3.7.1 DXT spiked into the central region of the soil chamber

The electrokinetics soil chamber was spiked (§ 2.3.6.2) with a 3000 µl contaminant mixture containing dodecane, xylene, and tetradecane, at concentrations of ~11 000, 9 100 and 4 000 mg kg^{-1}, respectively. 60 V was applied to the chamber for 14 days before sampling the chamber (§ 2.3.5.5). A control chamber was set up in exactly the same way except that a current was not applied. It was found, by performing a seven day pilot study, that as there was no movement of water into the control soil chamber (no electroosmosis) in the fume hood the soil dried out. To remedy this, in all experiments a light mist of water was applied to the surface of the soil chamber (both electrokinetic and control) twice during a 24 h period.

After 14 days xylene concentrations were significantly decreased in the electrokinetics chamber. A follow-up study was therefore conducted. 13 000 mg kg^{-1} of xylene was spiked into the electrokinetics chamber and sampling carried out at time points of < 0.05, 6, 12, 24, 36, and 48 h, using the sampling method described in section 2.3.5.6. A control chamber, without current, was treated and sampled in the same way.

2.3.7.2 The influence of volatilisation on the loss of xylene from the electrokinetic soil chamber

A series of experiments, using both the electrokinetics and control chambers, were set up in a sealed perspex box (designed and constructed to prevent loss of gases from the headspace) and air sample tubes, containing coconut shell charcoal (Anasorb® CSC, SKC Ltd. Dorset, UK), were used to sample the headspace surrounding the chambers to determine whether electrokinetics increased the volatilisation of xylene. Air was pumped into the chamber using a standard 4-psi aquarium pump (Rolf Hagen Ltd., Yorkshire, UK) and the air outlet pipe was filtered using the sorbent air sample tubes, which were replaced at 24 h intervals. The air sample tubes were analysed by Scientific Analysis Laboratories Ltd., Manchester, UK (Private Laboratory) for xylene using GC-FID.

2.3.7.3 DXT spiked homogenously through the soil chamber

The electrokinetics soil chamber (with 1 100 g Reading soil) was homogenously spiked with dodecane (3 405 mg kg^{-1}), xylene (2 679 mg kg^{-1}) and tetradecane (1 272 mg kg^{-1}) to determine if movement and/or enhanced degradation could be observed more effectively if the entire chamber was spiked rather than the central region alone.

2.3.7.4 Toluene mobilisation on application of electrokinetics

Toluene was spiked into the electrokinetics chamber at a concentration of 8 670 mg kg^{-1}, and mobility through electroosmosis was investigated (§ 2.3.6). 60 V (~10 mA) was applied for 14 days. The soil chambers (both control and electrokinetics) were sampled after 48 h and 14 days. Toluene was investigated as an example of a BTEX compound with a significantly higher solubility in water (530 mg l^{-1}) than the other target contaminants to determine whether low solubility had limited the movement of DXT through the electrokinetics chamber.

2.3.7.5 Movement of 3-chlorobiphenyl

3-Chlorobiphenyl (3-CB) was spiked into the electrokinetics chamber, at a concentration of 100 mg kg^{-1}, and mobility through electroosmosis was investigated (§ 2.3.6). Acetone was used as a carrier solvent to spike the soil at a concentration of 100 mg 3-CB ml^{-1} acetone. 60 V (~10 mA) was applied for 14 days, after which the electrokinetics chamber was sampled using the standard method (§ 2.3.5.5).

2.3.7.6 Movement of octadecylbenzene

Mobility of octadecylbenzene through electroosmosis was investigated. Acetone was used as a carrier solvent to spike octadecylbenzene (~1 000 mg kg^{-1}) into the electrokinetics chamber containing Reading soil (§ 2.3.6.2). 60 V (~10 mA) was applied for 14 days and the electrokinetics chamber was sampled after 14 days using the general method (§ 2.3.5.5).

The necessity for the use of a carrier solvent to facilitate the spiking of soil with either 3-CB or octadecylbenzene studies creates some variables and possible interferences when trying to interpret the data. Prior to reinstating the central spiked region into the soil chamber the acetone was allowed to vent in the fume hood for approximately 3 hours; however, the effect of remaining acetone on the adsorption and biodegradation of octadecylbenzene and 3-CB in the soil could not be investigated within the scope of this study. For the experiments it was assumed that after a 24 h period sufficient airflow and the movement of pore water into, and from, the spiked region would remove any excess acetone from the soil chamber and therefore possible interferences would be minimal.

2.3.8 Statistical analyses

Where applicable, data obtained from the electrokinetic chamber were compared to the control data using unpaired t-tests (two-sample, equal variance), and SE was calculated for all replicates using Microsoft Excel. Data were considered to be significantly different (*i.e.* the null hypothesis (H_0) was rejected) if $p \leq 0.05$, where p = the probability of H_0 being true; that there are no differences between the means of two sets of data.

2.4 Results

2.4.1 Feasibility studies for the electroosmotic mobilisation of the target contaminants

Vial (§ 2.3.3.1) and column (§ 2.3.3.2) studies were used to determine the likelihood of the target contaminants entering the aqueous phase from the soil phase, thus determining the likelihood of electroosmosis being a suitable remediation technique. As a result of the clay-rich nature of Wytham soil it was not possible to gravity feed water through the compacted soil column so consequently only Reading soil was used in the soil column experiments.

2.4.1.1 Transfer of the target hydrocarbons from the soil to the aqueous phase

The data plotted in Figures 2.6 and 2.7 (note x-axis is broken at 0.07 h) demonstrate the potential for the target DXT contaminant mix to enter the aqueous phase after being spiked onto the non-sterilised soil in the vial studies (§ 2.3.3.1). It was also observed that toluene partitioned into the aqueous phase from the soil phase (data summarised in Table 2.3).

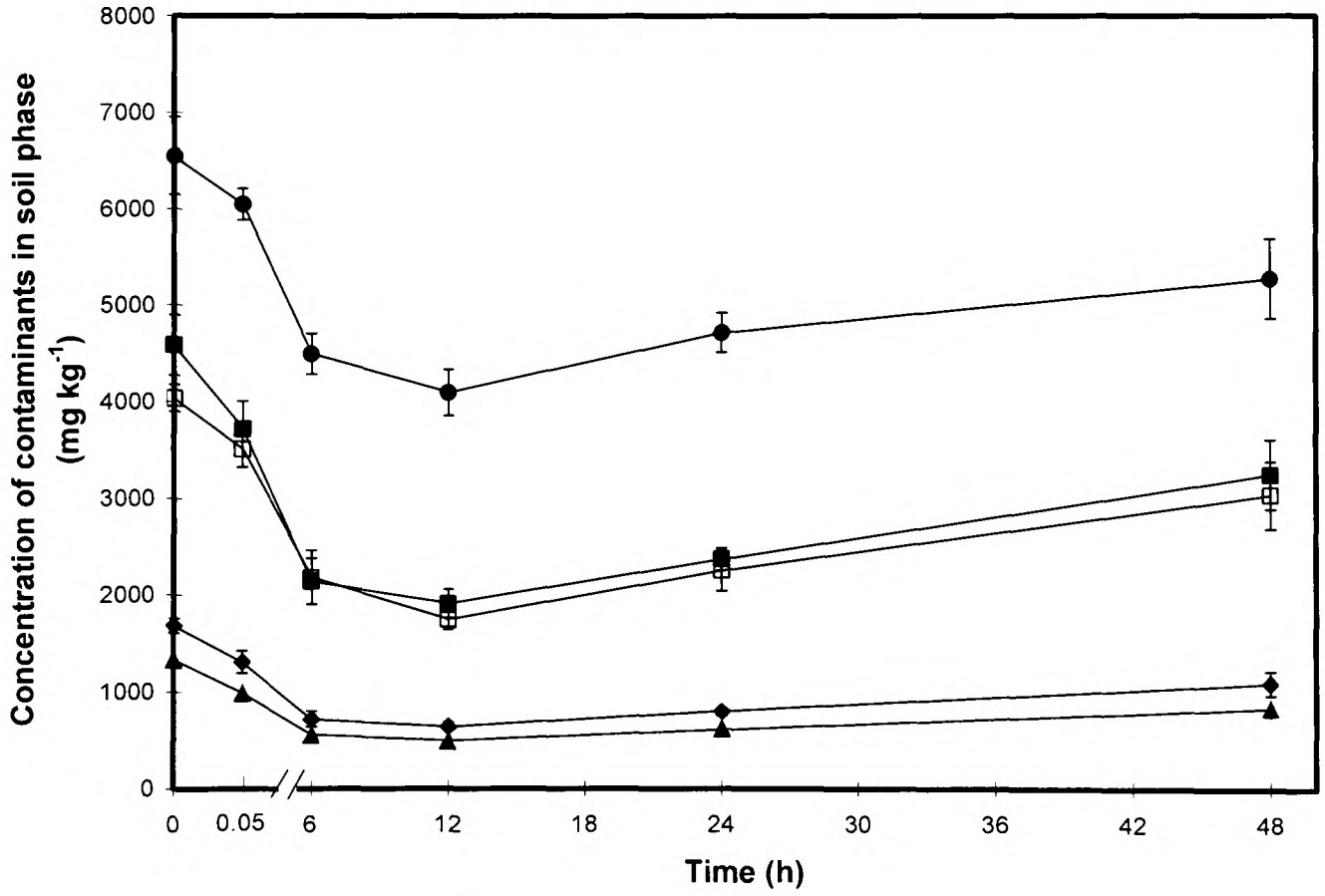


Figure 2.6 Partitioning of DXT contaminant mix from the soil into the aqueous phase. Concentration of DXT contaminant mix remaining in sterilised Reading soil (1 g) over 48 h. ● dodecane (11 000 mg kg^{-1}), ◆ *p*-xylene(1 900 mg kg^{-1}), ■ *m*-xylene (5 700 mg kg^{-1}), ▲ *o*-xylene (1 500 mg kg^{-1}), □ tetradecane (4 000 mg kg^{-1}). Data are means ± SE, n = 3, note the x-axis is broken at 0.07 h. (Concentrations in brackets are the approximate theoretical start concentrations).

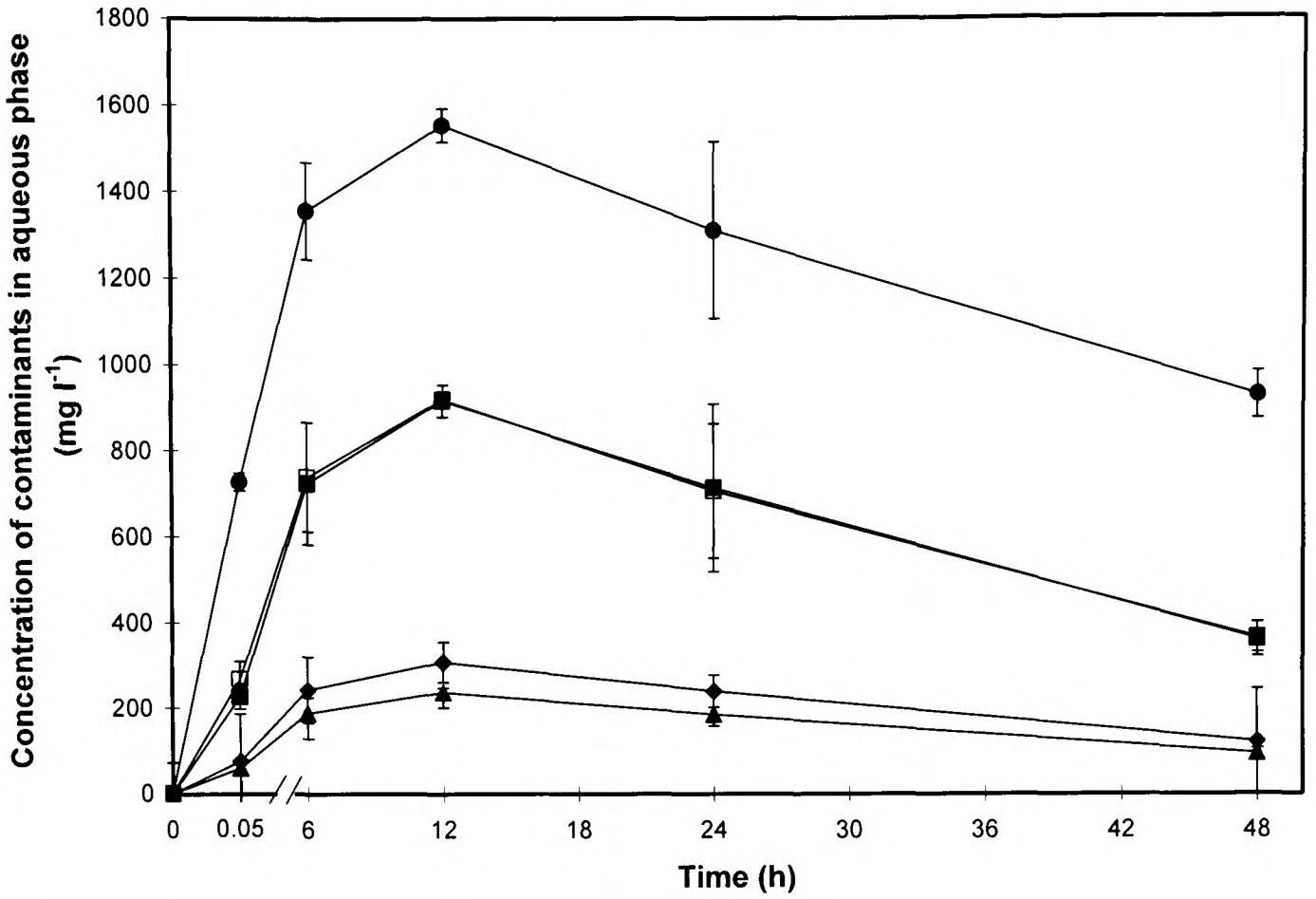


Figure 2.7 Partitioning of DXT contaminant mix from the soil into the aqueous phase. Concentration of DXT contaminant mix entering aqueous phase from sterilised Reading soil (compare with Figure 2.7). ● Dodecane, ◆ *p*-xylene, ■ *m*-xylene, ▲ *o*-xylene, and □ tetradecane. Data are means ± SE, n = 3, note the x-axis is broken at 0.07 h.

Table 2.3 Mass balance calculations for Reading soil with toluene at time point 24 h (data are means ± SE, n = 3). Toluene was spiked into the soil at the start of the experiment.

Approximate theoretical start concentration in soil phase	Contaminant in Soil phase	Contaminant in Aqueous phase	Mass balance
9 000 mg kg^{-1}	28.2 ± 10.9 %	18.7 ± 3.8 %	46.8 ± 5.1 %

2.4.1.2 Mobilisation of the target hydrocarbons from the soil under an hydraulic gradient

Column studies were used to assess the capacity of an advancing front of water to displace the target contaminants from the soil, which was considered representative of the likely displacement occurring in the electrokinetics chambers under the influence of electroosmosis (§ 2.3.3.2). Figure 2.8 provided some evidence that the xylene isomers could be mobilised with an advancing hydraulic gradient from the Reading soil. Dodecane and tetradecane had limited mobility from the soil column. The differences in mobility can in part be explained by the K_{ow} and C^s values of the contaminants (Table 2.1), with high K_{ow} and low C^s values indicating that a contaminant will preferentially partition to the soil. The percentage removal after 14 days for all of the target contaminants was extremely low.

Approximate removal efficiency through flushing, was 8.5%, 8.2% and 10.1% (*p*-, *m*-, *o*-xylene respectively), 0.3 % for dodecane and 0.9% for tetradecane, over a period of 14 days with 30.4 ml (~8 pore volumes) of filtered mains water flushed through the columns. The percentage efficiency quoted is relative to the start concentration and does not take into consideration loss through biodegradation, volatilisation or extraction efficiency. Table 2.4 details the average mass balance calculations performed from analysis of the column of soil on day 3, in triplicate. The experiment was designed to assess the concentration of contaminant in the aqueous phase, giving an indication of the concentrations likely to be mobile in the electrokinetics chamber under the influence of electroosmosis. The soil within the electrokinetics chamber, as in the soil column, was not sterile so would be susceptible to the same variables, including loss through biodegradation, volatilisation and extraction efficiency.

Table 2.4 Mass balance calculations for column study, day 3.

	In soil (mg)	Flushed in aqueous (mg)	Initial addition (mg)	% Flushed	% Recovery
p-xylene	11.36	0.30	29.48	1.02	39.6
m-xylene	37.1	0.88	89.99	0.98	38.1
o-xylene	10.30	0.30	25.44	1.18	41.7
Dodecane	86.6	0.08	179.76	0.04	48.3
Tetradecane	51.75	0.10	61.04	0.85	84.9

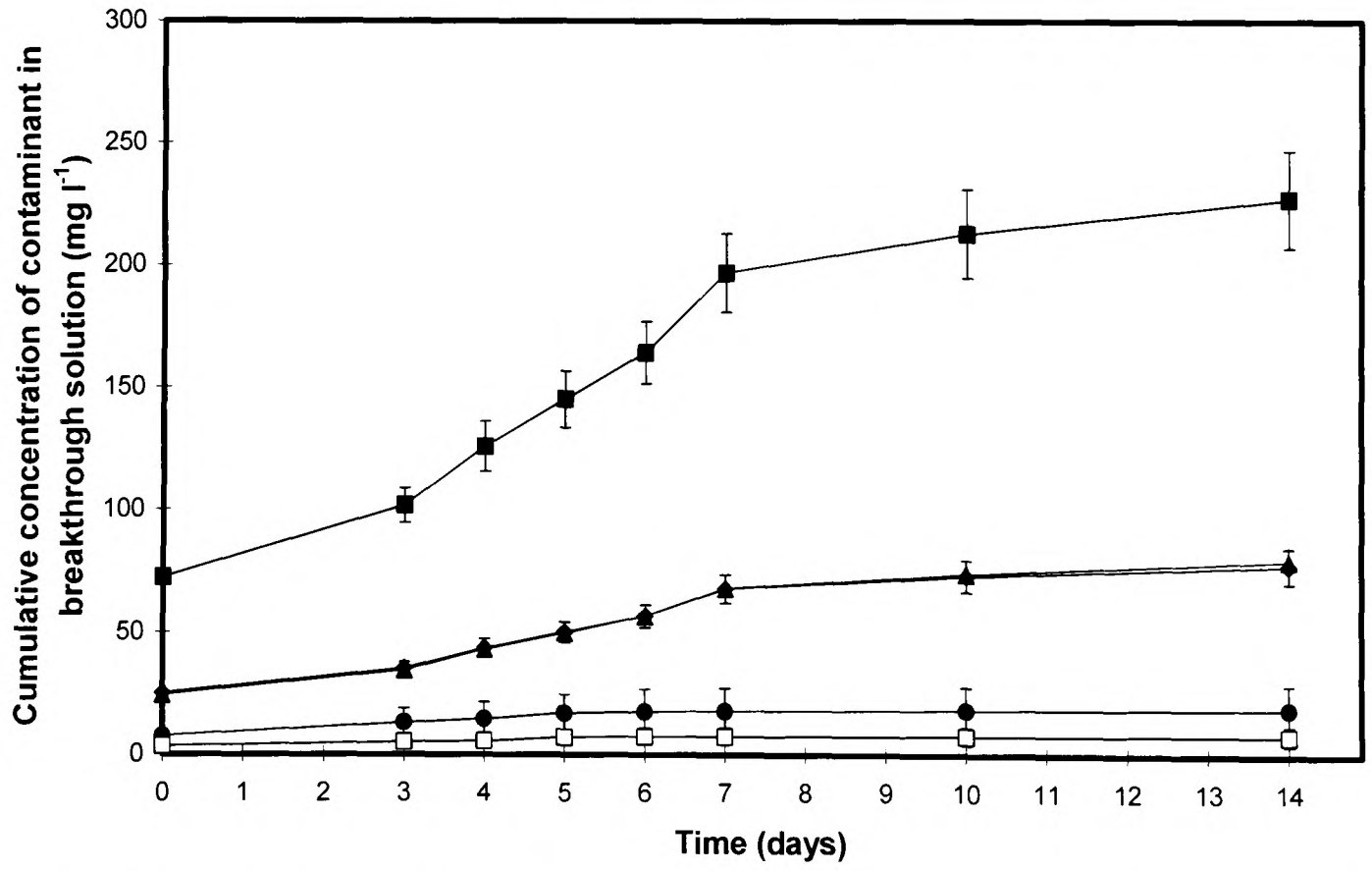


Figure 2.8 Mobilisation of DTX contaminant mix from Reading soil (non-sterilised) under a hydraulic gradient. Breakthrough of DXT contaminant mix from soil column (Reading soil) flushed with mains water, at one pore volume per day. ● dodecane, ◆ *p*-xylene, ■ *m*-xylene, ▲ *o*-xylene, □ tetradecane (data are means ± SE, n = 3).

2.4.1.3 Partitioning of the target hydrocarbons from the aqueous to the soil phase

Experiments using model soils (Wytham and Reading soil) and a DXT mix were performed to determine how the concentration of contaminant (sorbate) in the aqueous phase changed over time on addition of the aqueous contaminant mix to 'clean' soil (the sorbant) (§ 2.3.3.3). Reading soil was used as a model soil to investigate the partitioning behaviour of toluene from the aqueous to the soil phase. The aim of the experiments was not to calculate adsorption isotherms but to assess the feasibility of mobilising the target hydrocarbon contaminants through the soil; assessing the partitioning behaviour of the target contaminants if they were successfully flushed from the 'spiked' region of the soil into a 'clean' region of the soil.

A degree of sorption of the DXT contaminant mix from the aqueous phase to sterilised Reading soil occurred immediately (< 0.05 h, Figure 2.9), as compared to time point 0 h without soil. Thereafter, the concentration in solution remained relatively constant over the 48 h period. A mass balance analysis was performed on the samples collected at time point 6 h, Table 2.5. Without soil, the extraction efficiency from the aqueous phase was 98 ± 9 % (mean ± SE; n = 5), confirming that the decrease in concentration observed was as a result of biotic reactions and sorption to the soil and not as a result of sorption to the glass vial or volatilisation.

A comparison between the concentration of contaminants detected in the aqueous phase compared to their solubility limit (Table 2.1) revealed that there was a significantly higher concentration in the aqueous phase than would be expected. For instance, in the case of dodecane the solubility in water is given as 0.0037 mg l^{-1} and in the adsorption experiments an average of 1564 ± 81 mg l^{-1} of dodecane was detected. An extra 3 g of soil was added in a second experiment to assess if extra soil would result in a lower concentration of contaminants in the aqueous phase. There was no significant difference in the contaminant concentration detected in the aqueous phase in the presence of either 1 or 4 g ($p > 0.05$, df = 5) (compare Figures 2.9, 2.10 and Table 2.5). The concentration of contaminant detected above the solubility limit is likely to be attributable to the presence of free phase hydrocarbons in the aqueous phase above the saturation limit of both the soil and aqueous phases.

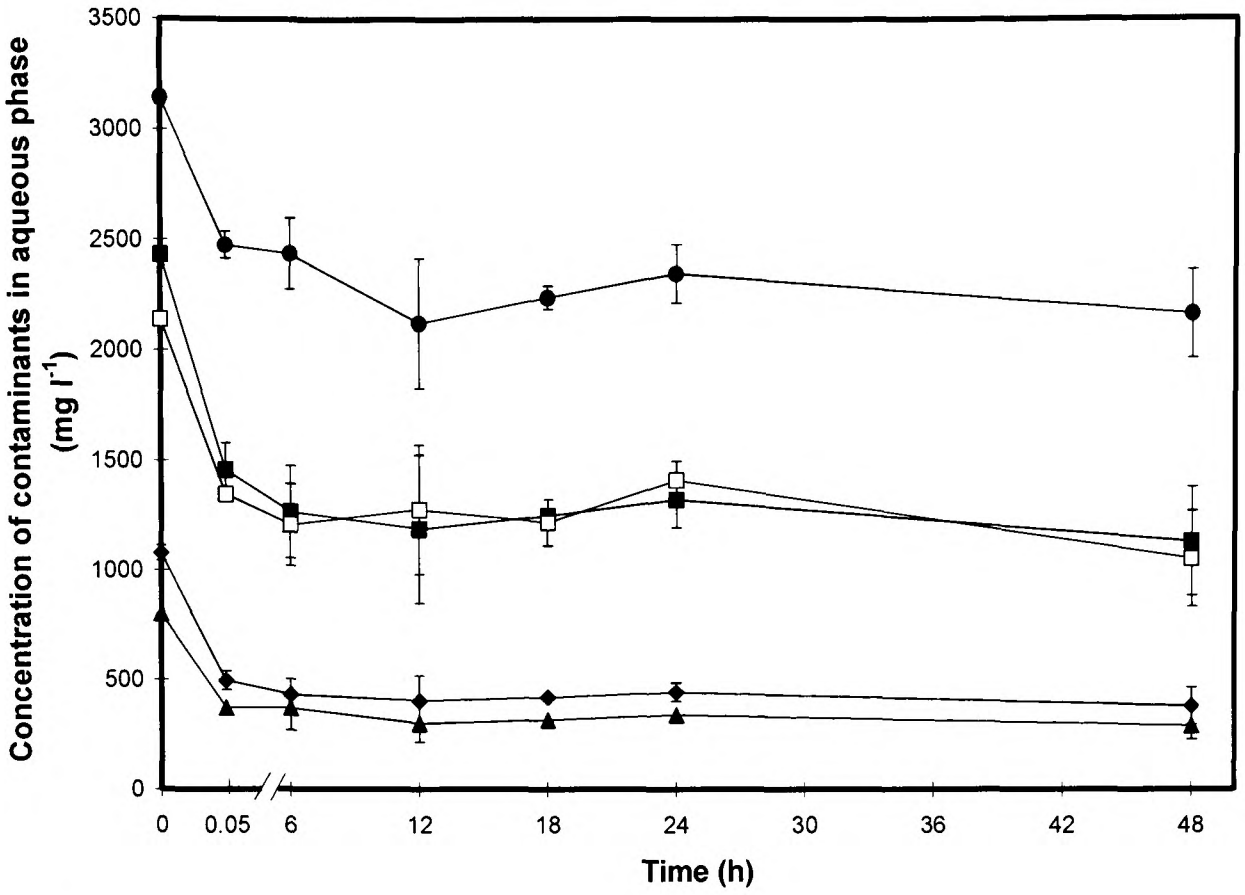


Figure 2.9 Concentration of DXT contaminant mix in the aqueous phase (mg l^{-1}) after the addition of the contaminant mix to 1 g of sterilised Reading soil at time point 0 h, investigation of DXT contaminant mix sorption from the aqueous to the soil phase over 48 h. ● Dodecane (4 500 mg l^{-1}), ◆ *p*-xylene (800 mg l^{-1}), ■ *m*-xylene (2 300 mg l^{-1}), ▲ *o*-xylene (600 mg l^{-1}), and □ tetradecane (1 600 mg l^{-1}) (data are means ± SE, n = 3). Note the x-axis is broken at ~0.07 h. (Concentrations in brakets are the approximate theoretical start concentrations).

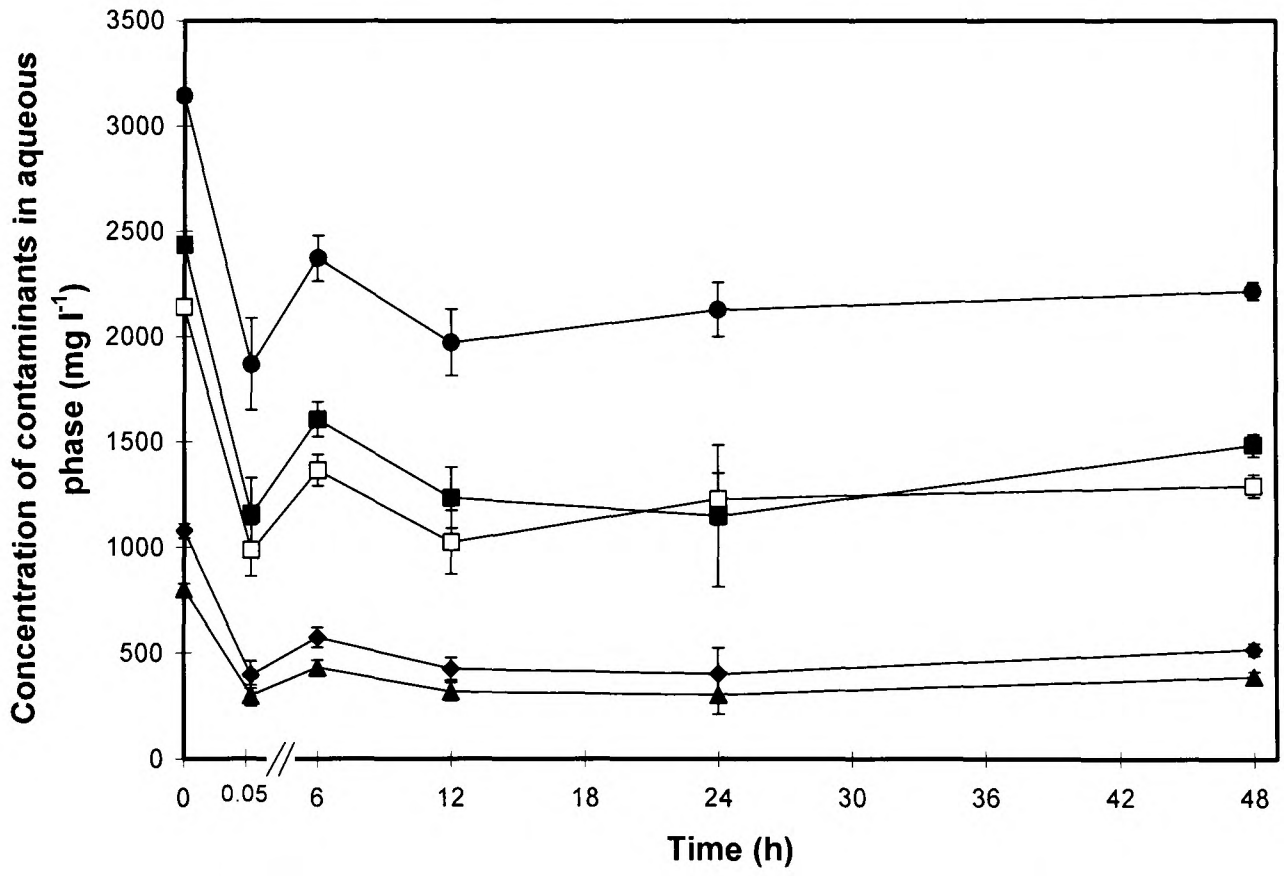


Figure 2.10 Concentration of DXT contaminant mix in the aqueous phase (mg l^{-1}) after the addition of the contaminant mix to 4 g of sterilised Reading soil at time point 0 h, investigation of DXT contaminant mix sorption from the aqueous to the soil phase. ● Dodecane (4 500 mg l^{-1}), ◆ *p*-xylene (700 mg l^{-1}), ■ *m*-xylene (2 300 mg l^{-1}), ▲ *o*-xylene (600 mg l^{-1}), and □ tetradecane (1 500 m l^{-1}) (data are means ± SE, n = 3). Note the x-axis is broken at ~0.07 h. (Concentrations in brakets are the approximate theoretical start concentrations).

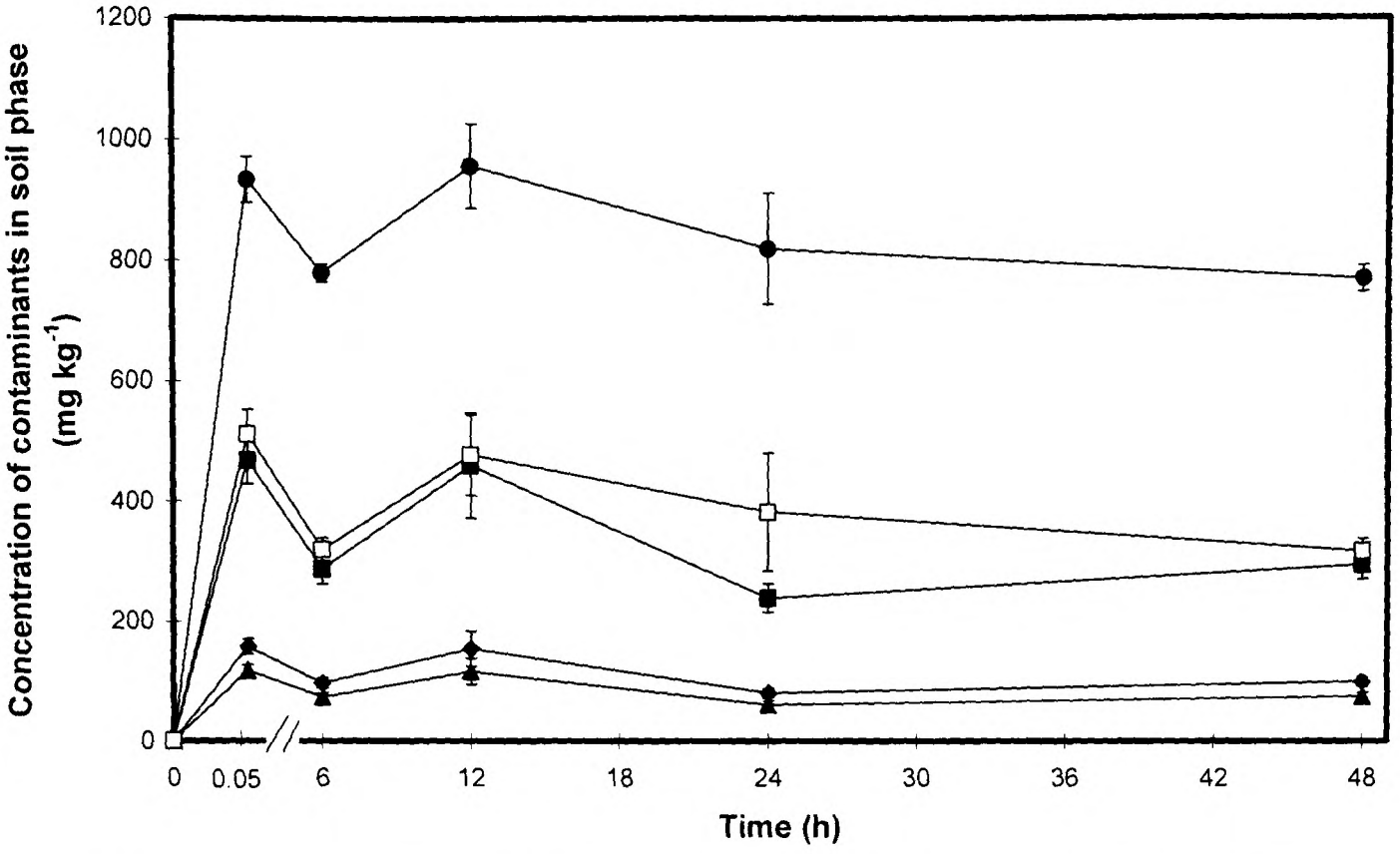


Figure 2.11 Concentration of DXT contaminant mix in the soil phase (mg kg^{-1}) after the addition of the contaminant mix to 4 g of sterilised Reading soil at time point 0 h, investigation of DXT contaminant mix sorption from the aqueous to the soil phase over 48 h. ● Dodecane, ◆ p-xylene, ■ m-xylene, ▲ o-xylene, and □ tetradecane. Data are means ± SE, n = 3, note the x-axis is broken at ~ 0.07 h.

Table 2.5 Mass balance calculations for sterilised Reading soil at time point 6 h (data are means ± SE, n = 3). 2.5 ml of DXT-spiked mains water with either 1 g or 4 g of sterilised Reading soil added.

	Contaminant	Theoretical start concentration in aqueous phase (mg l^{-1})	Contaminant in soil phase (%)	Contaminant in aqueous phase (%)	Mass balance (%)
1 g Reading soil	**Dodecane**	4494	35.7 ± 2.1	34.8 ± 1.8	70.5 ± 2.1
	***p*-Xylene**	737	33.1 ± 4.1	44.5 ± 6.1	77.6 ± 4.2
	***m*-Xylene**	2250	32.2 ± 4.0	41.8 ± 5.0	74.0 ± 3.0
	***o*-Xylene**	552	33.5 ± 4.1	45.0 ± 6.1	78.5 ± 4.1
	Tetradecane	1526	51.1 ± 2.2	59.9 ± 6.4	111.0 ± 4.9
4 g Reading soil	**Dodecane**	4494	38.0 ± 1.4	35.3 ± 1.4	73.2 ± 0.2
	***p*-Xylene**	737	28.8 ± 3.0	51.9 ± 3.9	80.7 ±1.3
	***m*-Xylene**	2250	28.1 ± 2.9	47.7 ± 2.1	75.7 ± 1.2
	***o*-Xylene**	552	29.1 ± 3.0	52.0 ± 3.8	81.1 ± 1.2
	Tetradecane	1526	45.9 ± 3.8	59.8 ± 2.8	105.7 ± 2.5

Further experiments were performed using sterilised Wytham as the model soil to compare the partitioning behaviour from the aqueous phase to the soil phase with the data from Reading soil (§ 2.3.3.3) (Figure 2.12). A mass balance calculation (Table 2.6) showed increased contaminant partitioning to the soil phase compared to sterilised Reading soil ($p < 0.05$; df = 4), but the mass balances were not significantly different ($p > 0.05$; df = 4).

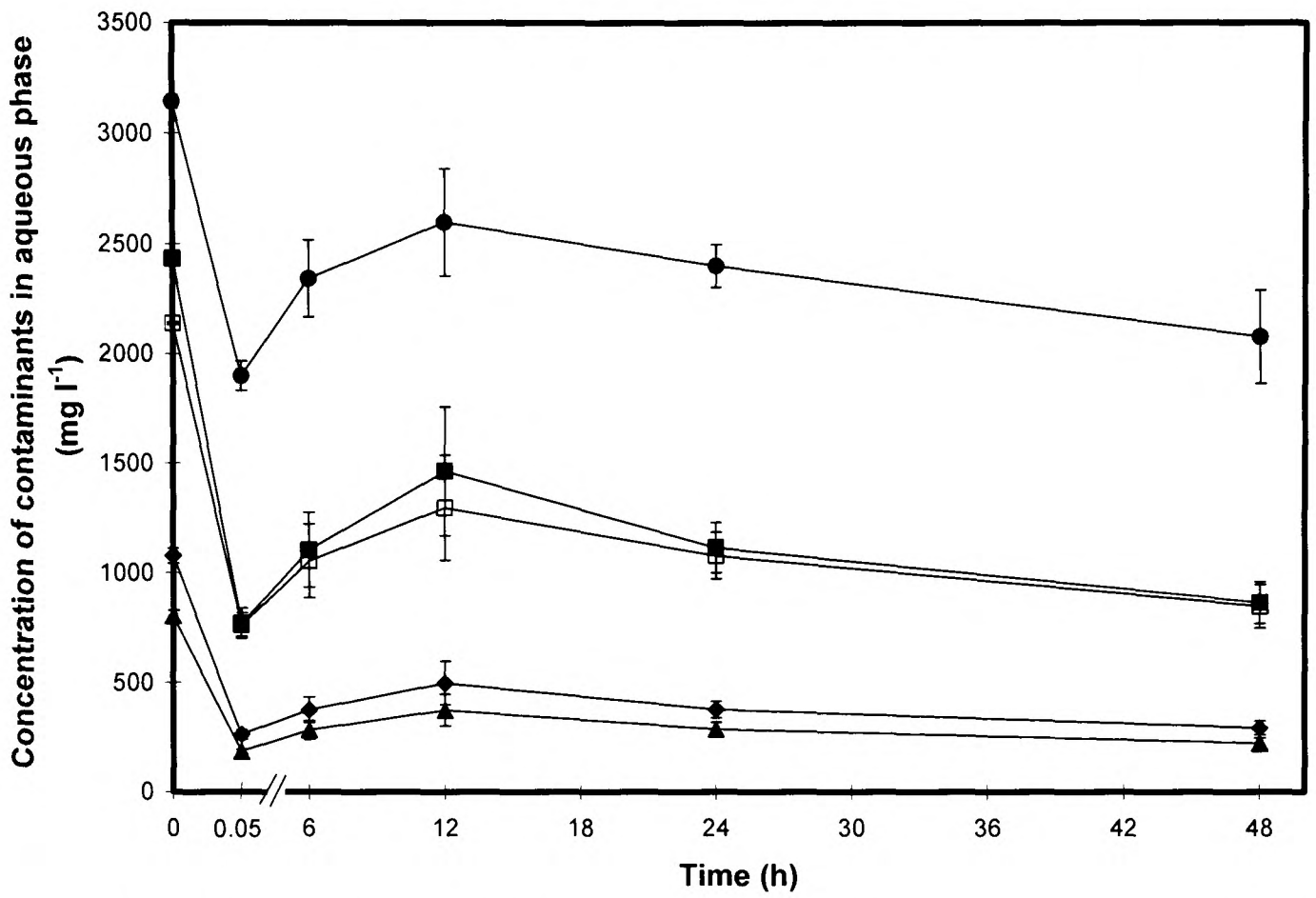


Figure 2.12 Concentration of DXT contaminant mix in the aqueous phase (mg l^{-1}) after the addition to 1 g sterilised Wytham soil at time point 0 h, investigation of DXT contaminant mix partitioning from the aqueous to the soil phase over 48 h. ● Dodecane (4 500 mg l^{-1}), ◆ *p*-xylene (700 mg l^{-1}), ■ *m*-xylene (2 300 mg l^{-1}), ▲ *o*-xylene (600 mg l^{-1}), and □ tetradecane (1 500 m l^{-1}). Data are means ± SE, n = 3, note the x-axis is broken at ~ 0.07 h. (Concentrations in brackets are the approximate theoretical start concentrations).

Table 2.6 Mass balance calculations for Wytham soil at time point 6 h.

Contaminant	Contaminant in Soil phase (%)	Contaminant in Aqueous phase (%)	Mass balance (%)
***p*-Xylene**	49.0 ± 5.9	25.5 ± 6.0	75.2 ± 1.7
***m*-Xylene**	48.1 ± 5.6	24.8 ± 5.7	73.0 ± 1.8
***o*-Xylene**	50.1 ± 5.7	26.2 ± 5.8	76.3 ± 1.9
Dodecane	44.2 ± 2.9	28.2 ± 2.4	72.3 ± 0.6
Tetradecane	70.1 ± 8.3	40.9 ± 6.6	111.0 ± 2.3

Since the mass balance for tetradecane exceeded 100% (Tables 2.5 and 2.6) it is likely that the elevated concentrations of tetradecane observed are attributed to high background readings on the GC-FID. For example, the analysis of 39 samples without any addition of tetradecane, and the use of new glassware, revealed an average concentration of 27.5 mg g^{-1} ± 1.0 (data is mean ± SE, n = 39). The source of tetradecane in the analysis remains unknown, but is considered in all further calculations proceeding the adsorption studies by assuming approximately 28 mg kg^{-1} is attributable to background noise on the GC-FID.

2.4.1.3.1 Partitioning of DXT to sterilised and non-sterilised Reading soil from the aqueous phase

To model the behaviour of the DXT contaminant mixture in the presence of non-sterilised soil, a second experiment was performed using Reading soil. Sterilising soil (§ 2.3.2.1) can affect the structure of the soil (Dao *et al.,* 1982), which is likely to influence the soil's adsorption capacity. Without sterilisation biodegradation would be an additional variable and lower concentrations of the contaminants maybe anticipated as a result. However, the degree of degradation observed after 6 h in a soil microcosm would be limited as the soil had been air dried for a period of one month before the start of the experiment and 6 h is not likely to offer a sufficient acclimation period for the soil microorganisms.

Figures 2.13 and 2.14 and Table 2.7 provide evidence that a greater concentration of the three xylene isomers had been removed from the aqueous phase in the presence of non-sterile soil, after 6 h, compared to removal from the aqueous phase with sterilised soil (p = 0.02; df = 4). The quantity of contaminants in the sterile soil compared to the non-sterile soil was not significantly different for any of the contaminants ($p > 0.05$; df = 4), which indicates that contaminants may have been biodegraded. There appeared to be no significant difference in removal of dodecane and tetradecane from the aqueous phase with the presence of either non-sterile or sterile Reading soil (p = 0.07, 0.2, respectively; df = 4). With regards to mass balance there was significantly less *m*- and *o*- xylene remaining in the vials containing non-sterilised soil compared to those containing sterilised soil (respectively p = 0.004, p = 0.008; df = 4). There appeared to be no significant difference between the mass balance calculations for *p*-xylene, dodecane or tetradecane in Reading soil compared to Wytham soil at time point 6 h ($p > 0.05$; df = 4).

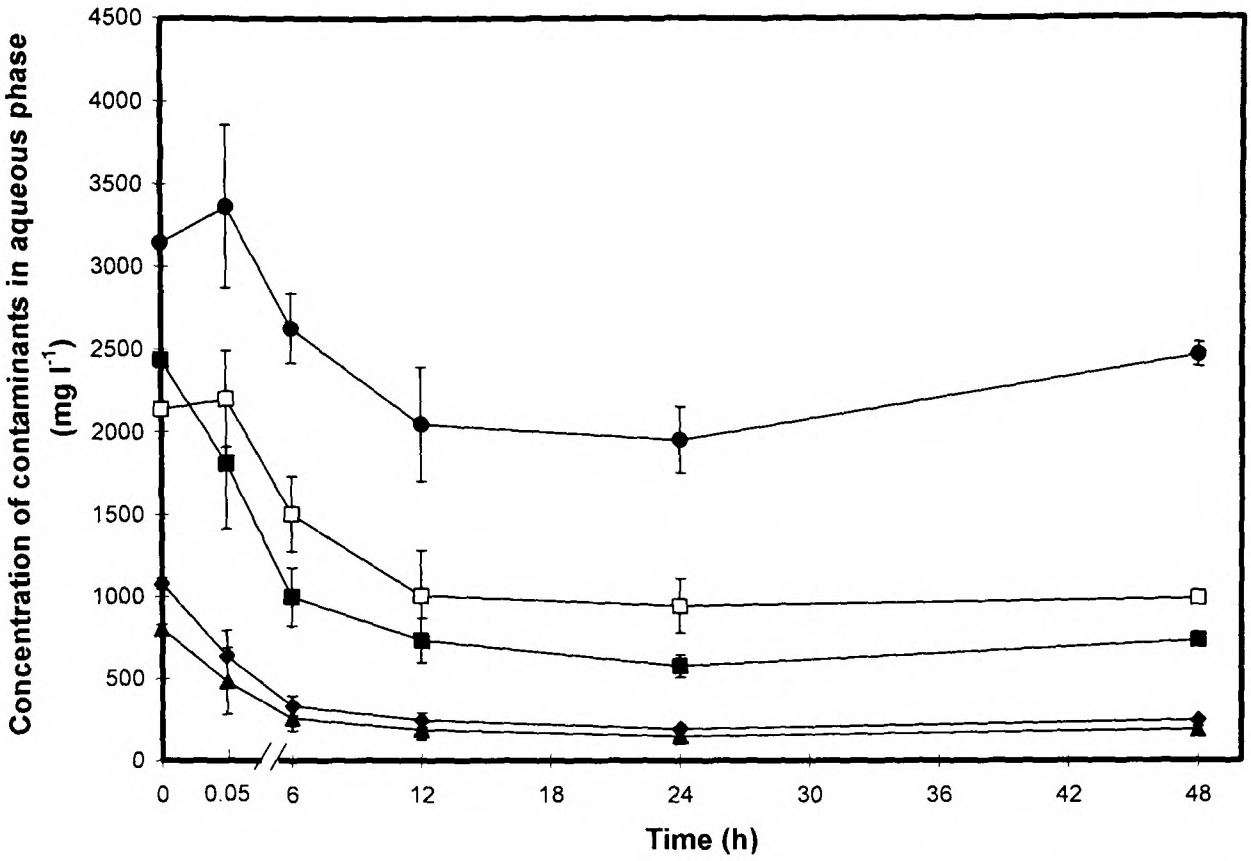


Figure 2.13 Concentration of DXT contaminants in the aqueous phase (mg l^{-1}) after addition to 1 g of non-sterilised Reading soil at time point 0 h, investigation of DXT contaminant mix partitioning from the aqueous to the soil phase. ● Dodecane (4 500 mg l^{-1}), ◆ *p*-xylene (700 mg l^{-1}), ■ *m*-xylene (2 300 mg l^{-1}), ▲ *o*-xylene (600 mg l^{-1}), and □ tetradecane (1 500 m l^{-1}). Data are means ± SE, n = 3, note the x-axis is broken at ~ 0.07 h. (Concentrations in brackets are the approximate theoretical start concentrations).

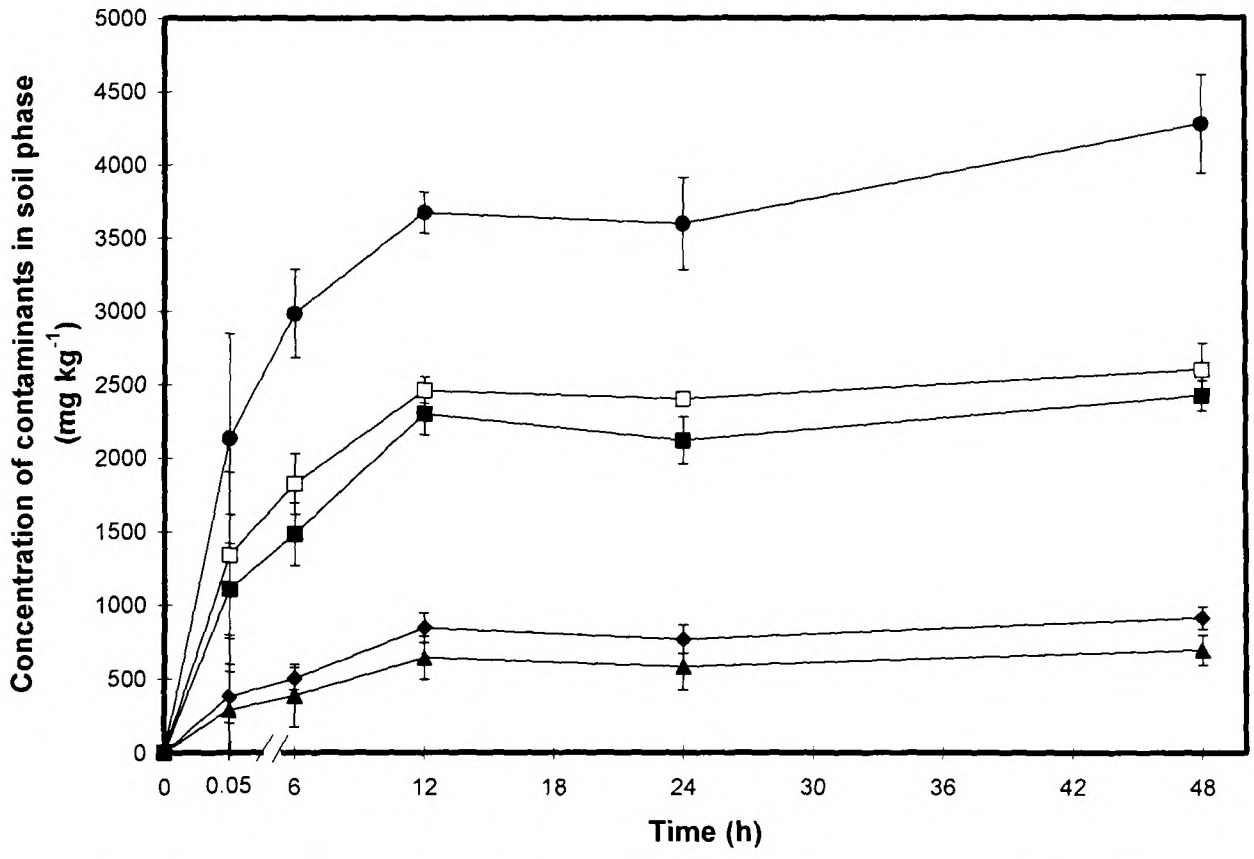


Figure 2.14 Concentration of DXT contaminant mix in the soil phase (mg kg^{-1}) after the addition of contaminant mix to 1 g of non-sterilised Reading soil at time point 0 h, investigation of contaminant mix (DXT) sorption from the aqueous to soil phase over 48 h. ● Dodecane, ◆ *p*-xylene, ■ *m*-xylene, ▲ *o*-xylene, and □ tetradecane. Data are means ± SE, n = 3, note the x-axis is broken at ~ 0.07 h.

Table 2.7 Mass balance calculations for Reading soil (non-sterilised) at time point 6 h (data are means ± SE, n = 3). 2.5 ml of DXT-spiked mains water with 1 g of Reading soil added.

	Contaminant	Theoretical start concentration in aqueous phase (mg l^{-1})	Contaminant in Soil phase (%)	Contaminant in Aqueous phase (%)	Mass balance (%)
1 g Reading soil	**Dodecane**	4494	34.2 ± 3.8	30.0 ± 1.9	64.2 ± 4.0
	***p*-Xylene**	737	50.8 ± 13.4	23.0 ± 3.8	73.7 ± 9.6
	***m*-Xylene**	2250	34.0 ± 5.3	22.7 ± 3.7	56.6 ± 2.1
	***o*-Xylene**	552	36.0 ± 5.7	23.9 ± 3.8	59.8 ± 2.4
	Tetradecane	1526	61.6 ± 7.9	50.3 ± 7.1	112.0 ± 5.2

2.4.1.4 Partitioning of toluene to sterilised and non-sterilised Reading soil from the aqueous phase

The partitioning of toluene to sterilised (autoclaved) and non-sterilised Reading soil was investigated using vial studies (§ 2.3.3.1). Sorption of toluene to Reading soil occurred immediately (< 0.05 h, Figure 2.15). Thereafter the concentration in solution remained relatively constant over the 48 h period. Mass balance calculations were carried out for the samples collected at 24 h (Table 2.8) providing evidence that a higher concentration of toluene had been removed from the system in the experiments containing non-sterilised soil compared to those with sterilised soil ($p = 0.02$; df = 4). As a result of the difference in mass balance calculations between sterile and non-sterile soils it was not possible to make inferences about the effect of autoclaving on the sorption capacity of the soil but suggests that biodegradation or volatilisation are important factors in experiments using toluene.

Table 2.8 Mass balance calculations for sterilised and non-sterilised Reading soil with toluene at time point 24 h (mean ± SE, n = 3).

Soil	Theoretical start concentration in aqueous phase (mg l^{-1})	Contaminant in Soil phase (%)	Contaminant in Aqueous phase (%)	Mass balance (%)
Sterilised	3468	45.0 ± 2.0	15.6 ± 0.4	60.5 ± 1.6
Non-sterilised	3468	20.2 ± 7.3	14.1 ± 1.9	34.3 ± 8.0

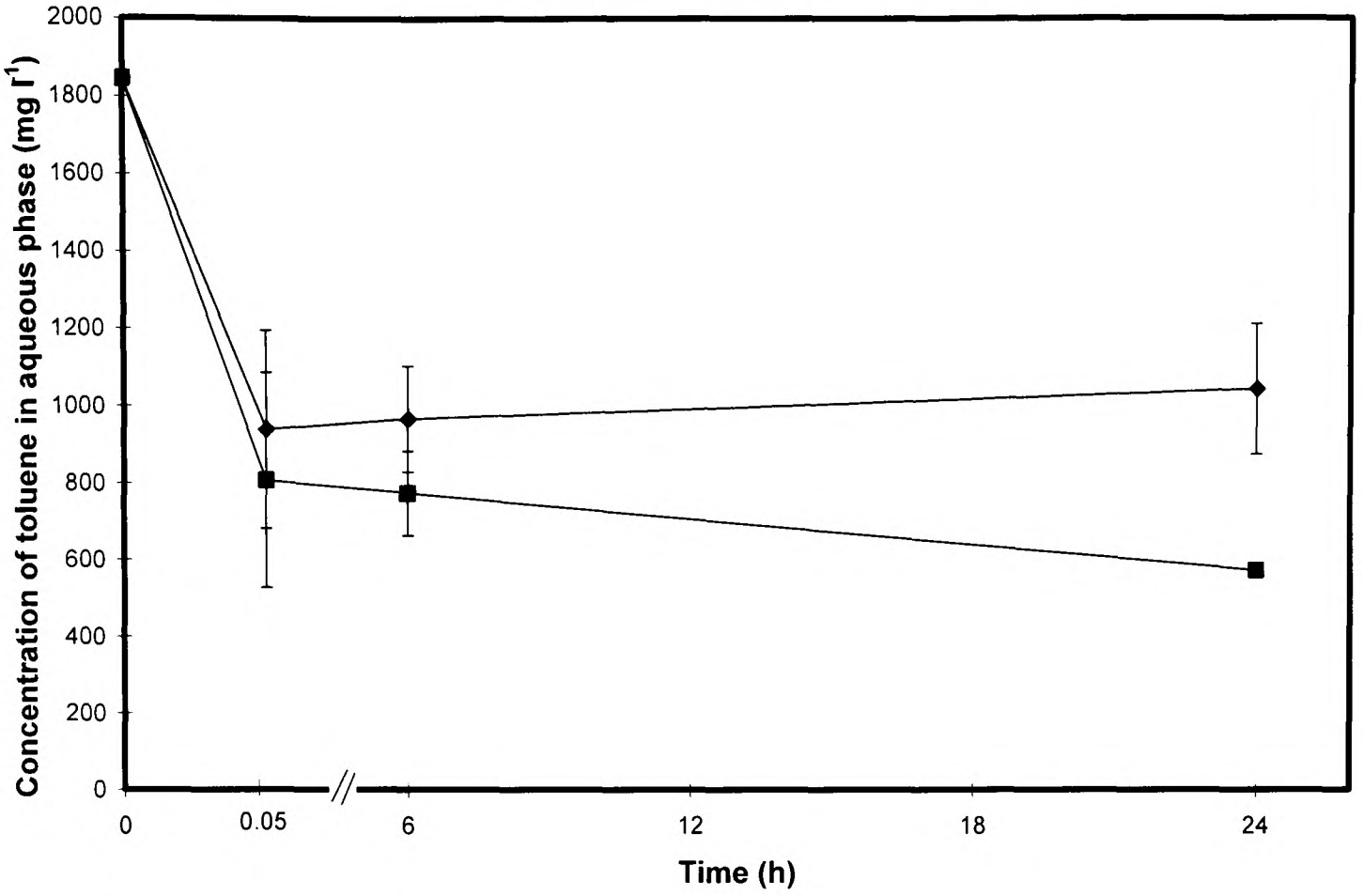


Figure 2.15 Concentration of toluene in the aqueous phase (mg l^{-1}) after addition of 1 g sterilised and non-sterilised Reading soil. Concentration of toluene in the aqueous phase (2.5 ml) in the presence of ◆ sterilised Reading soil (1 g) and ■ non-sterilised Reading soil over 24 h (data are means ± SE, n = 3). Note the x-axis is broken at ~ 0.07 h.

2.4.2 Determination of the electroosmotic flow rate

From triplicate experiments the electroosmosic flow through Reading soil was calculated to be 4.6 ml ± 0.25 within a 24 h period (data are means ± SE; n = 3). The flow through Wytham soil was calculated to be 5.9 ml ± 1.60 in a 24 h period (data are means ± SE; n = 3). During the desorption column studies (§ 2.4.2) it was not possible to pass water through a column of compacted Wytham soil using an hydraulic gradient; this experiment therefore showed that electroosmosis can facilitate the movement of water through compacted Wytham soil.

2.4.3 Temporal and spatial voltage variation

For the Reading soil (Figure 2.16) the voltage profile across the soil chamber remained approximately linear for the duration of 14 days with a constant current of approximately 10 mA. This gives evidence that there was no significant build up in resistance in the Reading soil on application of an electrical potential. Within 14 days of applying an electric potential to Wytham soil an increase in resistance was observed (Figure 2.17).

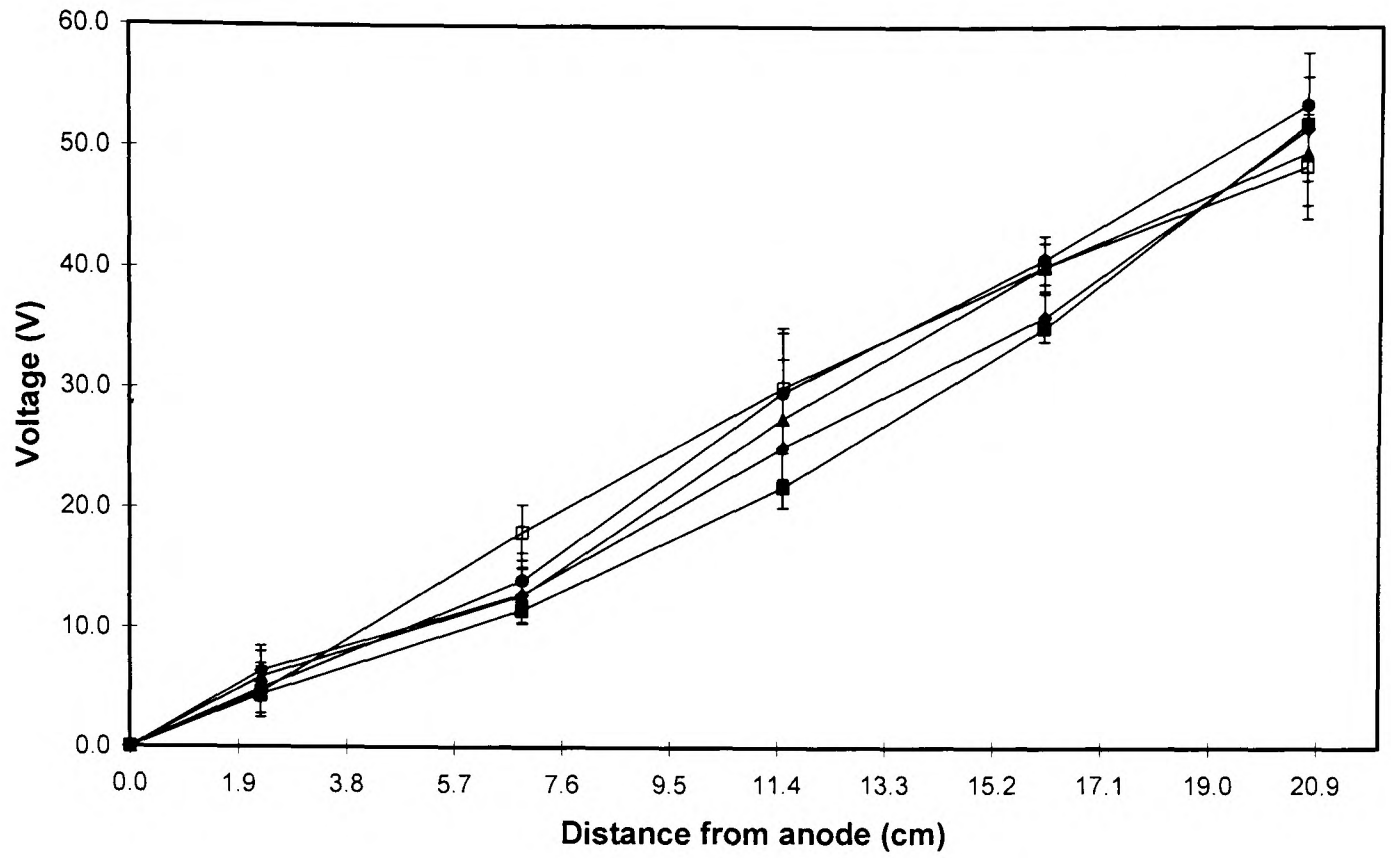


Figure 2.16 Voltage profile in electrokinetics chamber with Readng soil. Electric potential distribution across the electrokinetics soil chamber, containing Reading soil; ◆ 1 day; ■ 4 days; ▲ 7 days; ● 10 days; □ 14 days. 60 volts applied, ~ 10 mA. (Data are means ± SE; n = 2).

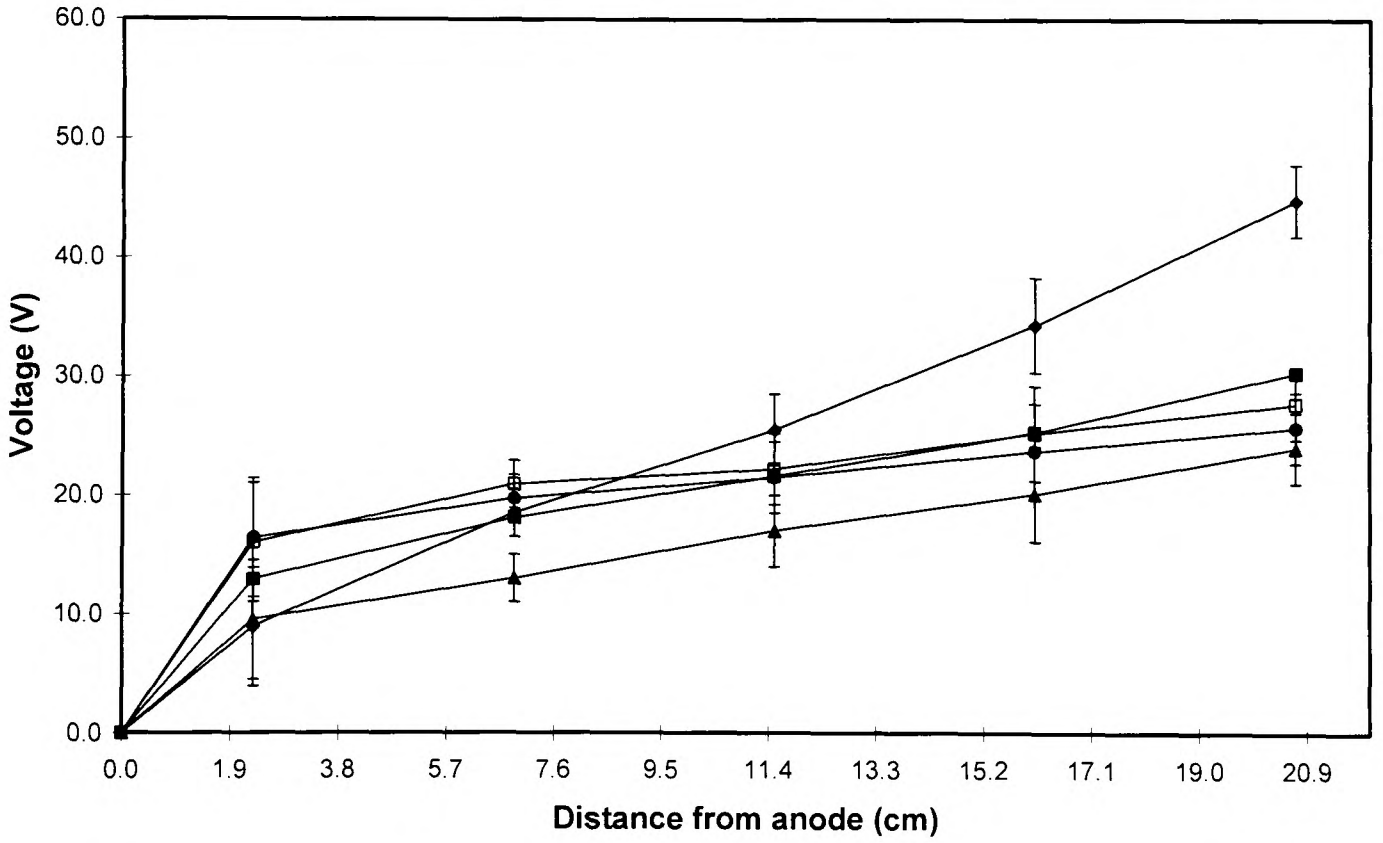


Figure 2.17 Voltage profile in electrokinetics chamber with Wytham soil. Electric potential distribution across the electrokinetics soil chamber, containing Wytham soil. ◆ 1 day; ■ 4 days; ▲ 7 days; ● 10 days; □ 14 days. 60 volts applied, ~ 10 mA. (Data are means ± SE; n = 2).

In both soil types (Reading and Wytham) at the end of the electrokinetics experiment a layer of white precipitate was found to accumulate predominantly at the cathode end of the soil chamber (see Appendix 8.5 for evidence of precipitation across the soil chamber). It is likely that this precipitate is calcium salts, which precipitates from the aqueous phase in

alkaline environments (pH > 7) (Figure 2.18). The distribution of iron and potassium in the electrokinetics soil chamber after two weeks of applying 60 V (~10 mA) did not appear to be influenced by the presence of the electric field (refer to Appendix 8.6).

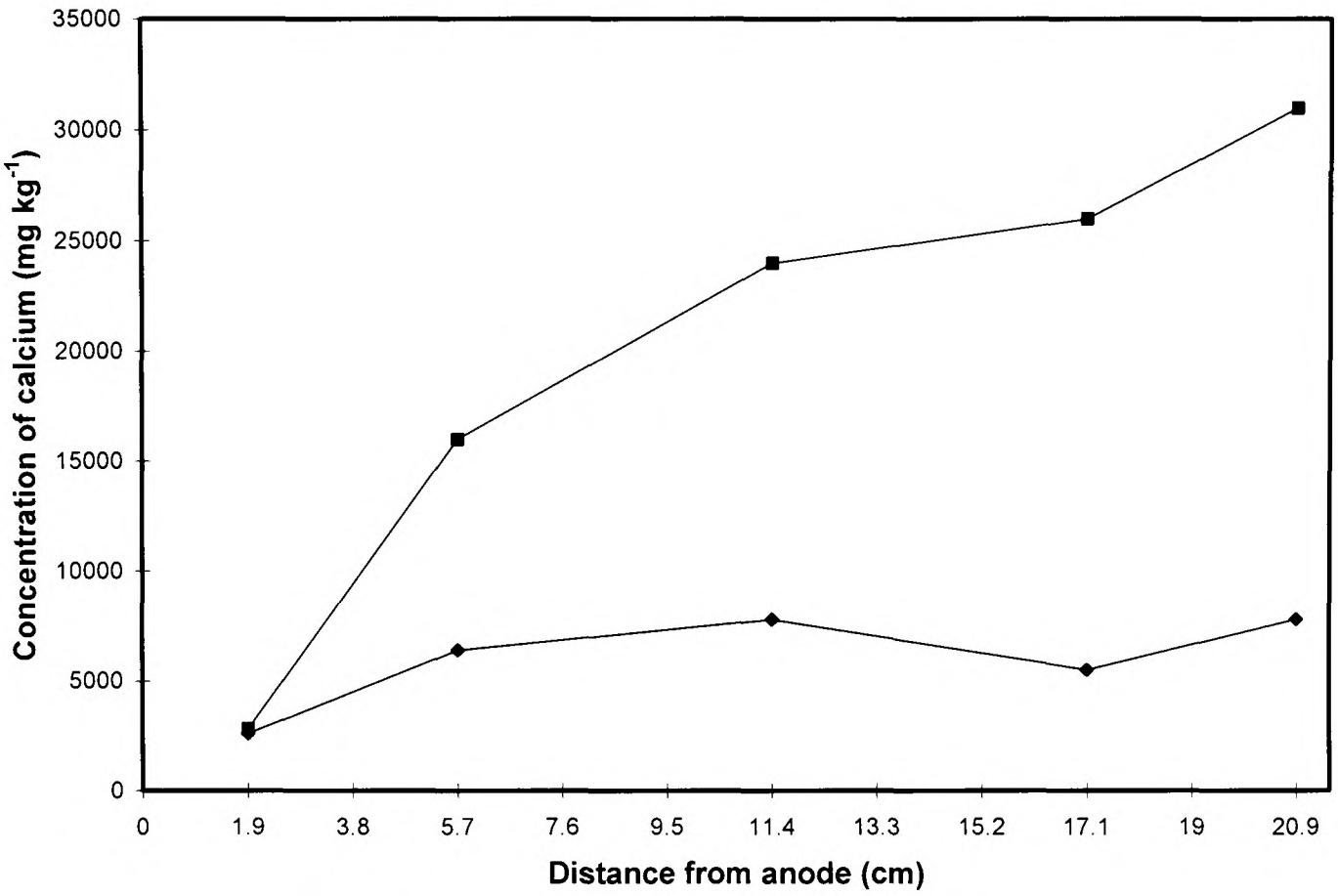


Figure 2.18 Distribution of Ca^{2+} (mg kg^{-1}) in the soil chamber after 2 weeks of applying an electric field (60 V, ~10 mA). ♦ Soil chamber packed with Reading soil; ■ soil chamber packed with Wytham soil. (Data points are based on analysis from one sample).

2.4.4 Temperature variation

Soil temperature was raised on application of the electric field (findings summarised in Table 2.9), caused by a partial conversion of the electrical energy to heat energy due to the resistive nature of the soils. In experiments carried out in the closed-box system (§ 2.3.7.2), observed temperature increases were significantly higher (Figure 2.19). It is considered likely that limited air flow across the surface of the soil led to an increase in the soil temperature in these experiments.

Table 2.9 Average temperature increase in tested soils with 60 V applied above that of the control soil (without electrokinetics) (data are means ± SE; n=3), over 14-day experiment.

Reading Soil (°C)			**Wytham Soil** (°C)		
Anode	Middle	Cathode	Anode	Middle	Cathode
1.7 ± 0.4	2.2 ± 0.4	2.2 ± 0.4	1.6 ± 0.3	1.5 ± 0.2	2.0 ± 0.4

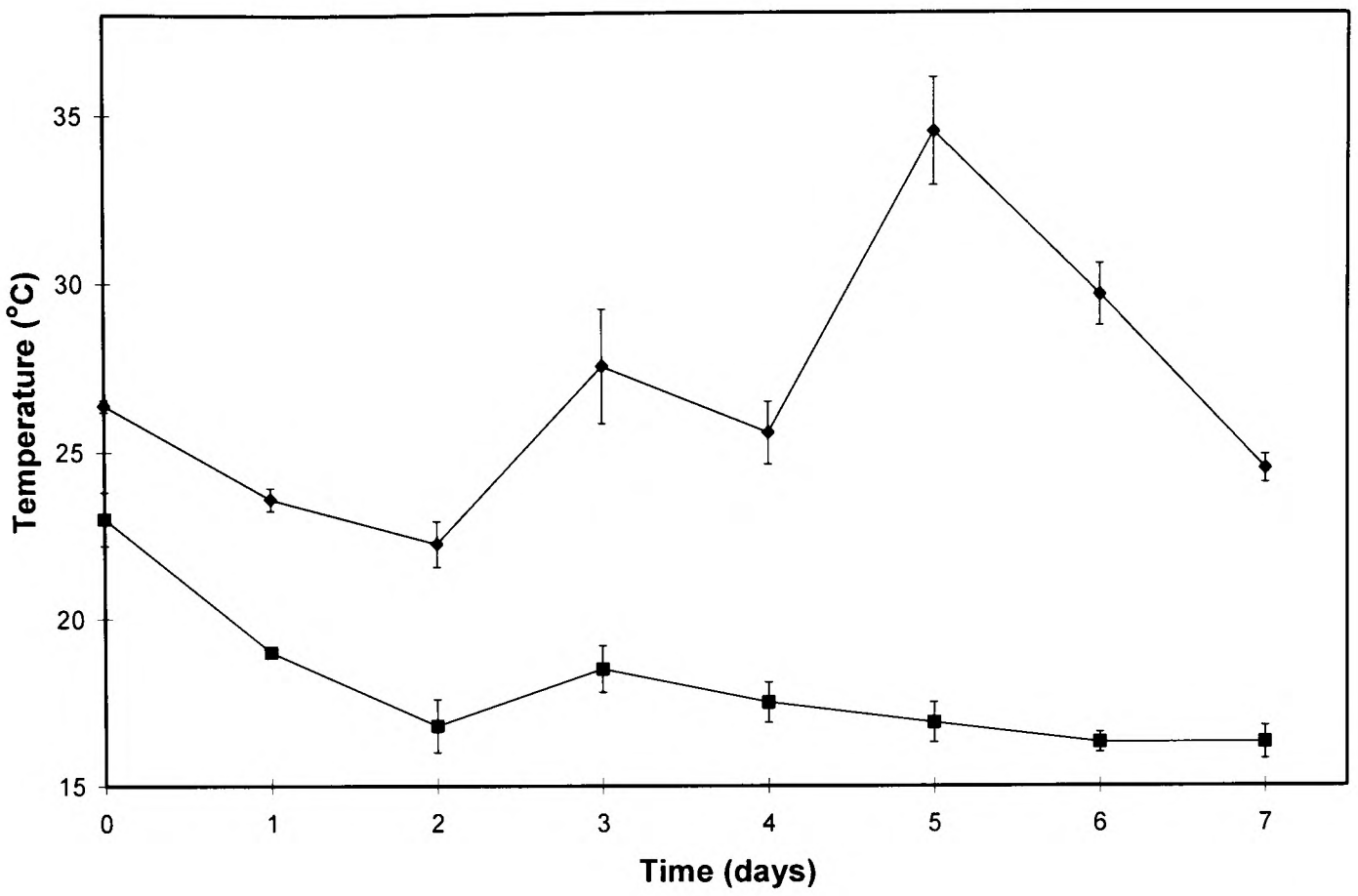


Figure 2.19 Difference in soil temperature (Wytham soil) with electrokinetics ◆ (60 V applied, ~10 mA), and without electrokinetics ■ (data are means ± SE; n = 3).

2.4.5 pH variation across the soil chambers

The pH of the electrode reservoirs was buffered using an automated pH management system (pH 6 in the catholyte [cathode solution] and pH 7 in the anolyte [anode solution]) in an attempt to maintain a near neutral pH throughout the soil system for biodegradation to occur. If pH is not adjusted, the anolyte and catholyte of the system rapidly become acidic and basic respectively as electrolysis products are formed at the electrodes. Under the influence of an electric field the electrolysis products (H^+ at the anode, OH^- at the cathode) are liable to migrate through the soil chamber creating a mobile pH front; the speed of migration is relative to the voltage applied and to the buffering capacity of the soil (concentration of exchangeable acids and bases). However, even with neutralisation in the reservoirs, some migration of H^+ and OH^- was observed (Figures 2.20 and 2.21). The cumulative addition of acid buffer to the Reading soil and Wytham soils over the 14 day duration of each experiment was 73 and 545 ml respectively, indicating that Reading soil had a greater buffering capacity than the Wytham soil.

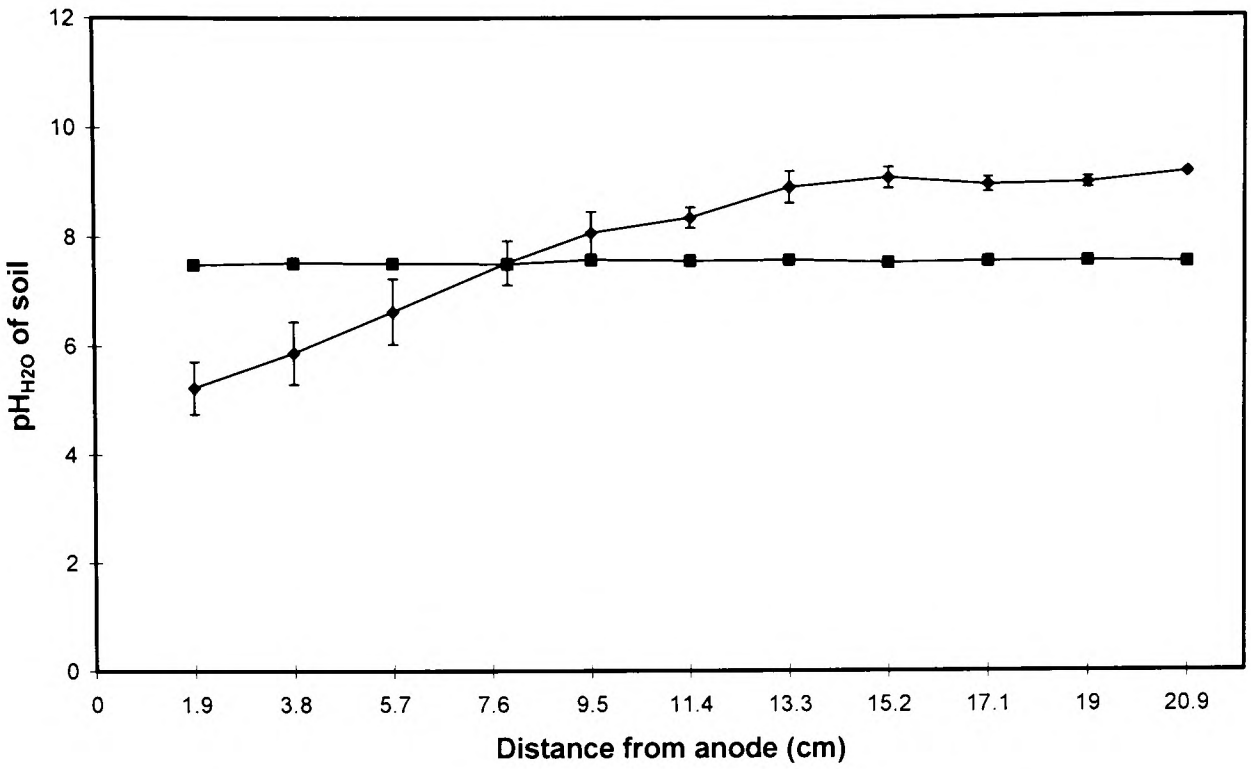


Figure 2.20 Comparison of pH profile across the soil chamber (Reading soi), with and without electrokinetics applied for 14 days. Electrokinetics Reading soil ◆ and control Reading soil ■. 60V applied for 14 days, pH control buffer 10:1 HNO_3:HPO_4 (0.1 M) (data are means ± SE; n= 4).

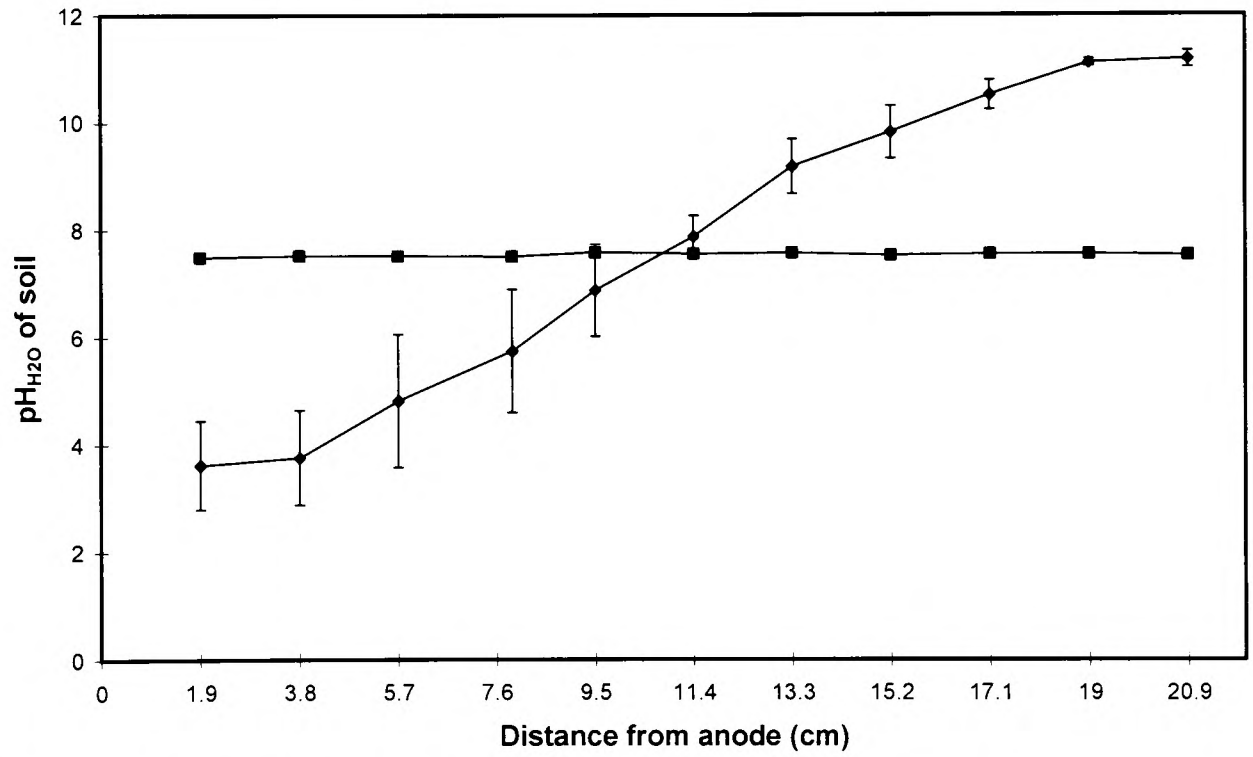


Figure 2.21 Comparison of pH profile across the soil chamber (Wytham soil), with and without electrokinetics applied for 14 days. Electrokinetics Wytham soil ◆ and control Wytham soil ■. 60V applied for 14 days, pH control buffer 10:1 HNO_3:HPO_4 (0.1 M) (data are means ± SE; n = 4).

2.4.6 Moisture content across the soil chambers

The moisture content of Reading soil with and without electrokinetics remained relatively uniform throughout the soil chamber (Figure 2.22) after a period of 14 days, whereas an increase in water content was observed with the Wytham electrokinetics soil chamber compared to the control (Figure 2.23). In the literature it is noted that water is drawn from the area surrounding the cathode and replenishment from the anode reservoir is not always

efficient enough to prevent drying (*e.g.* Segall & Bruell, 1992). One possible interference with results in this study could be attributed to condensation around the electrokinetics chamber as it was operated within a Teflon-lined Perspex box and in addition a light mist of water was sprayed onto the surface of the soil chambers, as described in Section 2.3.8.1.

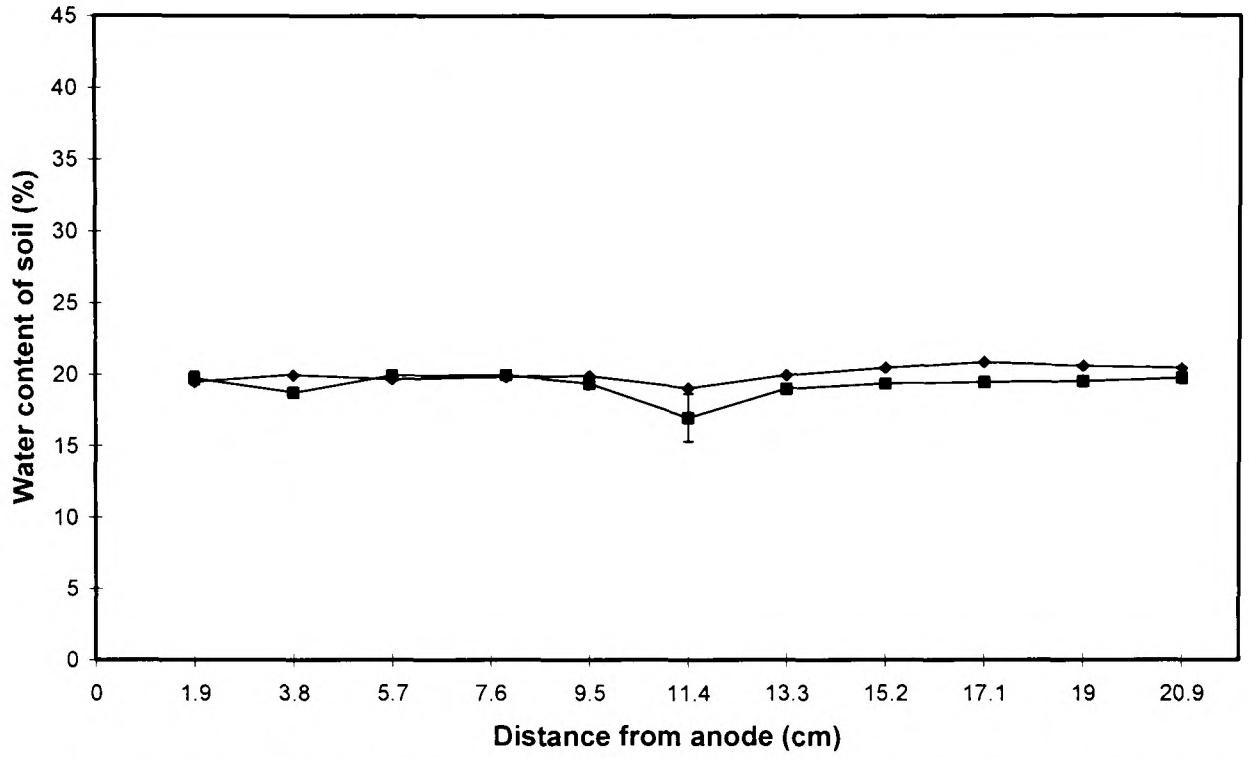


Figure 2.22 Comparison of water content (%) across the soil chamber (Reading soil), with and without electrokinetics applied for 14 days. Electrokinetics Reading soil ◆ and control Reading soil ■. 60V applied for 14 days (data are means ± SE; n = 4).

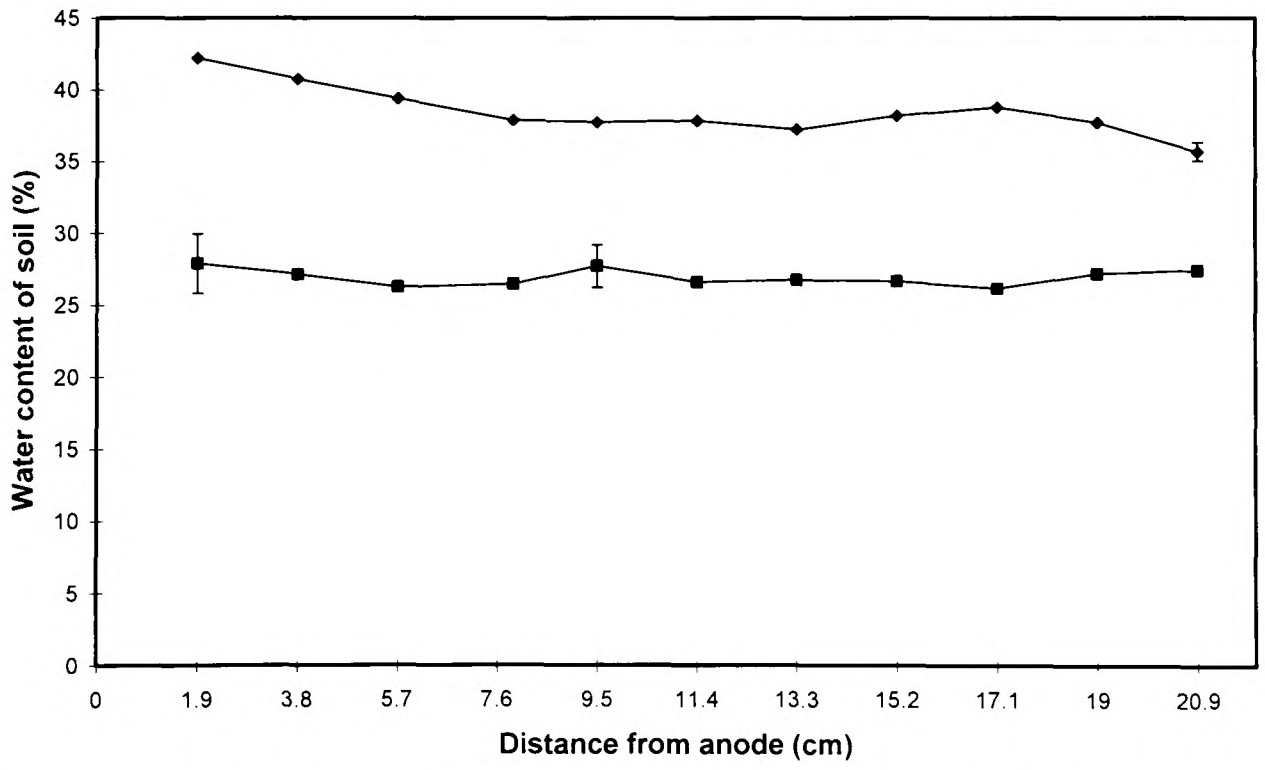


Figure 2.23 Comparison of water content (%) across the soil chamber (Wytham soil), with and without electrokinetics applied for 14 days. Electrokinetics Wytham soil ◆ and control Wytham soil ■. 60V applied for 14 days (data are means ± SE; n = 4).

2.4.7 Distribution of the target hydrocarbons in the soil chambers

Contaminant concentrations across the soil chambers are presented as bar charts with contaminant concentrations displayed on the y-axis with a large range of between 0 mg kg^{-1} to a maximum of 8 000 mg kg^{-1}. To ensure that low concentrations of the target contaminants were represented on the graphs when a concentration of contaminant was statistically significant the data was presented on a broken axis or a second inset graph used (*e.g.* Figure 2.30). The target contaminants were not detected in the electrolyte solutions in any of the electrokinetic chamber experiments.

2.4.7.1 Dodecane, xylene and tetradecane

To assess the potential of electroosmosis to mobilise the target contaminant mix (DXT) through the soil the contaminants were spiked into the central region of the soil chambers and 60 V was applied for 14 days (§ 2.3.8). The mobilisation of each of the target contaminants (DXT) was not observed to be significantly different compared to passive movement (*e.g.* diffusion) that had occurred in the control chamber with Reading soil ($p \geq 0.2$, df = 10, Figures 2.24 and 2.25) or with Wytham soil ($p \geq 0.2$, df = 5, Figures 2.26 and 2.27).

With regard to the final concentration of the contaminants in the soil chambers (Table 2.10) it was evident in both Wytham and Reading soils that overall concentrations of the 'spiked' contaminants had been reduced. In the soil chamber experiments containing Reading soil the concentration of *p*-xylene, *m*-xylene, and *o*-xylene had significantly been reduced in the soil chamber with an electric field applied compared to the concentrations remaining in the control chamber ($p = 0.001$, 0.003, 0.001, respectively, df = 10). There was no significant difference in the concentration of dodecane and tetradecane remaining in the soil chamber with an electric field applied compared with the control soil chamber ($p \geq 0.3$, df =10). In Wytham soil all five of the 'spiked' contaminants (DXT) decreased in concentration to a greater extent in the electrokinetics soil chamber compared with the control chamber (dodecane, $p = 0.005$; *p*-xylene, $p = 0.02$; *m*-xylene, $p = 0.03$; *o*-xylene, $p = 0.002$; tetradecane, $p = 0.002$; df = 7). By comparing the final concentration of the 'spiked' contaminants in the controls containing Reading and Wytham soil it was apparent that removal of the contaminants from Wytham soil by electrokinetics was significantly more effective (dodecane, $p = 0.07$; *p*-xylene, $p = 0.04$; *m*-xylene, $p = 0.06$; *o*-xylene, $p = 0.05$; tetradecane, $p = 0.03$; df = 4).

Table 2.10 Mass balance analyses for Reading and Wytham soil (data are means ± SE; n = 3). Contaminants were not detected in the electrode reservoirs.

Soil	Test	Contaminant	Initial Conc. (mg kg^{-1})		Final Conc. (mg kg^{-1})		Extractable contaminant, of initial spike (%)
Reading	**EK**	Dodecane	6941.0	±143.9	6690.0	± 1010.3	96.4
	Control	Dodecane	6941.0	± 143.9	7877.0	± 986.6	101.1
	EK	*p*-xylene	1054.0	± 137.7	217.4	± 101.0	20.6
	Control	*p*-xylene	1054.0	± 137.7	932.1	± 261.2	88.4
	EK	*m*-xylene	3253.9	± 365.5	687.8	± 315.2	21.1
	Control	*m*-xylene	3253.9	± 365.5	2696.5	± 768.6	82.9
	EK	*o*-xylene	920.7	± 96.6	191.0	± 78.9	20.7
	Control	*o*-xylene	920.7	± 96.6	776.2	± 208.8	84.3
	EK	Tetradecane	3769.5	± 101.5	3286.8	± 550.1	87.2
	Control	Tetradecane	3769.5	± 101.5	3905.5	± 566.3	103.6
Wytham	**EK**	Dodecane	8229.4	± 88.7	1683.6	± 750.3	20.5
	Control	Dodecane	8229.4	± 88.7	4474.2	± 459.6	54.4
	EK	*p*-xylene	768.3	± 19.3	83.2	± 34.7	10.8
	Control	*p*-xylene	768.3	± 19.3	233.3	± 102.2	30.4
	EK	*m*-xylene	2573.8	± 67.0	274.4	± 116.4	10.7
	Control	*m*-xylene	2573.8	± 67.0	724.1	± 319.6	28.1
	EK	*o*-xylene	727.7	± 19.7	85.6	± 32.5	11.8
	Control	*o*-xylene	727.7	± 19.7	191.2	± 84.2	26.3
	EK	Tetradecane	4805.7	± 105.2	560.1	± 241.6	11.7
	Control	Tetradecane	4805.7	± 105.2	1911.4	± 182.5	39.8

EK = Electrokinetics

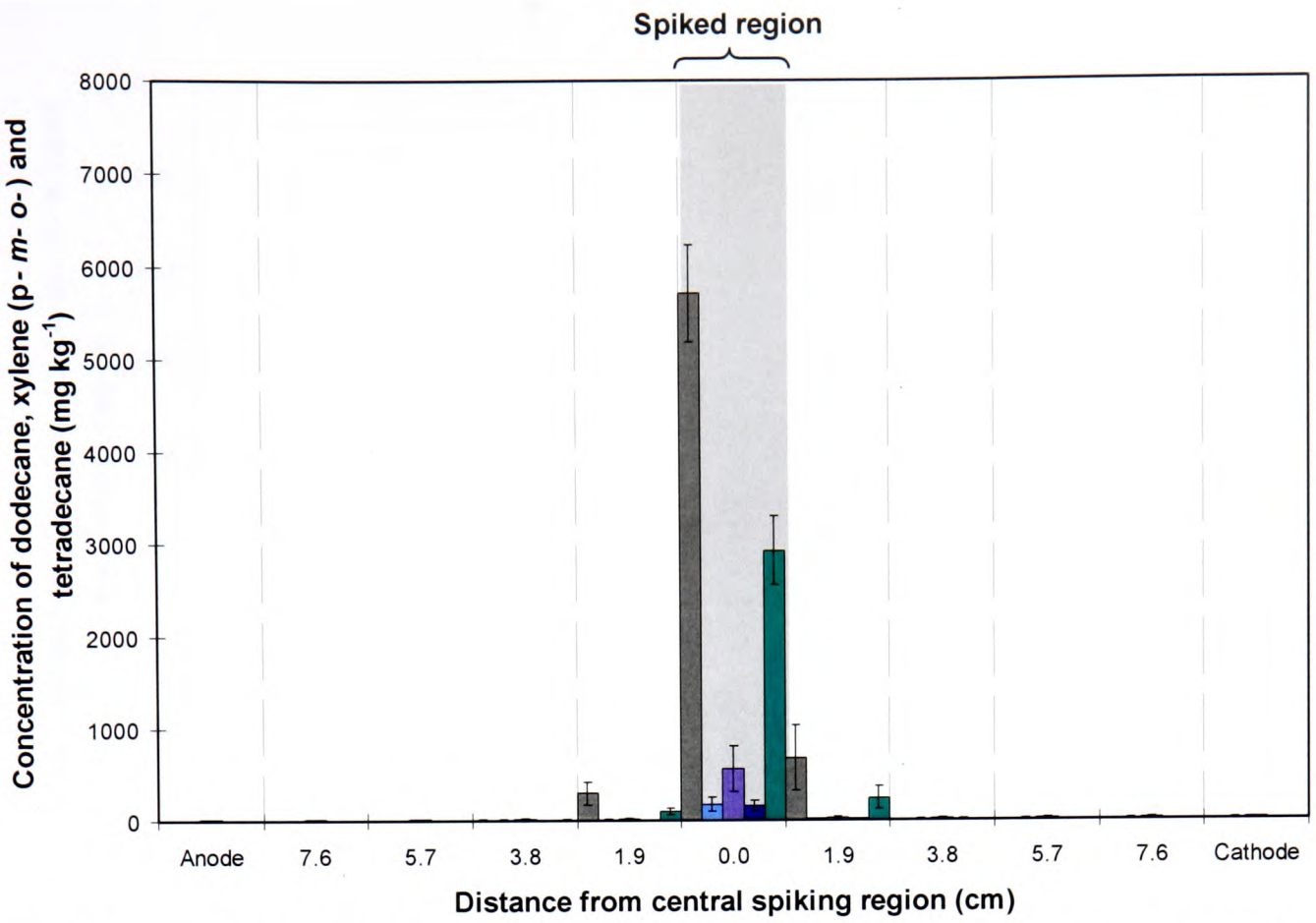


Figure 2.24 **Electrokinetics** chamber with Reading soil spiked with DXT contaminant mix. Distribution in soil chamber of ■ dodecane (11 000 mg kg^{-1}), ■ *p*-xylene (1 900 mg kg^{-1}), ■ *m*-xylene (5 700 mg kg^{-1}), ■ *o*-xylene (1 500 mg kg^{-1}), and ■ tetradecane (4 000 mg kg^{-1}) after 14 days with 60 V applied (~10 mA) (data are means ± SE; n = 9). (Concentrations in brackets are the approximate theoretical start concentrations).

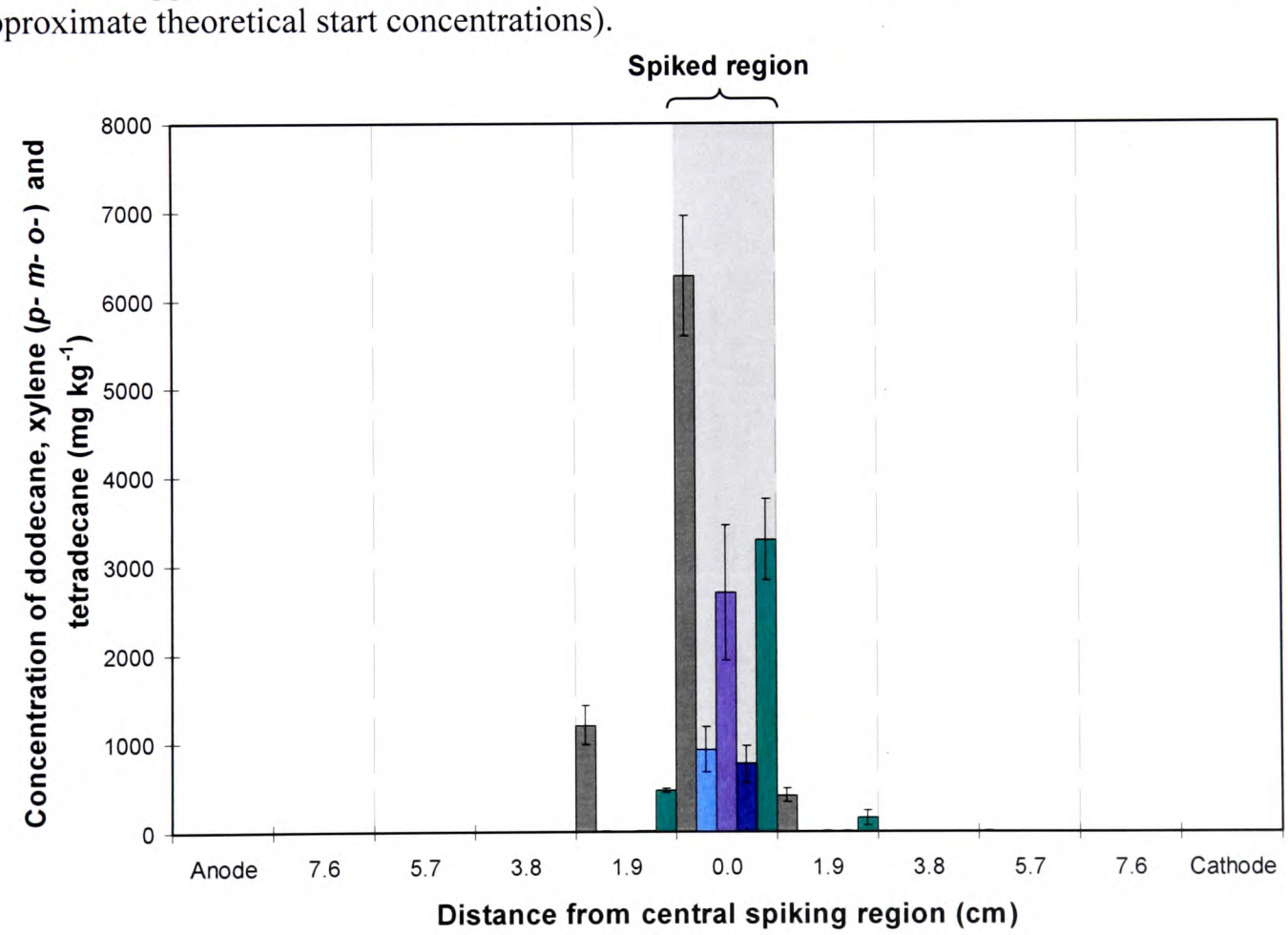


Figure 2.25 **Control** chamber with Reading soil spiked with DXT contaminant mix. Distribution in soil chamber of ■ dodecane (11 000 mg kg^{-1}), ■ *p*-xylene (1 900 mg kg^{-1}), ■ *m*-xylene (5 700 mg kg^{-1}), ■ *o*-xylene (1 500 mg kg^{-1}), and ■ tetradecane (4 000 mg kg^{-1}) after 14 days (data are means ± SE; n = 3). (Concentrations in brackets are the approximate theoretical start concentrations).

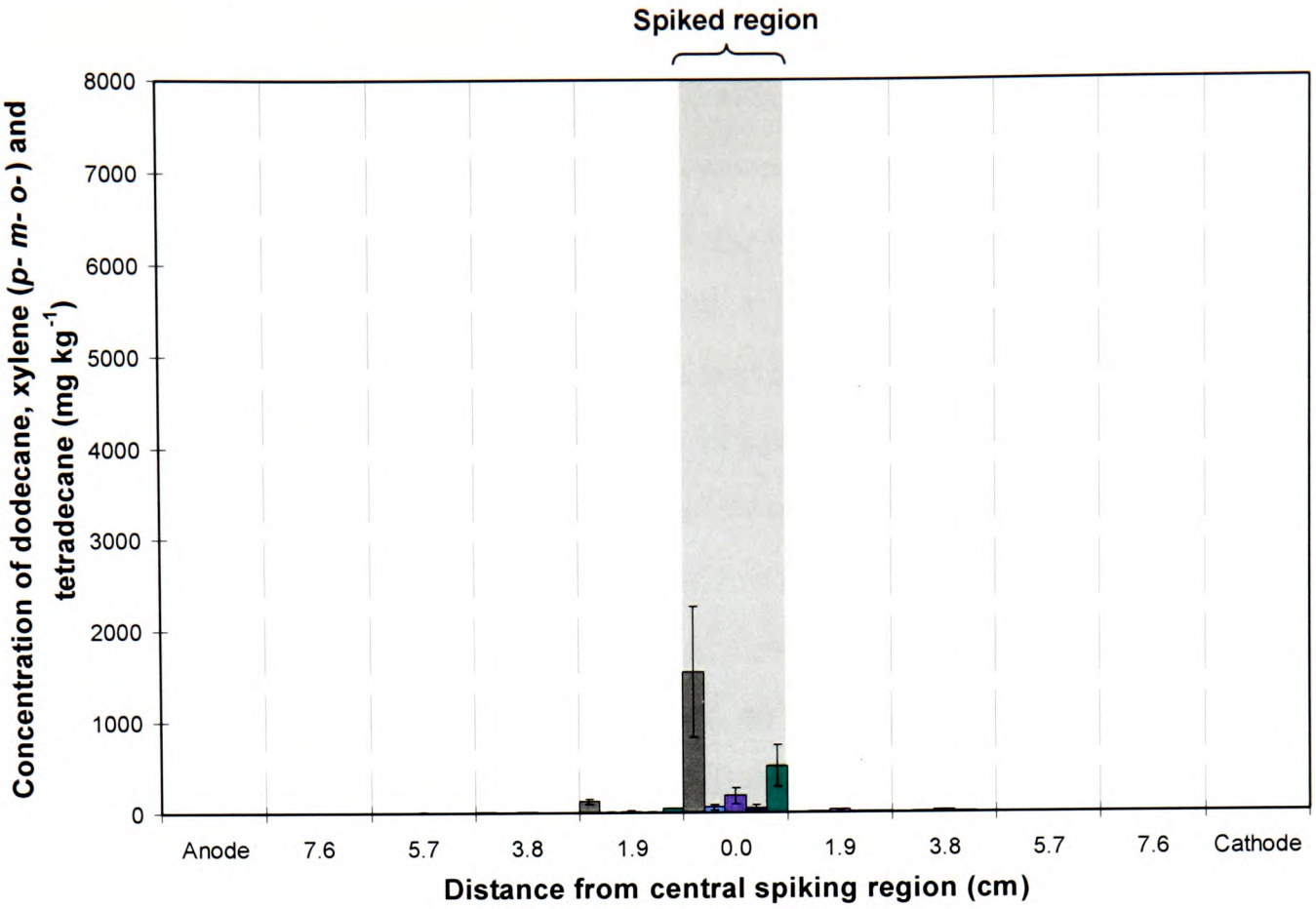


Figure 2.26 **Electrokinetics** chamber with Wytham soil spiked with DXT contaminant mix. Distribution in soil chamber of ■ dodecane (11 000 mg kg^{-1}), ■ *p*-xylene (1 900 mg kg^{-1}), ■ *m*-xylene (5 700 mg kg^{-1}), ■ *o*-xylene (1 500 mg kg^{-1}), and ■ tetradecane (4 000 mg kg^{-1}) after 14 days with 60 V applied (~10 mA) (data are means ± SE; n = 3). (Concentrations in brackets are the approximate theoretical start concentrations).

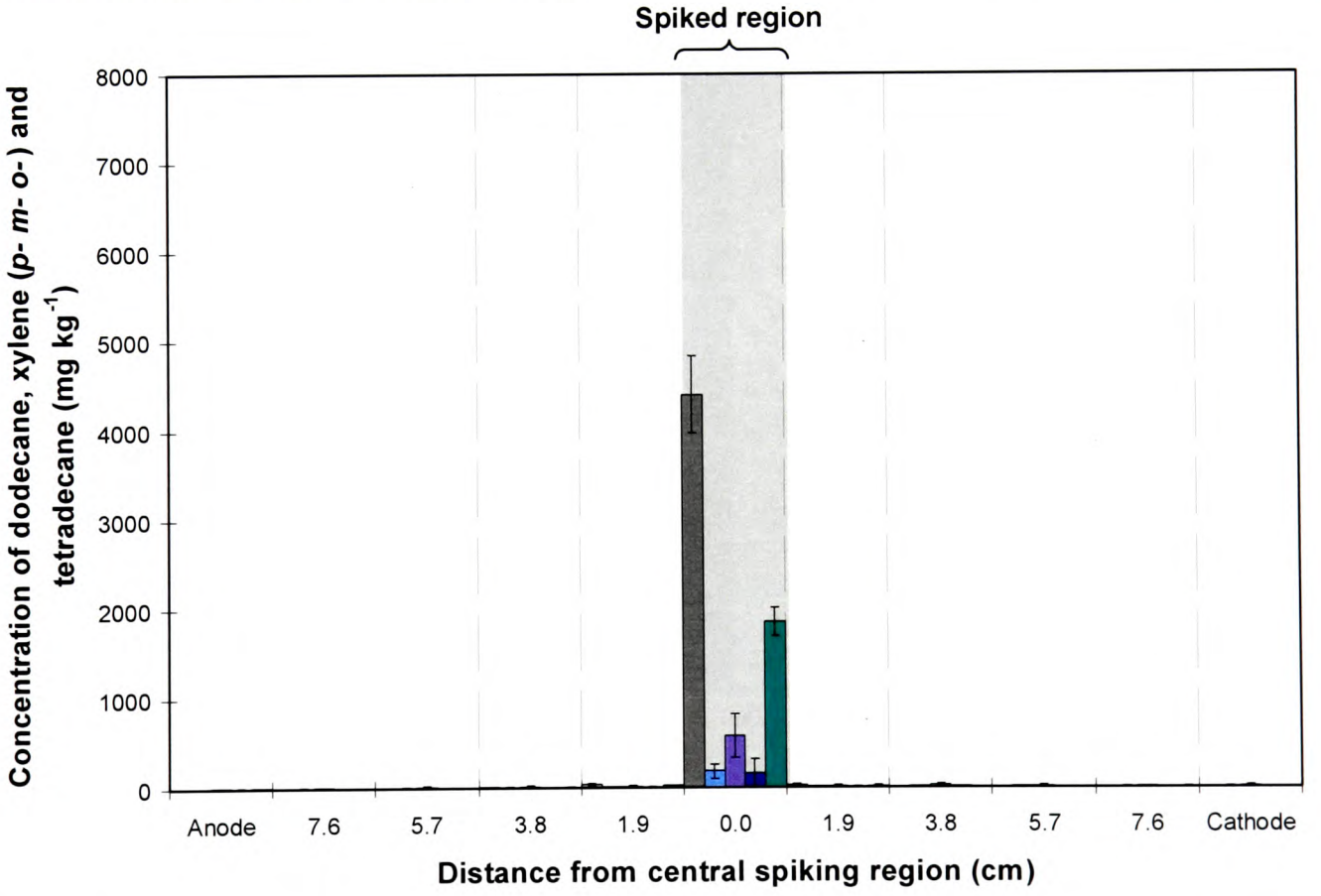


Figure 2.27 **Control** chamber with Wytham soil spiked with DXT contaminant mix. Distribution in soil chamber of ■ dodecane (11 000 mg kg^{-1}), ■ *p*-xylene (1 900 mg kg^{-1}), ■ *m*-xylene (5 700 mg kg^{-1}), ■ *o*-xylene (1 500 mg kg^{-1}), and ■ tetradecane (4 000 mg kg^{-1}) after 14 days (data are means ± SE; n = 3). (Concentrations in brackets are the approximate theoretical start concentrations).

2.4.7.2 The effect of electrokinetics (48 h) on xylene mobilisation in soil

Xylene was significantly reduced in the electrokinetics chamber over a 14-day period. Consequently 48 h experiments were set up to observe potential xylene mobilisation in the electrokinetics chamber (with Reading soil) before depletion from the soil below GC-FID detection levels had occurred. Figures 2.28 and 2.29 give further evidence of enhanced removal of *p*-xylene ($p = 0.04$, df = 4) in the electrokinetics chamber, compared to the control chamber, but lateral movement was not observed. The concentration of *m*- and *o*-isomers also reduced in the soil but there was no significant difference between the control and electrokinetics soil chamber ($p > 0.05$, df = 4). Analysis of air forced across the surface and surrounding the electrokinetics and control chambers indicated that loss of xylene in the electrokinetics chamber was not as a result of increased volatilisation. Analysis of the air sample tubes, to UKAS accreditation, indicated that there was no significant difference in the quantity of xylene in the headspace of the control compared to the electrokinetics chamber, approximately 0.16 mg compared to 0.11 mg xylene l^{-1} air 24 h^{-1}, respectively.

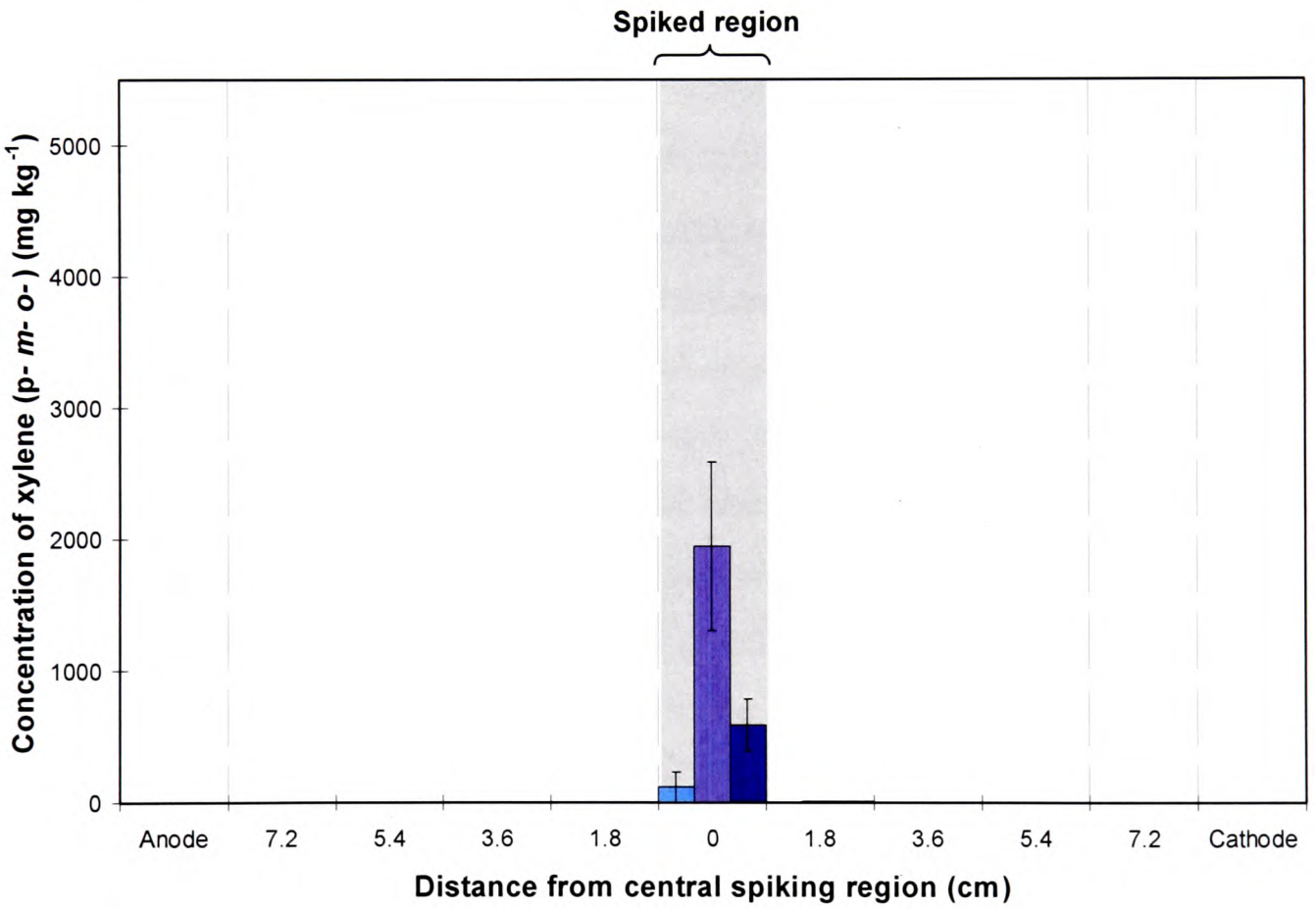


Figure 2.28 **Electrokinetics** chamber with Reading soil spiked with xylene. Distribution in soil chamber of ■ *p*-xylene (~2 800 mg kg^{-1}), ■ *m*-xylene (~8 200 mg kg^{-1}), ■ *o*-xylene (~2 100 mg kg^{-1}) after 48 h with 60 V applied (~10 mA) (data are means ± SE; n = 6). (Concentrations in brackets are the approximate theoretical start concentrations).

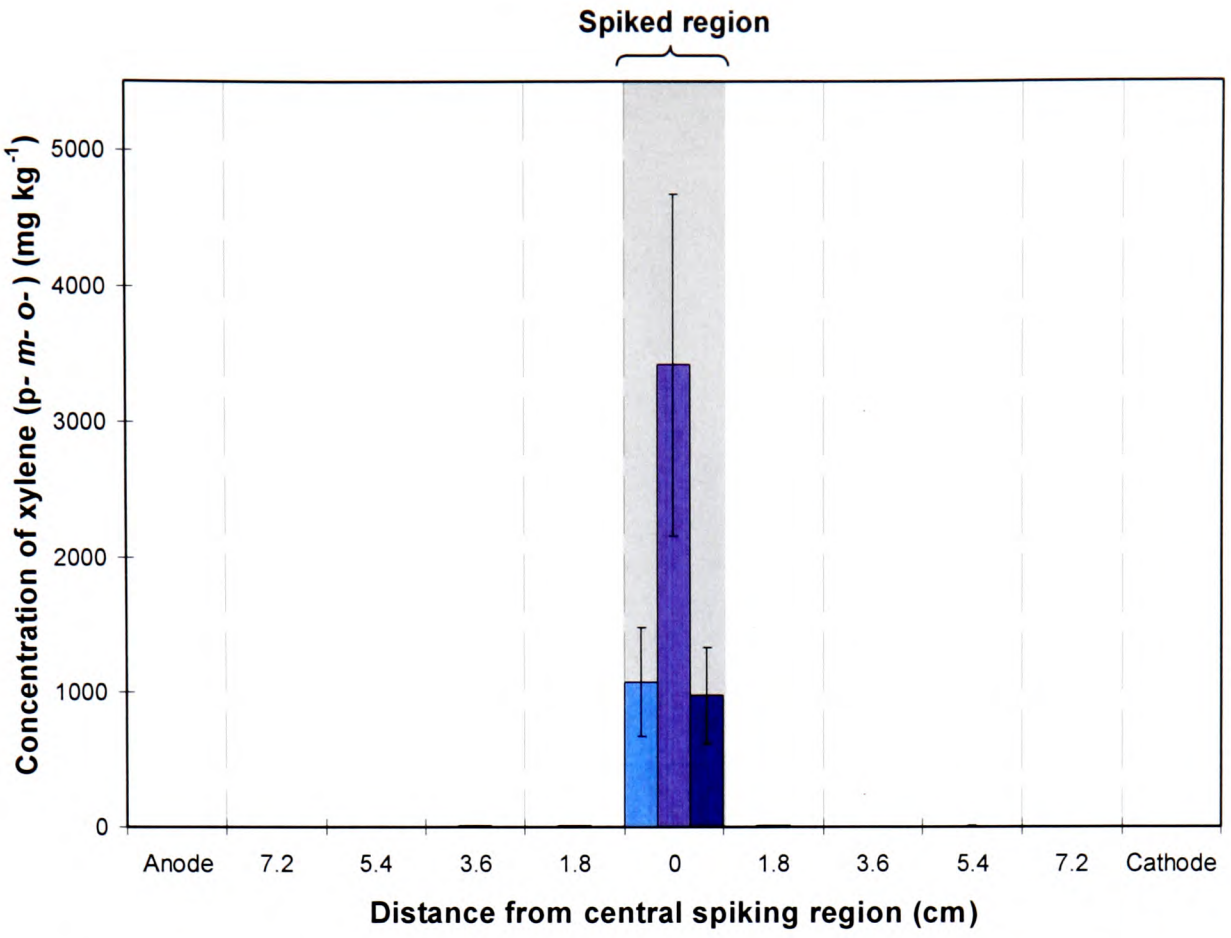


Figure 2.29 **Control** chamber with Reading soil spiked with xylene. Distribution in soil chamber of ▪ *p*-xylene (~2 800 mg kg^{-1}), ▪ *m*-xylene (~8 200 mg kg^{-1}), ▪ *o*-xylene (~2 100 mg kg^{-1}) after 48 h (data are means ± SE; n = 3). (Concentrations in brackets are the theoretical start concentrations).

2.4.7.3 Evidence of xylene movement on addition of nitrate

Experiments carried out in § 4.6.4.3 to investigate the delivery of NO_3^- into the 'spiked' central region to enhance biodegradation of the target contaminant mix (DXT) fortuitously made it possible to observe sequential movement of xylene through the soil chamber towards the cathode in the presence of an electric field. The addition of 12 000 mg l^{-1} of NH_4NO_3 into the soil chamber (delivered by electromigration) appeared to have inhibited microbial degradation of the target contaminant mix (DXT). After 14 days of applying 60 V to the soil chamber (packed with Reading soil) there was evidence to suggest that the three xylene isomers had migrated towards the cathode of the circuit, which could be attributed to the influence of electroosmosis (Figure 2.30) as compared to the distribution of the contaminant mix in the control (Figure 2.25). No xylene was detected in the catholyte of the system.

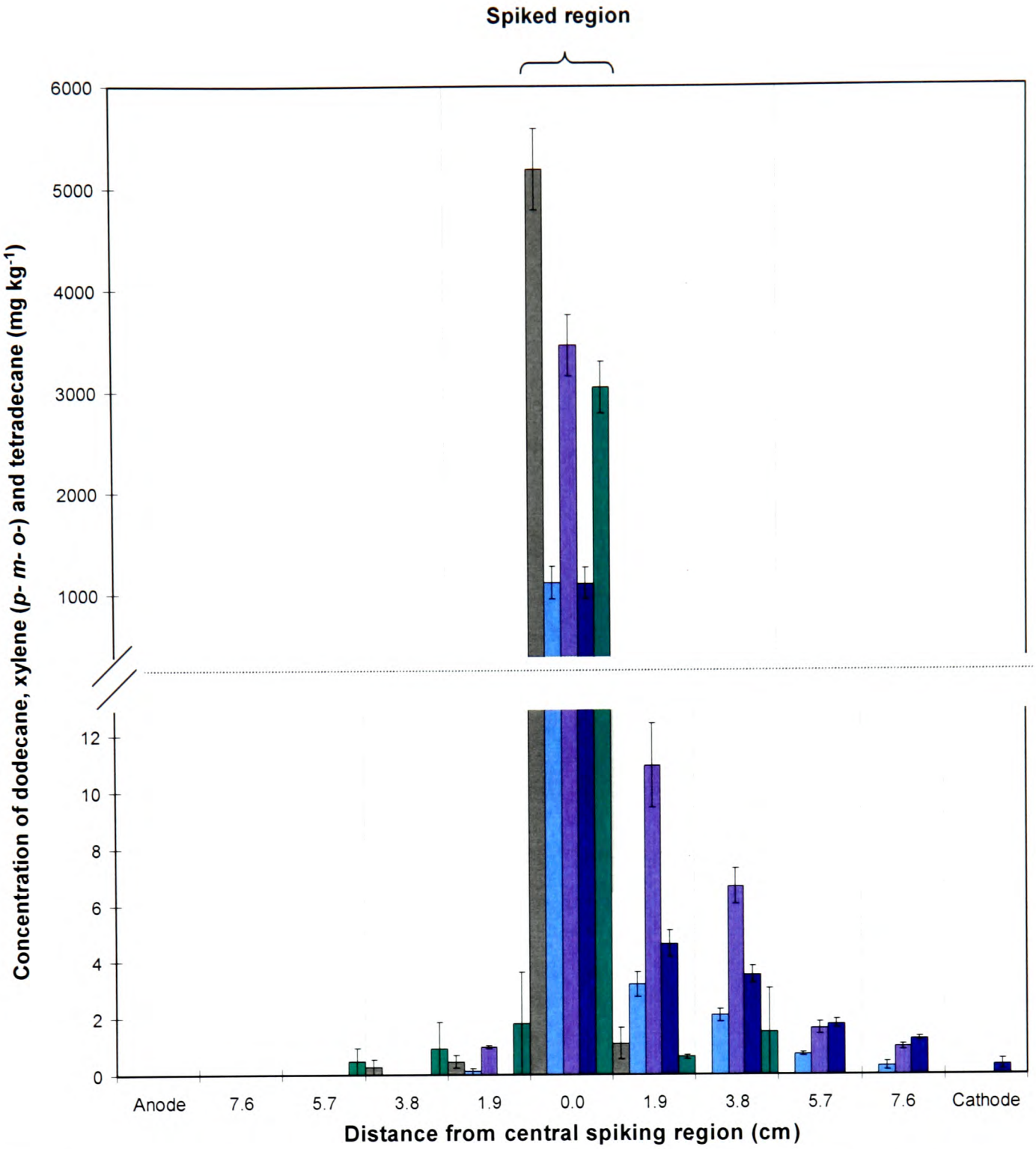


Figure 2.30 **Electrokinetics** chamber with Reading soil spiked with DXT contaminant mix. Distribution in soil chamber of ■ dodecane (11 000 mg kg^{-1}), ■ *p*-xylene (1 900 mg kg^{-1}), ■ *m*-xylene (5 700 mg kg^{-1}), ■ *o*-xylene (1 500 mg kg^{-1}), ■ tetradecane (4 000 mg kg^{-1}) after 14 days with 60 V applied (~10 mA) with NH_4NO_3 (~12 000 mg l^{-1}) added to the cathode reservoir (data are means ± SE; n=3). (Concentrations in brackets are the theoretical start concentrations).

2.4.7.4 The effect of electrokinetics (48 h) on toluene mobilisation in soil

After 14 days, toluene (8 670 mg kg^{-1}) had disappeared from the soil chamber with and without electrokinetics. Consequently (as discussed for xylene) 48 h experiments were performed. Figure 2.31 provides evidence that lateral movement towards the cathode was achieved using electroosmosis. A second experiment (data not shown) showed similar rates of disappearance of toluene from the electrokinetics chamber but failed to clearly show movement. Toluene was present in the sections towards both the anode and cathode but the maximum concentrations (which were observed in the cathode region) of 2.04 mg kg^{-1} were below the confidence levels ($p > 0.05$) of the calculable concentrations. In

the experiments performed there was a significant reduction of toluene in the electrokinetics soil chamber compared with the control chamber ($p = 0.01$, df = 4).

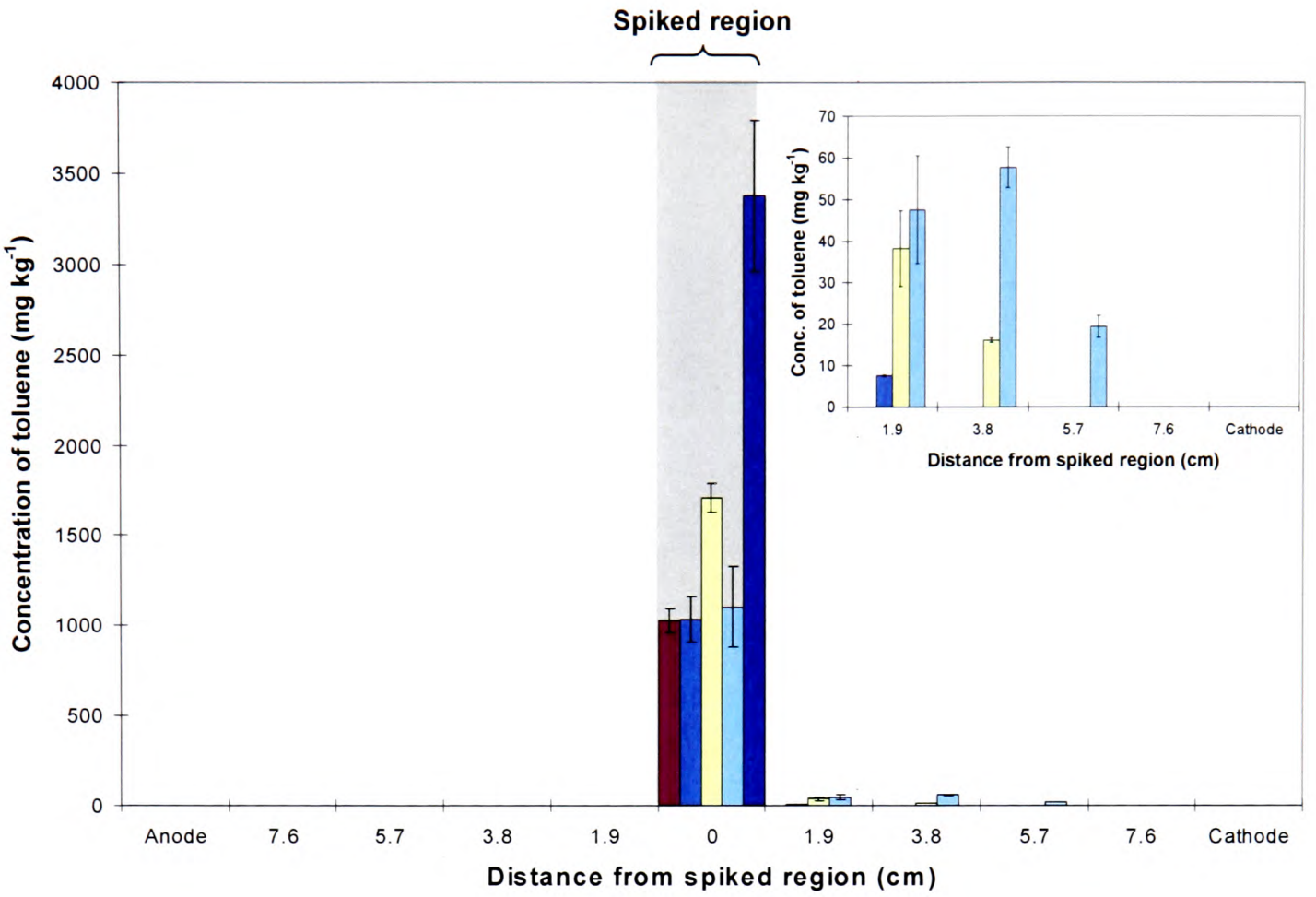


Figure 2.31 Distribution of toluene (8 670 mg kg^{-1}) in soil chamber (Reading soil) with and without electrokinetics. Concentration after ■ 6 h EK; ■ 12 h EK; ■ 24 h EK, ■ 48 h EK (60 V, 10 mA) and ■ 48 h without EK (data are means ± SE; n = 3). Inset shows toluene distribution between 1.8 cm to 7.2 cm from the central region towards the cathode. (The concentration in brackets is the approximate theoretical start concentration).

2.4.7.5 The effect of electrokinetics on 3-CB mobilisation in soil

The potential to mobilise 3-Chlorobiphenyl (3-CB) using electroosmosis in a soil system was investigated by spiking 3-CB into the central region of the electrokinetics soil chamber containing Reading soil. The distribution of 3-CB in the soil chamber after 14 days of applying 60 V (~ 10 mA) provides evidence that electroosmosis may be used to promote movement of the compound in the direction of the cathode (Figure 2.32). In addition there was a significant reduction in the concentration remaining in the electrokinetics soil chamber compared to the control chamber ($p = 0.01$, df = 4).

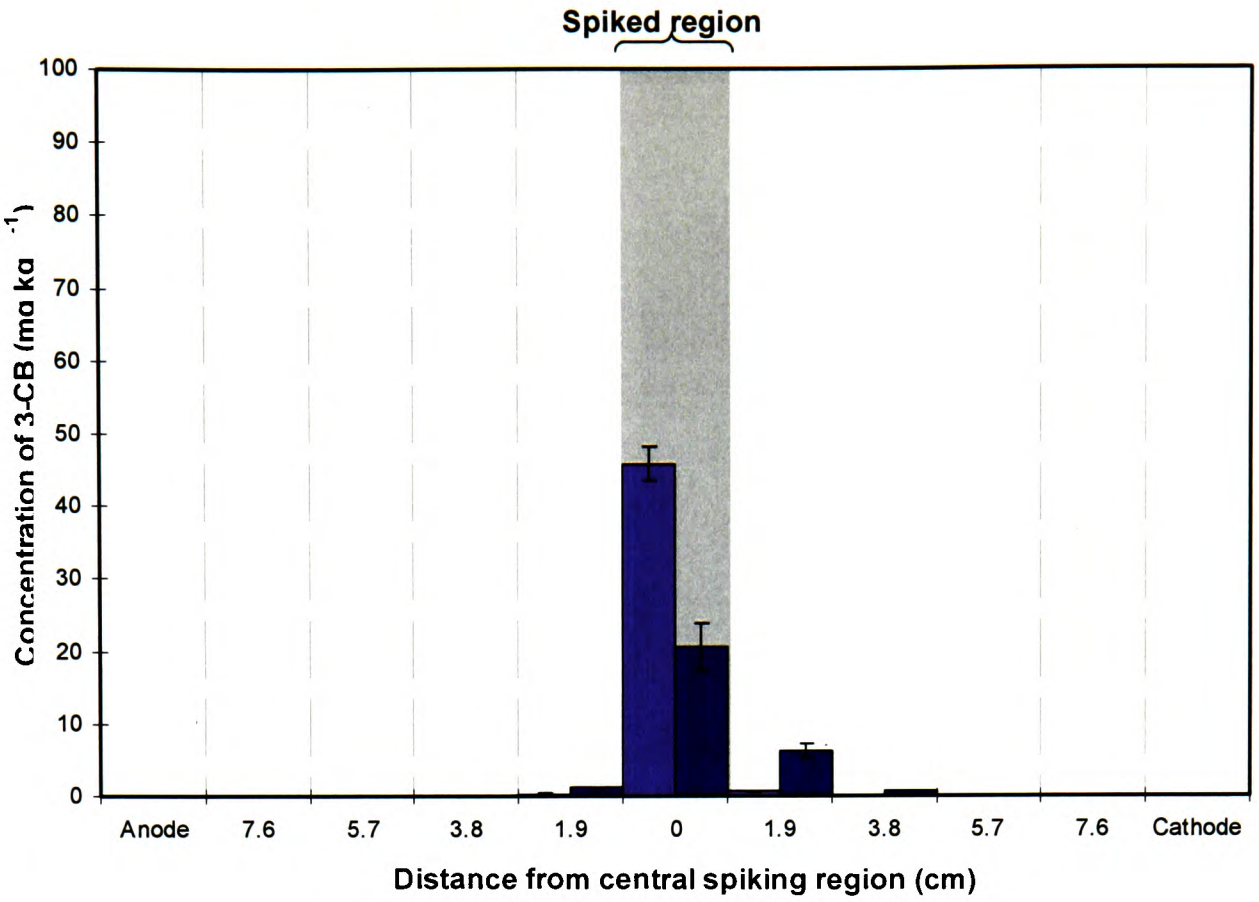


Figure 2.32 Distribution of 3-CB (100 mg kg^{-1}) in the soil chamber (Reading soil) with and without electrokinetics. Concentration and distribution after ■ 14 day EK (60 V, ~10 mA) and ■ 14 day control without EK (data are means ± SE; n = 6). (The concentration in brackets is the theoretical start concentration).

2.4.7.6 The effect of electrokinetics on octadecylbenzene mobilisation in soil

The use of electroosmosis to flush octadecylbenzene (a constituent of transformer oil) was investigated by spiking the compound into the central region of the electrokinetics soil chamber containing Reading soil. After 14 days of applying 60 V (~10 mA) to the soil chamber mobilisation of octadecylbenzene was not observed through the soil chamber (Figure 2.33). There was no significant difference observed between the concentrations remaining in either the electrokinetics or control chamber ($p > 0.05$, df = 10).

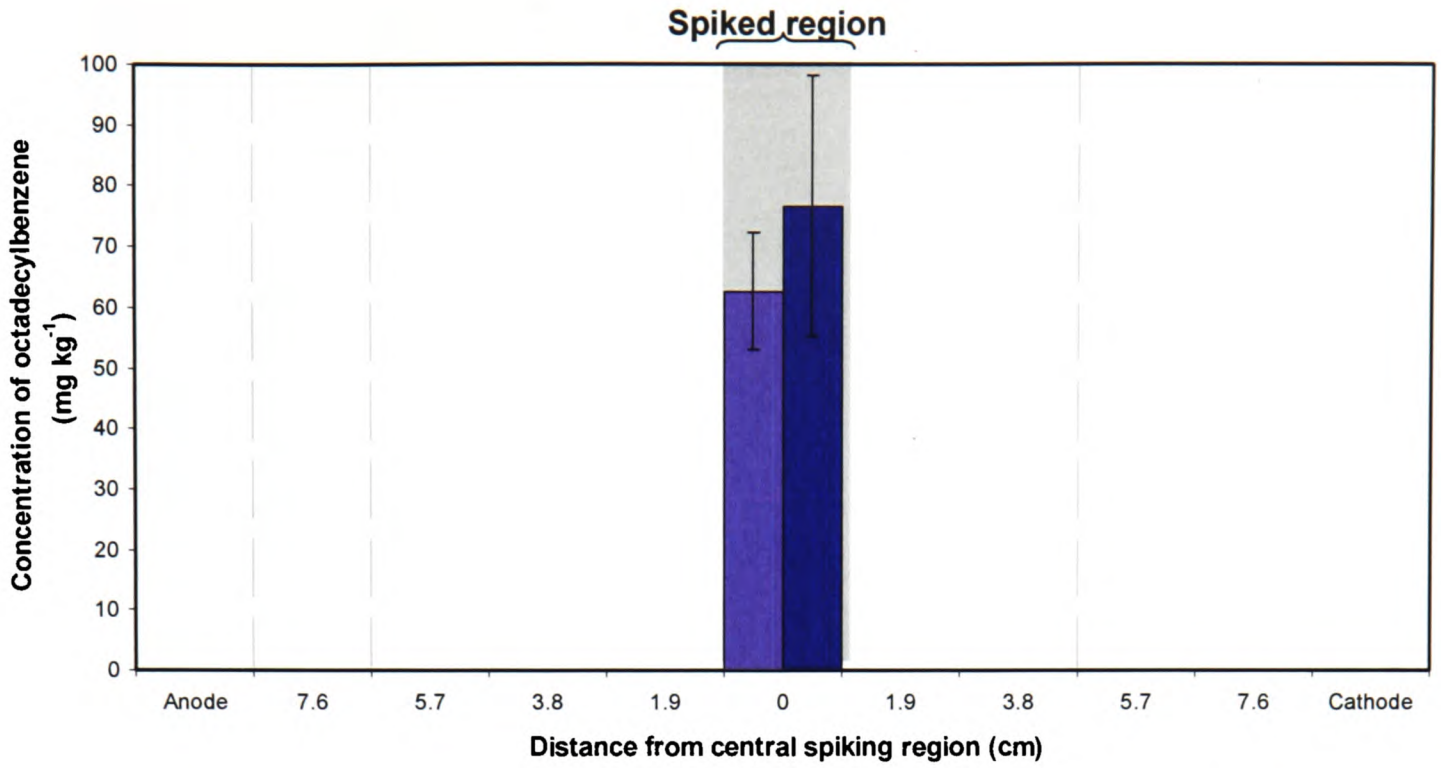


Figure 2.33 Distribution of octadecylbenzene (100 mg kg^{-1}) in the soil chamber (Reading soil) with and without electrokinetics. Concentration and distribution after ■ 14 day EK (60 V, ~10 mA) and ■ 14 day control (data are means ± SE; n = 6). (The concentration in brackets is the approximate theoretical start concentration).

2.4.8 Distribution of chloride, nitrate, phosphate and sulphate in the electrokinetic soil chamber

After 14 days of applying an electric field (60 V, ~10 mA), with the anolyte and catholyte not recirculated together the concentration of Cl^-, PO_4^{3-}, and NO_3^- was depleted. The anions had been removed from the soil via electromigration, refer to Table 2.2 for the start concentrations. There was also evidence to show that SO_4^{2-} had been mobilised through the soil under the influence of the electric field but it had not been depleted from the soil as it was being periodically supplied to the catholyte in the form of H_2SO_4 to neutralise the products of electrolysis (Figure 2.34). Approximately 500 ml of 0.1 M H_2SO_4 was used in 14 days to maintain the catholyte to approximately pH 6.

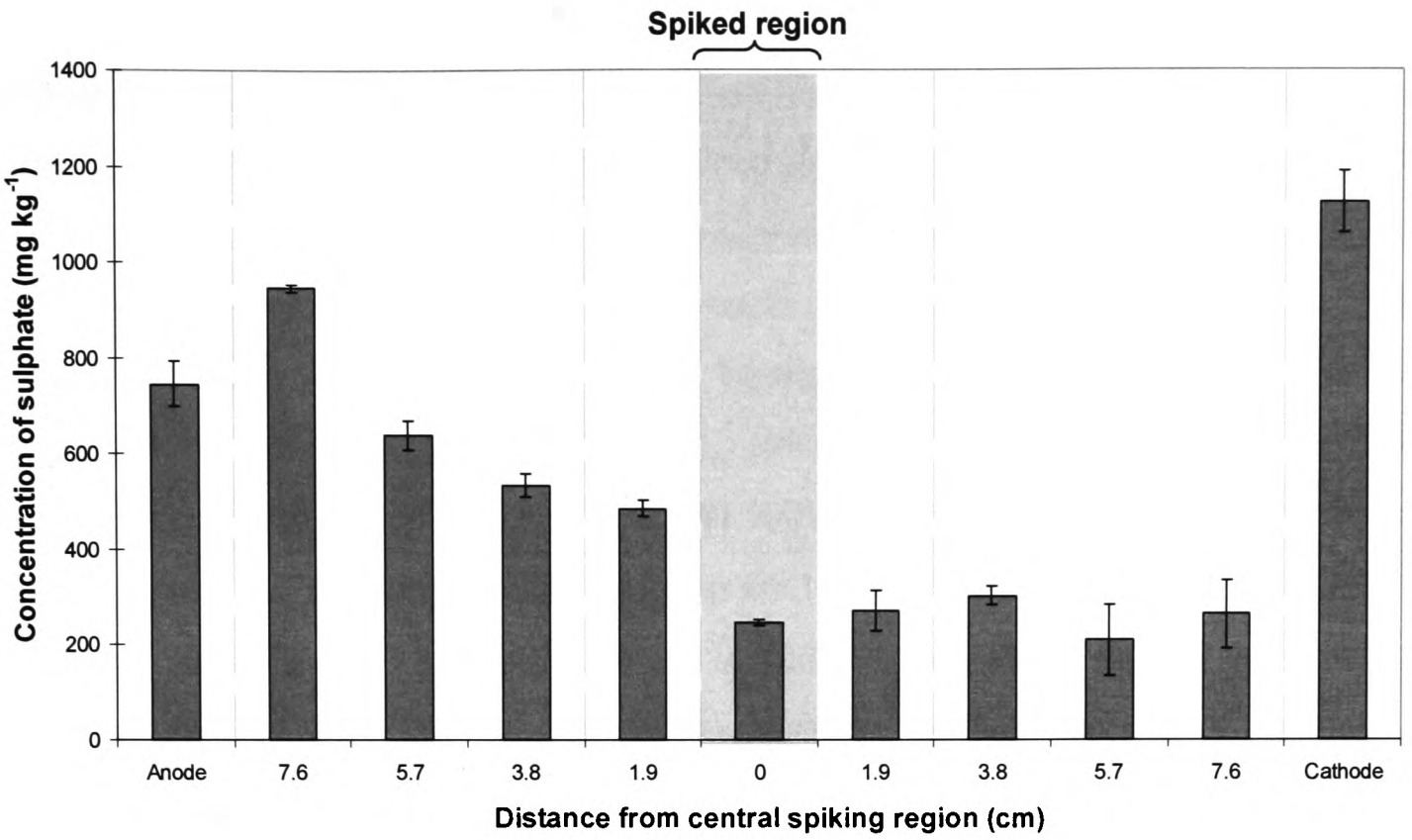


Figure 2.34 Delivery of SO_4^{2-} into the Reading soil chamber using electrokinetics (60 V, ~10 mA). H_2SO_4 (0.1 M) added to the catholyte as a buffering solution. Samples taken at end of experiment, after 14 days of applying an electric field (data are means ± SE, n = 3).

2.5 Discussion

The objective of the investigation described in this chapter was to determine the feasibility of mobilising hydrocarbons from the sub-surface using electroosmosis induced by the application of electrokinetics to the soil (§ 1.7, Objective 1). Electrokinetics experiments were run for a maximum of 14 days, to provide time to observe movement and/or degradation and also enable sufficiently numerous experiments to be undertaken.

2.5.1 Transfer of the target hydrocarbons between the soil and aqueous phases

Experiments where 2.5 ml of mains water was added in a vial containing 1 g of soil spiked with either the contaminant mix (DXT) or toluene provided evidence that the target contaminants could enter the aqueous phase from the soil. The concentration of contaminants used in the feasibility studies were the same as used in the electrokinetic soil chamber studies (§ 2.3.7.1). Figures 2.6 and 2.7 show the immediate release of the target contaminants from the soil into the aqueous phase on addition of water above the contaminant's water solubility (it is likely that the contaminants appear at a higher concentration than their water solubility as they are present as hydrocarbon droplets on the water surface rather than fully dissolved in the aqueous phase). The mass balance calculations for the experiments, performed at 24 h, indicate that significant concentrations

of contaminants were removed from the system. This loss in concentration could be attributed to microbial degradation of the contaminants. If loss of the contaminant were as a result of volatilisation, inefficient solvent extraction or sorption to the glass vials, low mass balance results would also have been observed in the adsorption experiments where sterile soil was used (*e.g.* Table 2.5). Differences observed between the adsorption of the target contaminants in the presence of either Wytham or Reading soil may be attributed to the organic content of the soil (Means *et al.*, 1980) and the WHC. Means *et al.* (1980) reported that adsorption of PAHs to different soils was greatest with the highest organic concentration. The WHC of Wytham soil was 66.3 ± 2.3% compared with a WHC of 17.0 ± 1.4% for Reading soil. The results of the feasibility studies indicate that partitioning of the target contaminants into the soil phase, with or without adsorption, reduced the effectiveness of soil flushing. It is therefore likely that the higher the WHC the greater the partitioning of the contaminant to the soil phase.

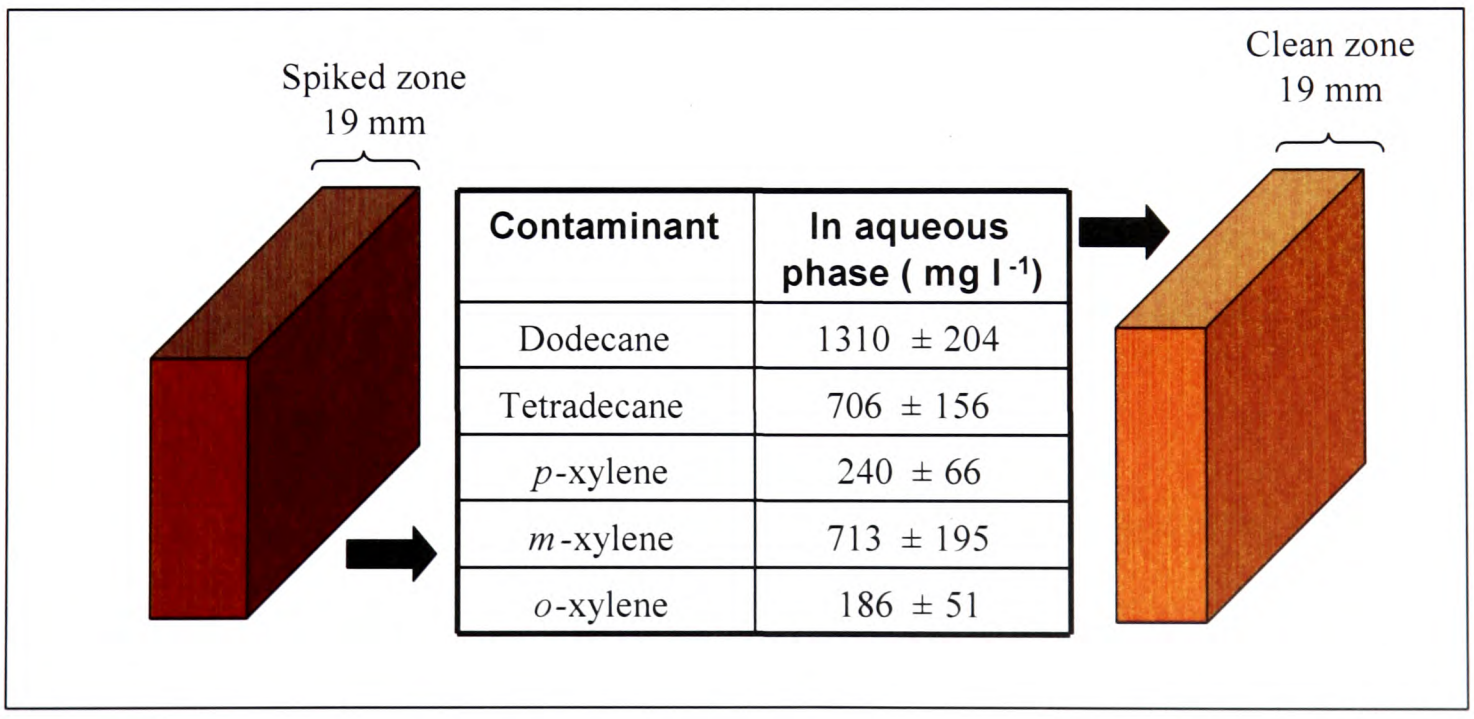
In the desorption studies it appeared that flushing spiked soil with approximately eight pore volumes of mains water was ineffective at mobilising significant concentrations of the contaminants from the soil system (Figure 2.8 and Table 2.4). Overall it appeared that the xylene isomers were more mobile than dodecane and tetradecane, which is consistent with the lower K_{ow} and higher water solubility of the aromatic hydrocarbons compared with the aliphatic hydrocarbons, which have a greater tendency to partition to the soil phase.

Lower concentrations of contaminants were flushed from the soil column than were expected based on the results of the desorption experiments using vial-based studies (§ 2.3.3.1). The spiked vials were placed on a laboratory rotor throughout the experiment and therefore the soil was dispersed increasing the surface area and therefore theoretically providing a larger surface area for adsorption of the target contaminants. Within the column experiments the soil was compacted and therefore there was a greater potential for the hydrophobic contaminants to partition within the pores and between soil particles and to bind to the organic and inorganic fractions of the soil. The experiments performed were subject to many variables including preferential pathways through the soil column, with all soil systems being subject to differences in tortuosity. Tortuosity is a measure of the path length of an element flowing through a soil (or other matrix) relative to its actual length. For example in a soil column packed with clay the size of the particles will influence flow through the column; the solution will not be able to move directly through and is likely to take the easiest pathway, which may not result in the even flushing of contaminants from the soil. However, the high concentrations of the target contaminants observed in the

aqueous phase above the solubility limit indicated that contaminants would be present in the aqueous phase to be flushed from the soil (§ 2.4.1.3).

It would be of value to carry out column experiments where the entire column could be extracted in slices so that the vertical concentration gradient of the spiked contaminants could be identified. This would be particularly relevant if it were found that the top region of the contaminated soil column had been effectively flushed and the contaminants were now concentrated further down the soil column.

The adsorption experiments provided evidence showing that if contaminants are mobilised from the spiked region of the soil, uptake (adsorption and/or sequestration) of the contaminants in clean regions of the soil chamber would occur but a percentage of contaminant may remain available in the aqueous phase for further mobilisation through the soil. Figure 2.35 depicts the concentration of the target contaminants available in the aqueous phase, based on the desorption studies using Reading soil and the DXT mix. The concentration (mg l^{-1}) in the aqueous phase is available to move away from the spiked region either through diffusion or under the influence of electroosmosis. Upon movement into a clean region of soil the contaminants would then be likely to become sorbed, degraded or volatilised. For further movement there would have to be a sufficient concentration of contaminant present in order to exceed the sorption capacity of the soil. Contaminants would then be present in the aqueous phase and be susceptible to remediation through electroosmosis.



Contaminant	In aqueous phase (mg l^{-1})
Dodecane	1310 ± 204
Tetradecane	706 ± 156
p-xylene	240 ± 66
m-xylene	713 ± 195
o-xylene	186 ± 51

Figure 2.35 Conceptual model to highlight potential for electroosmosis to be used as in situ flushing technique. Example based on desorption studies using Reading soil at time point 24 h.

Although there was no clear evidence gained in the desorption column studies to demonstrate that electroosmotic flushing of the contaminants would be effective, the data warranted further investigation using electrokinetic soil chambers. It is reported (*e.g.* Shapiro & Probstein, 1993) that electroosmosis results in a more uniform flow of water through soil as it is relatively independent of the size of the soil pores. It therefore may be more effective at flushing than where water is pressure-driven through a column of soil.

2.5.2 Electroosmotic flow rate

Where electroosmosis is observed in the 'U-tube' apparatus (Figure 2.2), the hydraulic pressure of the head of water in the electrode reservoirs has been overcome by the rate of electroosmosis. In this way the calculated flow rate always includes the reverse-acting hydraulic pressure, in the direction of the anode. The experiments revealed that water could be effectively flushed through both Reading and Wytham soil columns. This was of particular importance in the case of Wytham soil, as it was observed in the desorption column studies that it was not possible to pass water passively through the Wytham soil column with the use of an hydraulic gradient (§ 2.4.10).

A paper recently published by Heister *et al.* (2005) describes a method for using a permeameter to monitor electroosmosis alongside streaming potential (pressure-driven flow). The technique they describe overcomes the problems associated with the swelling and shrinkage of clay, which could give rise to preferential flow and unaccounted for movement in the apparatus used in this investigation, and also controls for evaporation. However, the equipment designed for this study is likely to serve as a rapid method for characterising a site for the feasibility of using electrokinetics as a remediation technique. The apparatus used in this study could be improved significantly by reducing the diameter of the reservoir chambers, allowing for the observation of smaller volumes of water displaced, thus allowing for the electroosmosis rate per hour to be calculated instead of per day.

2.5.3 The use of electroosmosis to mobilise the target hydrocarbons

Movement of dodecane, tetradecane and octadecylbenzene was not detected under the influence of electroosmosis in the performed experiments. Using the described methods (§ 2.3.8.1) movement of the hydrocarbons would have to be in centimetre increments to be detected. For electrokinetics to be used for the physical extraction of contaminants from

the soil significant movement would have to be achieved so that the contaminants could be collected from the electrolyte.

2.5.3.1 Mobilisation of xylene, toluene and 3-CB

In the initial electrokinetics experiments it was found that xylene and toluene was lost from the soil chamber in less than 14 days of applying 60 V. In order to further assess the fate of xylene and toluene, further investigations were carried out to ascertain whether contaminant loss was as a result of volatilisation, biodegradation, migration or a combination of these removal mechanisms. The results recorded in Figures 2.30 and 2.31 provide evidence that the directed movement of xylene and toluene towards the cathode could be achieved using electroosmosis. The movement of xylene was only observed where biodegradation appeared to be inhibited. There was also some evidence to show that movement of 3-CB towards the cathode could be promoted using electroosmosis (Figure 2.32). The removal of toluene and xylene from the soil system using electrokinetics showed that it may be possible to mobilise the other two BTEX compounds, benzene and ethylbenzene, through electroosmosis. Solubility of BTEX compounds is approximately 1 770, 530, 169 and 170 mg l^{-1} respectively, (van Stempvoort *et al.*, 2002) with xylene being the least soluble.

The potential to promote the movement of BTEX compounds and PCBs in soil systems using electroosmosis is highly significant for sub-surface remediation. BTEX compounds and PCBs are toxic contaminants (§ 1.2.1). One of the major limitations to the use of bioremediation *in situ* is the inefficiency of the process. Remediation programmes are operated within a risk-based framework and consequently if there are sensitive receptors at the contaminated site (*e.g.* householders at a domestic property) the time-frame available for remediation may be too short for bioremediation to be used. However, if electrokinetics could be used to rapidly remove the toxic compounds (*e.g.* BTEX and PCBs) from a site, for subsequent *ex situ* treatment or disposal, the time-frame for complete remediation of less toxic contaminants in the sub-surface could be increased making it possible for bioremediation to be used in place of intrusive technologies, such as 'dig and dump'.

2.5.3.2 Removal of the target hydrocarbons from the soil chamber

All of the target contaminants were depleted from the soil in the electrokinetics soil chamber to a greater extent than from the control chamber after 14 days (p <0.05). A reduction of contaminants in the soil system may be attributed to biodegradation with complete/incomplete mineralisation, volatilisation and/or the contaminants may have become irreversibly sorbed to the soil matrix.

An increase in soil temperature in the electrokinetics chamber due to ohmic heating may have promoted increased volatilisation of contaminants compared to the control soil chamber. However, evidence from headspace analysis during the 48 h xylene experiment did not show a significant difference between the concentrations of xylene in the headspace between the control and the electrokinetics soil chamber (§ 2.4.7.2). Amendments to the apparatus to limit volatilisation included the use of a Teflon sheet to cap the spiked region (§ 2.3.5.2).

It is likely that the increased removal of contaminants from the electrokinetics soil chamber compared with the control can be attributed to enhanced biodegradation. One of the major limitations to biodegradation is limited bioavailability (Harms & Bosma, 1997). Bacteria can be spatially discrete from the contaminants and frequently the soil matrix impedes diffusion of the contaminants. It is possible that electrokinetics may have increased bioavailability. Relatively small movements of the contaminants or the action of electroosmotic flow could render contaminants more bioavailable and thus biodegradation may take place more readily. Mobilisation of contaminants on the micron scale could be sufficient to enhance biodegradation (*e.g.* Lear *et al.,* 2004). Furthermore, electroosmosis and electromigration influence water and ion movement through the soil chamber respectively, which could result in optimisation of the environment for more efficient biodegradation if ions and water are recirculated through the soil.

To determine with a higher degree of confidence whether the increased removal of the contaminants from the electrokinetics chamber compared to the control was the result of enhanced biodegradation (as opposed to abiotic reactions) further studies are required. With the resources available it was not possible to run an entirely sterile electrokinetic experiment for the duration of two weeks; once soil is sterilised it is practically impossible to maintain a sterile soil for experiments longer than 48 h, as a result of inoculation from the air and sporulation of resistant spores. In addition, autoclaving or gamma-irradiating

soils can significantly alter the structure and thus sorption characteristics of soil (*e.g.* Sandoli *et al.*, 1996), which may have given misrepresentative results when trying to relate the findings from laboratory studies to field studies. Within Chapter 4 (Figure 4.3) it was shown that the contaminant mix (DXT) when spiked to autoclaved Reading soil and incubated at 25°C for 14 days there was no significant reduction in concentration observed. Thus, providing some evidence that disappearance of the target contaminant mix (DXT) from the soil chambers could be attributed to biotic processes.

Within the constraints of this study it was not possible to make inferences about the differences in sorption capacity between sterile and non-sterile soil as apparent degradation of the contaminants in the non-sterile soils is likely to have been attributable to the lower concentration of contaminants observed in the aqueous phase (compare Figure 2.9 and Figure 2.13).

2.5.4 Electrical resistance and pH control

In the electrokinetics chamber containing Reading soil after 14 days of applying an electric field (60 V, ~10 mA) the voltage profile remained relatively linear and there was a relatively small increase in the concentration of Ca^{2+} towards the cathode (Figure 2.18). Conversely, in Wytham soil a noticeable increase in resistance was observed across the soil chamber, which may be attributed to the accumulation of Ca^{2+} towards the cathode (Figure 2.18). Calcium phosphates, for example, are highly insoluble and precipitation of the Ca^{2+} can occur at pH elevated above neutral. Although a method of pH control was in place (pH 6 in the catholyte and pH 7 in the anolyte) a uniform neutral pH across the soil chamber could not be achieved (Figures 2.20 and 2.21) and the products of electrolysis (OH^- and H^+) migrated from the electrode reservoirs before they were neutralised by the buffering solutions. The precipitation of Ca^{2+} towards the cathode would have directly increased resistance within the soil chamber.

In subsequent experiments (see Chapter 4), modifications were made that overcame the inefficiency in buffering within the electrokinetics chamber, including: increasing the flow rate of the delivery of electrolyte from a speed of 48 ml min^{-1} to 68 ml min^{-1} and mixing the electrode reservoirs with a magnetic plate stirrer and follower.

2.5.4.1 Temperature increase

A slight increase in temperature was observed in the electrokinetics chamber compared to the control (Figure 2.19, Table 2.8). This can be explained by the influence of the ohmic

heating (Longchamp, 1989 cited in Baraud *et al.*, 1999). With increased resistance, the temperature of the soil will increase. It is reported that where higher currents are involved and where the duration of experiments is longer, temperature increase, as a result of the joule effect, can be more significant (Lageman *et al.*, 1989). The low current (10 mA) used in the electrokinetics experiments is likely to have had little impact on the joule heating effect, as reported elsewhere (*e.g.* Baraud *et al.*, 1999). As discusssed previously (§ 2.5.3.1), an increase in temperature may result in the enhanced mobilisation of volatile (and semi-volatile) organic contaminants and can also change the interfacial tension of contaminants resulting in increased solubility and volatilisation (Rosen, 1989).

2.6 Interpretive summary

1. There is evidence to support the use of electroomosis as an *in situ* flushing technique for the mobilisation of the three xylene isomers (*p*-, *m*- and *o*-), toluene, and 3-CB. Movement towards the cathode of the soil chamber was observed to be more significant than diffusion and dissolution reactions that may have resulted in movement in the control soil chamber.

2. The results of this study provide evidence that electrokinetics is not an effective *in situ* flushing technique for hydrocarbon contaminant mixtures containing straight chain alkanes and organics with low water solubilities (*e.g.* dodecane, tetradecane and octadecylbenzene). Sorption of the hydrophobic contaminants to the soil appears to limit effectiveness of the use of electroosmosis to flush the soil.

3. Increased removal of all contaminants investigated was observed in the electrokinetics chamber compared to the controls, providing evidence that electrokinetics may be able to enhance biodegradation. It was apparent that the removal of the target contaminants from Wytham soil in the presence of electrokinetics was more significant than from Reading soil (§ 2.4.8.1).

4. In all of the electrokinetic experiments, after a duration of 14 days, no contaminants were detected in the electrolyte solutions. Biodegradation rates may have been higher (or equal to) the dissolution rate, so that as contaminants were released into the aqueous phase they were degraded. As a result, movement of the contaminants as a consequence of electrokinetics could not be detected effectively using the described protocols. The mass transfer rate for the contaminant entering the aqueous phase would have to exceed the biodegradation rate to facilitate movement from the central

spiked region to the electrolyte. Furthermore, if the contaminant entered the aqueous phase and was mobilised from the spiked region, on entry into clean soil the contaminant would be susceptible to sorption restricting further movement.

5. Although water flow through clay-rich Wytham soil could not be achieved with the use of an hydraulic gradient alone, electroosmosis was shown to facilitate the movement through a column of Wytham soil (§ 2.5.3). This demonstrated the potential of electrokinetics for flushing low-permeability soils via electroosmosis.

6. A lack of homologous pH control in the electrokinetics chamber resulted in the precipitation of Ca^{2+}, which appeared to affect resistance and the permeability of the soil. In addition a small increase in temperature was noted on addition of the electric field.

3 The electrokinetic delivery of surfactants to promote mobilisation of hydrocarbons

3.1 Introduction

One of the major obstacles in remediating hydrocarbon contaminants using electroosmosis is their limited aqueous solubility (Chapter 2). Many enhancing agents (*e.g.* synthetic surfactants (surface-active agents), biosurfactants, polymers, cosolvents and commercial humic products) have been investigated for their potential to increase the solubility of hydrocarbons and thus improve the efficiency of soil remediation. In particular, there have been many studies examining the use of synthetic surfactants for the remediation of hydrocarbons from soil systems (*e.g.* Thibault *et al.*, 1996; Pennel *et al.,* 1997; Christofi & Ivshina, 2002). This chapter details an investigation of the effectiveness of using surfactants and electrokinetics to release the target contaminants (dodecane, tetradecane and xylene isomers) from the soil into the aqueous phase. Once in the aqueous phase the contaminants may be mobilised through electroosmosis and/or electromigration and could also be more bioavailable for subsequent biodegradation.

3.1.1 Surfactants

As discussed (§ 1.5.3), surfactants are generally amphiphatic molecules that can be used to lower the interfacial tensions between non-aqueous phase liquids (NAPLs) and the aqueous phase. Surfactants may be anionic, cationic, zwitterionic (contain both a negative and positive charge) or non-ionic. Surfactant molecules can aggregate to form micelles that facilitate the partitioning of NAPLs from the soil phase into the aqueous phase (Figure 3.1). Each micelle requires approximately 50 to 100 surfactant molecules (monomers), and for micelle formation to occur the surfactant must be at (or above) its critical micelle concentration (CMC); the CMC level depends on the surfactant (Christofi & Ivishina, 2002). Micelles have a hydrophilic outer surface and an inner hydrophobic core ('hydrophobic pseudo-phase'). It is within this inner core that hydrophobic NAPLs can reside and consequently enter the aqueous phase. For example, in the case of the anionic surfactant sodium dodecyl sulphate (SDS) the dodecyl group forms the hydrophobic region and the sulphate group the hydrophilic moiety.

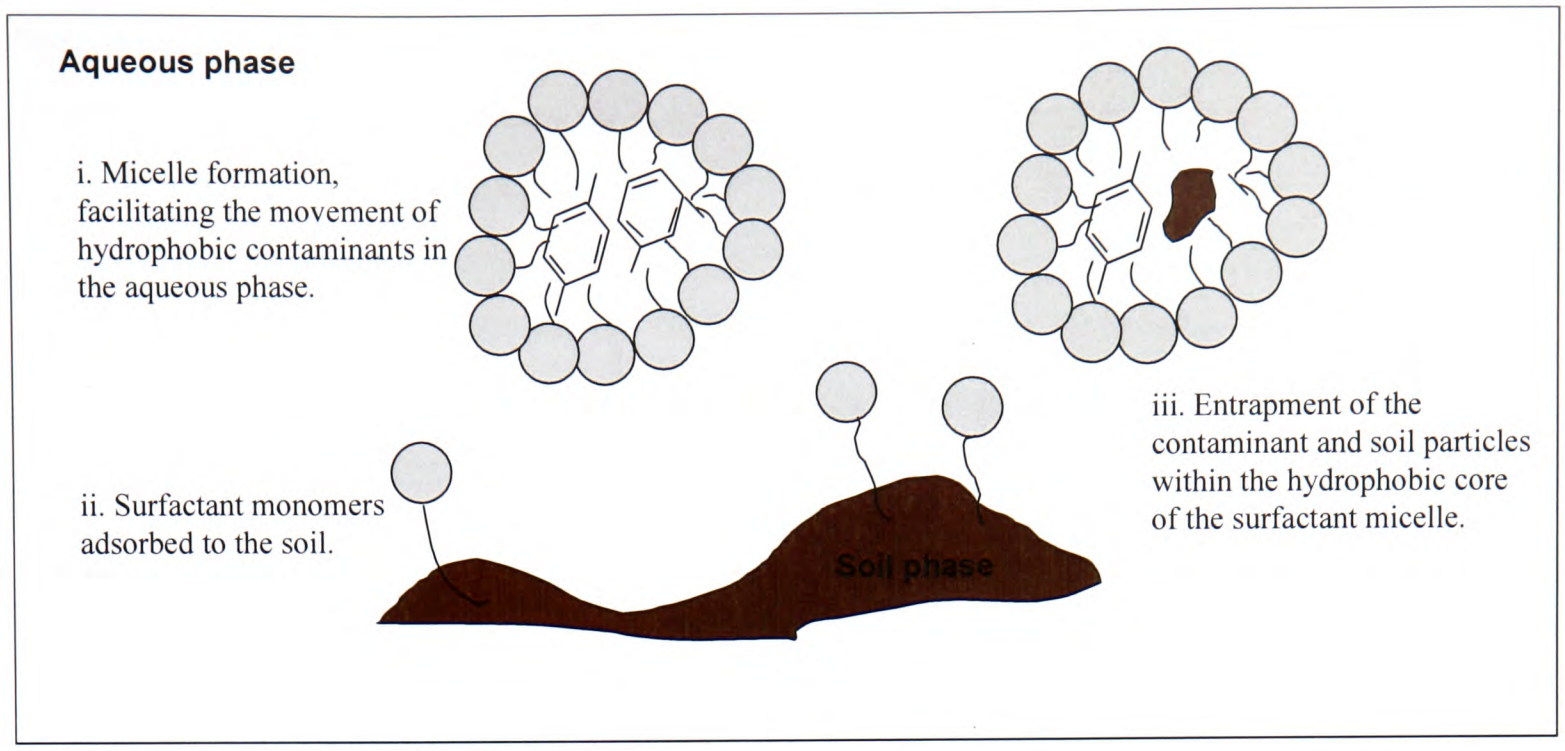


Figure 3.1 The interaction of surfactants with contaminants and the soil. (i) Micelle formation (50-100 surfactant molecules); (ii) adsorption of surfactant monomers to the soil surface; (iii) entrapment of soil and contaminants in the hydrophobic core of the micelle.

Surfactant monomers can become associated with the soil surface causing disruption to the soil surface by pulling soil particles into the aqueous phase or by affecting the zeta potential (the potential difference between the static liquid at the surface of the soil and the mobile liquid phase in contact with it) of the soil surface (depending on the charge of the surfactant). Some surfactants, particularly cationic surfactants, form strong bonds with the soil and consequently have been used in both laboratory studies and the field to immobilise contaminants onto soil surfaces (*e.g.* Boubonais *et al.,* 1995; Xu & Boyd, 1995; Moon *et al.,* 2003; Yang *et al.,* 2005). This immobilisation technique can be coupled with biodegradation to remediate hydrophobic contaminants from the soil. The cationic surfactant hexadecyltrimethylammonium (HDTMA) has been used successfully in studies to promote the immobilisation of contaminants in soil systems.

3.1.2 Biosurfactants

Many microorganisms are able to produce biosurfactants. Although there are a limited number of studies detailing the use of biosurfactants with *in situ* bioremediation there are numerous technologies that could feasibly incorporate them. The significant advantages to the use of biosurfactants *in situ*, compared to synthetic surfactants, include biodegradability (limited toxicity) and a greater tolerance of the surfactant to salt solutions (ionic strength tolerance), meaning that the ionic biosurfactants do not precipitate so readily from aqueous solution (Urum *et al.,* 2006). The main drawbacks to the use of biosurfactants *in situ* include a high cost (associated with production costs) and the limited number of feasibility studies undertaken; as a result confidence in their field use is limited.

Enrichment studies described in Chapter 4 show that bacteria directly enriched from Reading soil demonstrated the ability to produce biosurfactants using kerosene as a multiple carbon source (Figure 3.2 [ii]). This illustrates the potential of microorganisms indigenous to a contaminated site to promote bioavailability and thus aid remediation efficiency, albeit on a small-scale. The production of surfactants by microorganisms increases the bioavailability of the target contaminant by solubilising the contaminant into the aqueous phase and/or using the surfactant to facilitate adherence to the surface of the contaminant.

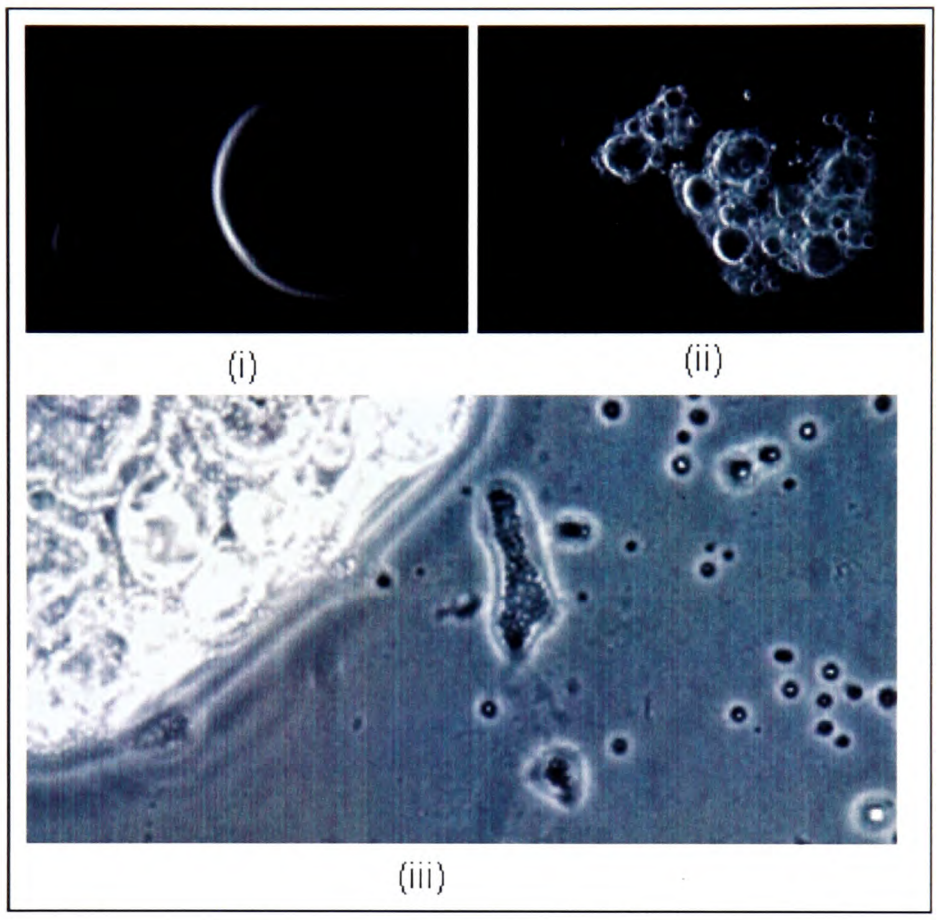


Figure 3.2 Microscopic images of hydrocarbon enrichments using 100 x oil emersion lens, total magnification x 1 000. (i) Dodecane in abiotic conditions; (ii) dodecane enrichment culture (from Reading soil), oil droplets emulsified in the presence of biosurfactants; (iii) microcolonies in the presence of kerosene.

3.1.3 Surfactants and biodegradation

The addition of surfactants can have positive and/or negative consequences to a microbial population. At concentrations equal to and less than the CMC of a surfactant, bioavailability of contaminants is likely to be increased as the interface between the NAPL and the water is increased. However, there is an upper threshold where the concentration of surfactant is so high that the contaminants become entrapped within the micelles and therefore bioavailability is reduced (Guha & Jaffe, 1996; Christofi & Ivshina, 2002). Many synthetic surfactants are also inhibitory to bacteria (Rouse *et al.*, 1994). SDS is used in protein studies, due to its ability to disrupt membranes and proteins effectively by increasing their negative surface charge, such that the surface of the proteins repel each

other. Triton X-100 and other alkylphenol ethoxylate-type surfactants may be converted to toxic alkylphenol monoethoxylates and diethoxylates or alkylphenols in anaerobic conditions (Edwards *et al.,* 1994). Generally, the shorter the side chains of the surfactant, the more toxic the compound is to microorganisms. In addition, surfactants may be used as a preferential carbon source in comparison to the target contaminant, so care has to be taken not to limit biodegradation of contaminants with the addition of surfactants (*e.g.* Thiem, 1994).

3.1.4 The electrokinetic delivery of surfactants and enhancing agents

The ionic range of surfactants (anionic, cationic, non-ionic and zwitterionic) infers that both electromigration and electroosmosis could be important mechanisms for the delivery of surfactants into a contaminated soil to aid remediation. The delivery of non-ionic surfactants into the electrokinetcs soil system will be dependent on electroosmosis, which in turn will be dependent on the charge density of the soil and the amount of pore fluid. Delivery of ionic surfactants could be achieved via electromigration towards the surfactants respective electrode (*e.g.* the movement of an anionic surfactant towards the anode). This is advantageous since the rate of injection can be controlled and movement is not dependent on the surface charge of the soil; in addition the electromigration of ions is generally considerably faster than the flux induced by electroosmosis (Acar & Alshawabkeh, 1993). As described previously, cationic surfactants are generally used to decrease the mobility of hydrophobic contaminants into the aqueous phase so they are not considered in this study. Furthermore, cationic surfactants can impede electroosmosis in the conventional direction, from the anode to the cathode, by affecting the zeta potential of the soil; the sorption of the cationic surfactant to the soil surface could result in reverse electroosmosis (§ 1.6.4.2). Nevertheless, it may be feasible to consider the use of electromigration as a technique to deliver cationic surfactants into contaminated soils to immobilise contaminants, for example to prevent them from entering the groundwater. However, using surfactants to stabilise contaminants is not likely to be a permanent solution as the surfactants may be eventually degrade, releasing the contaminants from the soil surface.

The key to the use of electroosmosis as a flushing technique, is either to target contaminants that are already soluble in the aqueous phase or to incorporate an enhancing agent to mobilise the contaminant into the aqueous phase for subsequent flushing. To date a number of studies have been undertaken to investigate the use of surfactants with

electrokinetics to remediate soils of contaminants. Yang *et al.* (2005) described the use of the non-ionic surfactant alkyl polyglucosides (APG) and anionic surfactant Calfax 16L-35 for the electrokinetic remediation of phenanthrene, showing increased phenanthrene removal from soil using APG, compared to experiments without the surfactant. However, this work did not investigate the feasibility of using electromigration to deliver the anionic surfactant into the soil and subsequently the surfactant's effectiveness upon addition to the anolyte was limited. Reddy & Saichek (2003) investigated the use of Tween 80 and ethanol, again showing increased desorption of phenanthrene, but unknown factors resulted in re-adsorption during electrokinetic treatment. Yuan & Weng (2004) investigated the use of a surfactant mix, containing SDS and Pannox 110, to promote the removal of ethylbenzene from the soil using electroosmosis. The surfactants were added to the soil using a combination of electroosmosis and electromigration and their results (without repeats) gave some evidence that the surfactants enhanced solubility and thus aided remediation by electroosmosis.

Other enhancing agents such as co-solvents (*e.g. n*-butylamine [Li *et al.* 2000]), chelating agents (*e.g.* EDTA [Wong *et al.,* 1997]) and compounds such as humic acid have been noted in the literature for their ability to aid *in situ* flushing of contaminants from soils. Humic acid is mentioned in the literature as a potential organic carrier in soil systems (Cho *et al.,* 2002; Van Stempvoort *et* al., 2002). In their studies, Van Stempvoort *et al.* (2002) tested the ability of humic acid (1 g l^{-1}) to enhance the remediation of diesel. They concluded that humic acids, whilst not enhancing the flushing rate of BTEX compounds, increased the removal of methylated naphthalenes. Sawada *et al.* (2003) used humic acid as a surfactant for the electrokinetic remediation of copper(II)-oxinate, increasing the removal of the contaminant from the soil by up to three-fold. Although humic acids have a negative charge at neutral pH, Sawada *et al.* (2003) found that electroosmotic movement of humic acids dominated over electromigration, so migration was predominately to the cathode of the circuit. It is also recognised (Chiou *et al.,* 1984) that the presence of high molecular weight humic substances could greatly enhance solubility of non-ionic organics by providing a hydrophobic region (a 'partition medium') (Chiou, 1989). Wong *et al.* (1997) demonstrated the ability to deliver the chelating agent EDTA (negatively charged ethylenediaminetetraacetic acid) into soil through electromigration, for the laboratory-scale remediation of lead and zinc by electromigration towards the anode of the system.

3.1 Chapter objectives

The objective of the investigations detailed in this chapter was to determine whether electrokinetics could be used as a delivery system for surfactants to subsequently increase the mobilisation of the target contaminants (dodecane, xylene and tetradecane) from the soil using electroosmosis (§ 1.7, Objective 2) (Figure 3.3). The non-ionic surfactants Span 85 (sorbitan trioleate) and Triton X-100 (polyoxyethelyene) and the ionic surfactant SDS were investigated: firstly for their mobility within an electric field, and secondly for their ability to mobilise hydrocarbons.

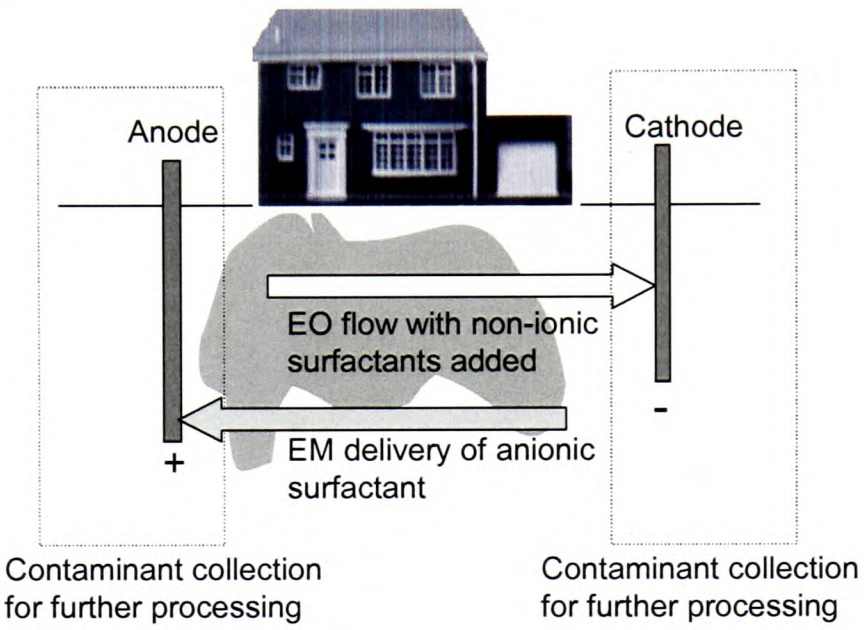


Figure 3.1 Objective 2. Schematic showing electrokinetic delivery of surfactants to promote removal of hydrocarbons from the soil.

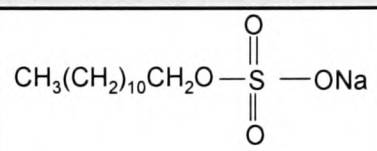
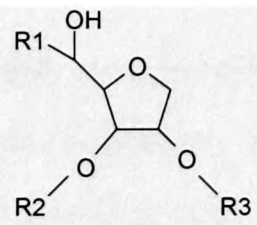
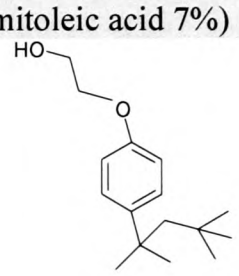
3.2 Materials and methods

Reading and Wytham soils (§ 2.3.2) were used in the investigations. The general physical characteristics of the surfactants are detailed in Table 3.1.

3.2.1 Surfactant column studies

Column studies were carried out to determine the suitability of the chosen surfactants to mobilise the target contaminants from the soil system. The experiments were set-up, as described in Section 2.3.3.2, using Reading soil. Triton X-100, Span 85 and SDS were tested at a concentration of 0.4%. One column pore volume (3.8 ml) of surfactant solution was passed through the columns daily on seven consecutive days. The breakthrough solutions were collected and analysed for hydrocarbons using the general method for GC-FID (§ 2.3.6.3). As a control, one column was flushed with mains water to evaluate the effect of dissolution on the movement of hydrocarbons. A higher surfactant concentration (1%) was also tested using the described methods.

Table 3.1 Physical characteristics of the surfactants used.

Surfactant	Molecular Formula	Structure	MW	CMC	Ref.
SDS (anionic)	$CH_3(CH_2)_{11}OSO_3Na$		288	*2380.0 mg l^{-1}	Sigma Aldrich; *Sanchez-Camazano *et al.*, 2000
Span 85 (non-ionic)	$C_{60}H_{108}O_8$	 (R1, R2 & R3 = Oleic acid 74%; linoleic acid 2%; linolenic acid 2%; palmitoleic acid 7%)	957	90.0 mg l^{-1}	Sigma Aldrich.
Triton X-100 (non-ionic)	$C_8H_{17}C_6H_4(OC_2H_4)_{10}OH$		625	13.6 mg l^{-1}	Kim *et al.*, 2001

3.3.2 The use of electrokinetics to deliver surfactants into the soil

The rate of movement of SDS, Triton X-100 and Span 85 into the soil system from the reservoir chamber was monitored over 72 h with 60 V applied (~10 mA). Initially Dr Lange™ cuvette tests (Hach Lange Ltd., Manchester, UK) were used to measure the movement of the test surfactants. However, these tests for both anionic and non-ionic surfactants (LCK 332 and 333, respectively) appeared to give erroneous results from soil samples due to humic material affecting the colorimetric tests. Furthermore, Span 85 could not be analysed using the test kits because the test kits are dependent on the presence of ethoxylates, which are not found in Span 85.

The U-tube apparatus (designed to measure electroosmotic flow [§ 2.3.4]) was used to determine if electrokinetics could be used to deliver surfactants into the soil (Reading soil was used). After addition of the target surfactant to the anode or cathode (anode for non-ionic surfactants for electroosmotic delivery, cathode and anode for the anionic surfactant SDS for delivery via electroosmosis and/or electromigration) the electrolyte solutions were monitored for 72 h. The movement of surfactants into the reservoirs was followed quantitatively using chemical oxygen demand (COD) tube-tests (LCK 514, Dr Lange). The movement of surfactant into the electrolyte would raise the COD as surfactants are

carbon-rich (Table 3.1). The tube-tests were heated using a Thermostat LT 100 (Dr Lange) at 148°C for 2 h and analysed using a photometer (Lasa 100, Dr Lange) at 605 nm. In the tube-tests dichromate oxidises organic matter to end products of H_2O and CO_2 while being reduced to Cr^{3+} in the presence of a silver sulphate catalyst. The amount of dichromate reduced is proportional to the COD of a sample (Palintest Ltd., Tyne on Wear, UK).

3.3.3 Movement of target hydrocarbons with an anionic surfactant

SDS, an example of an anionic surfactant, was investigated for its ability to aid in the mobilisation of the target contaminants dodecane, xylene isomers (*p*-, *m*-, *o*-) and tetradecane (DXT) in both Wytham and Reading soil. The electrokinetics soil chamber was spiked with the standard contaminant mix (DXT) at concentrations of 11 000, 9 100 and 4 000 mg kg^{-1}, respectively (§ 2.3.5.2). A 0.4% SDS solution was added to the cathode reservoir each day for a period of 14 days with 60 V applied with the aim of delivering it to the central 'spiked' region via electromigration. The soil chambers were destructively sampled after 14 days (§ 2.3.5.5) to identify the distribution and concentrations of the target contaminants throughout.

3.3.4 Movement of target hydrocarbons with non-ionic surfactants

Triton X-100 and Span 85, non-ionic surfactants, were investigated individually for their ability to aid in the mobilisation of the standard contaminant mix containing dodecane, xylene, and tetradecane (DXT), at concentrations of 11 000, 9 100 and 4 000 mg kg^{-1}, respectively (§ 2.3.5.2). In each of the experiments a 0.4% surfactant solution was added to the anode reservoir daily for a period of 14 days with 60 V applied with the aim of delivering it to the central 'spiked' region via electroosmosis. The electrokinetics chambers were destructively sampled at the end of the experiment (§ 2.3.5.5). Reading soil was used as the model soil for investigating the use of the non-ionic surfactants.

3.4 Results

3.4.1 Surfactant column studies

Column studies using the surfactants SDS, Triton X-100 and Span 85 as flushing agents at a concentration of 0.4% yielded analogous profiles to those observed when mains water was used as the purging solution through a glass column containing Reading soil. For logistical reasons mass balance analysis was not undertaken but the results (Table 3.2) give an indication of the feasibility of using the selected surfactants for the mobilisation of the target contaminant mix (DXT) from Reading soil. It was not possible to compare the efficiency of the three surfactants with water as loss of the contaminant via biodegradation, preferential migration pathways in the soil column, and volatilisation were not controlled in the experiments.

Table 3.2 The concentration of contaminant (DXT) flushed from the soil column (Reading soil) on day 7. Data are means ± SE; n = 3.

	Concentration flushed from soil column (mg l^{-1})			
Contaminant	**Mains water**	**SDS (0.4%)**	**Span 85 (0.4%)**	**Triton X-100 (0.4%)**
p-Xylene	67.5 ± 5.8	36.2 ± 1.5	13.5 ± 1.9	10.3 ± 1.6
m-Xylene	196.7 ± 16.1	109.0 ± 2.0	39.9 ± 6.0	32.0 ± 4.3
o-Xylene	67.7 ± 4.8	38.6 ± 8.3	13.6 ± 2.2	11.3 ± 1.3
Dodecane	17.8 ± 9.2	43.6 ± 18.5	11.1 ± 6.1	38.6 ± 7.7
Tetradecane	7.5 ± 3.5	19.6 ± 2.7	3.9 ± 2.1	14.2 ± 2.7

It was found that where concentrations of the surfactants used were 1% or higher the permeability of the soil was lowered. For example, the first three pore volumes of 1% SDS flushed through the soil column in approximately 20 min. Thereafter, for one pore volume of 1% SDS to be flushed through the soil column it took longer than 24 h. Consequently, in this study only surfactant concentrations of 0.4% were investigated in the electrokinetics soil chamber experiments (§ 3.4.3).

3.4.2 The use of electrokinetics to deliver surfactants into the soil

COD concentrations were measured in the anolyte (anode reservoir) and catholyte (cathode reservoir) over a 72 h (3 day) time period to qualitatively identify the directed movement of surfactant through the soil column using electrokinetics. Visually, it was evident that mobilisation of the surfactants through the soil column had occurred: an emulsion appeared on the surface of electrolyte in the opposite electrode to that in which the surfactant had

been introduced. Furthermore, where the anionic surfactant SDS was delivered into the soil via electromigration from the cathode, after 48 h SDS was observed to accumulate on the front edge of the soil column from the catholyte (Figure 3.4).

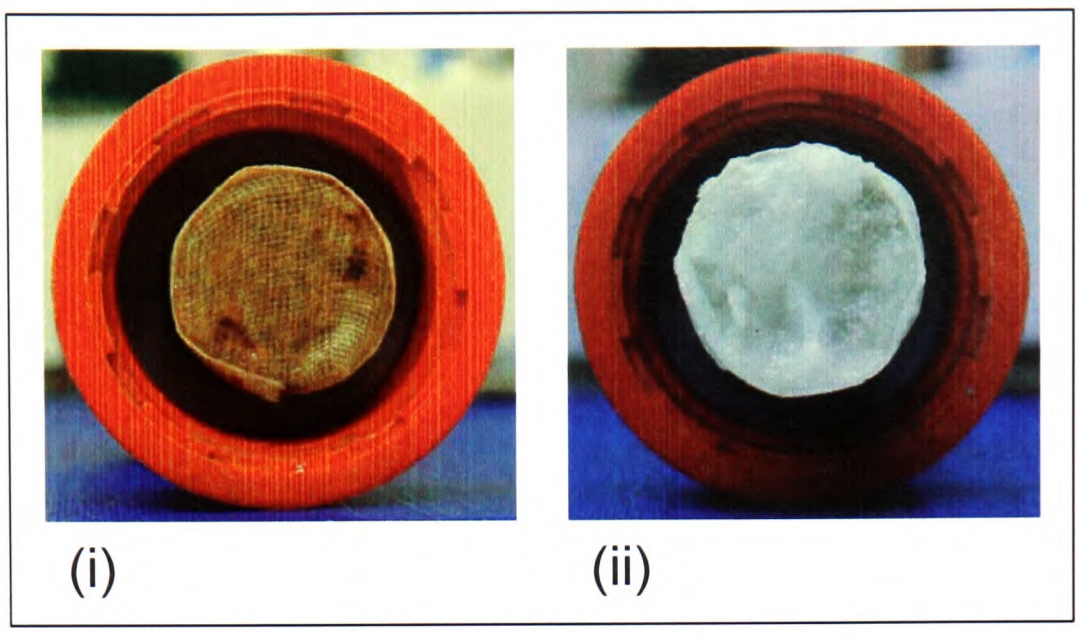


Figure 3.4 Accumulation of SDS from catholyte solution (0.4% SDS) onto the cathode membrane after 48 h electrokinetics. Compare the cathode membrane before (i) and after application of 60 V for 48 h (ii). (Refer to Figure 2.2 for a photograph of the complete apparatus).

Both electroosmotic and electromigratory delivery of 0.4% SDS was achieved in the investigation (Figures 3.5 and 3.6) by applying 60 V (~10 mA) across the soil column. The electroosmotic delivery of SDS from the anode to the cathode (Figure 3.5) was evidently more efficient than delivery via electromigration (Figure 3.6), with 0.4% added to the cathode at the start of the experiment as indicated by the change in COD concentration between the electrolytes.

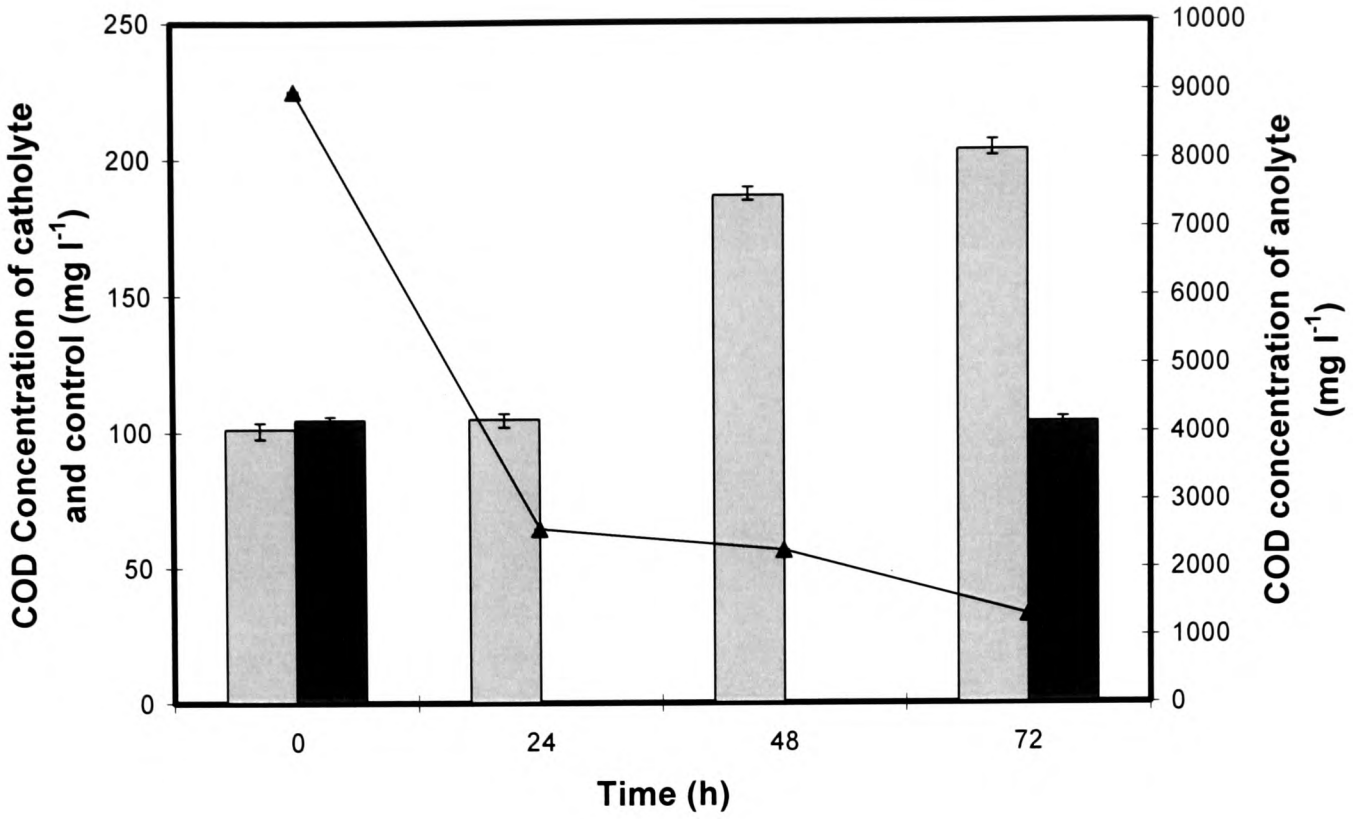


Figure 3.5 Changing concentration of SDS in the electrolytes on application of an electric field (60 V) after 0.4% SDS was added to the anode. Delivery of SDS via electroosmosis investigated over 72 h through a soil column containing Reading soil. COD (mg l^{-1}) of catholyte; ■ COD (mg l^{-1}) of control reservoir; and ▲ COD (mg l^{-1}) of anolyte (data are means ± SE, n = 3).

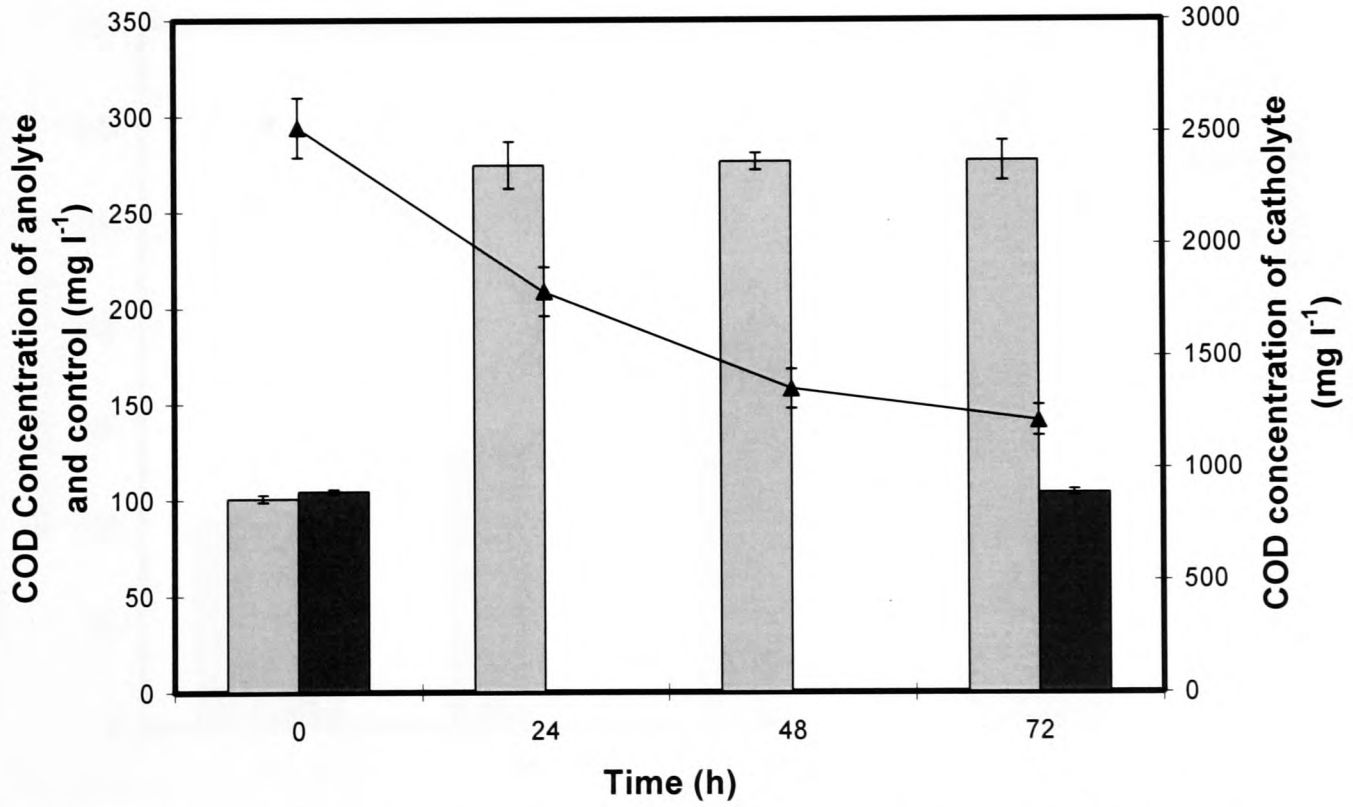


Figure 3.6 Changing concentration of SDS in the electrolytes on application of an electric field (60 V) after 0.2% SDS was added to the cathode. Delivery of SDS via electromigration investigated over 72 h through soil column containing Reading soil. COD (mg l^{-1}) of anolyte; ■ COD (mg l^{-1}) of control reservoir; and ▲ COD (mg l^{-1}) of catholyte (data are means ± SE, n = 3).

The electroosmotic delivery of the non-ionic surfactants Span 85 and Triton X-100 was achieved using electroosmosis, as shown in Figures 3.7 and 3.8, respectively.

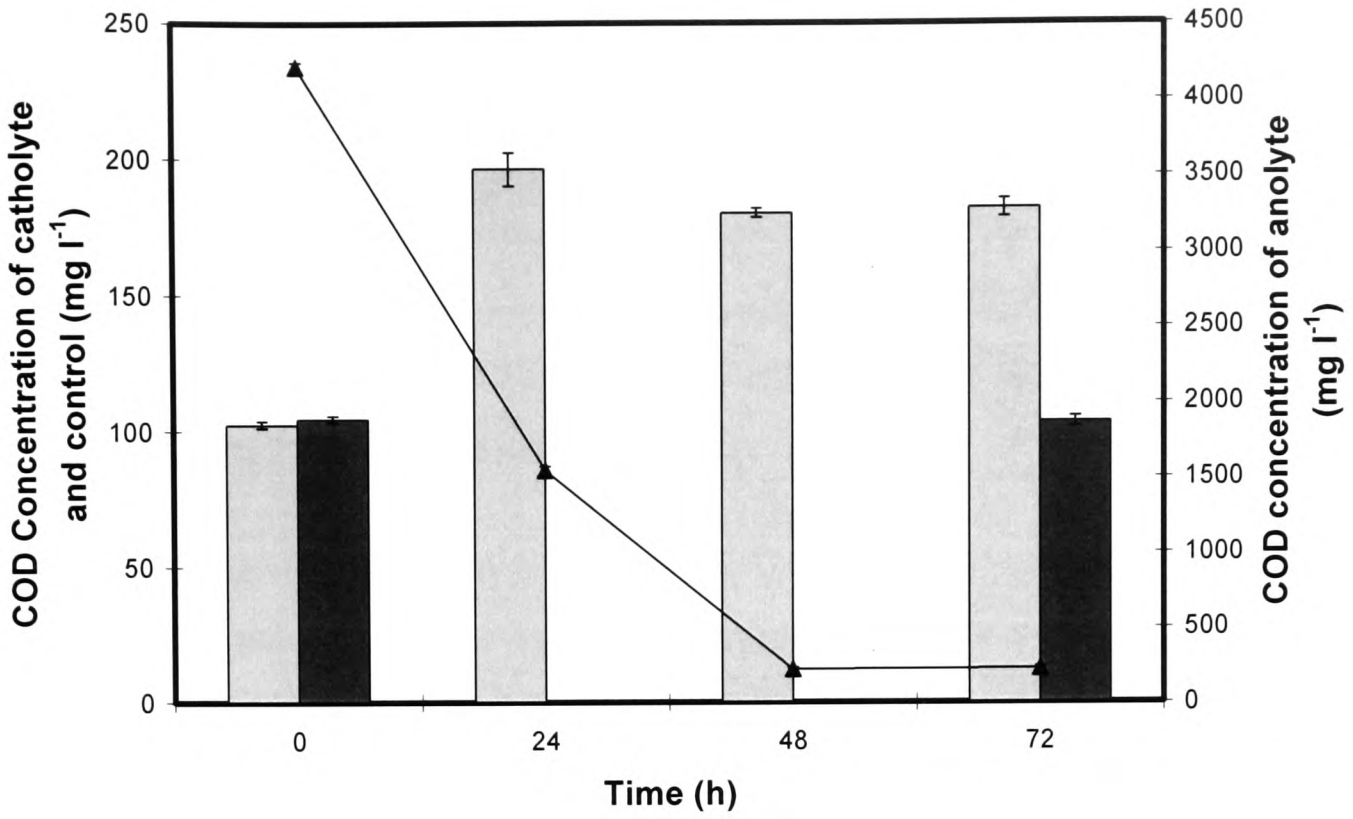


Figure 3.7 Changing concentration of Span 85 in the electrolytes on application of an electric field (60 V) after 0.4% Span 85 was added to the anode. Delivery of Span 85 via electroosmosis investigated over 72 h through a soil column containing Reading soil. COD (mg l^{-1}) of catholyte, ■ COD (mg l^{-1}) of control reservoir, and ▲ COD (mg l^{-1}) of anolyte (data are means ± SE, n = 3).

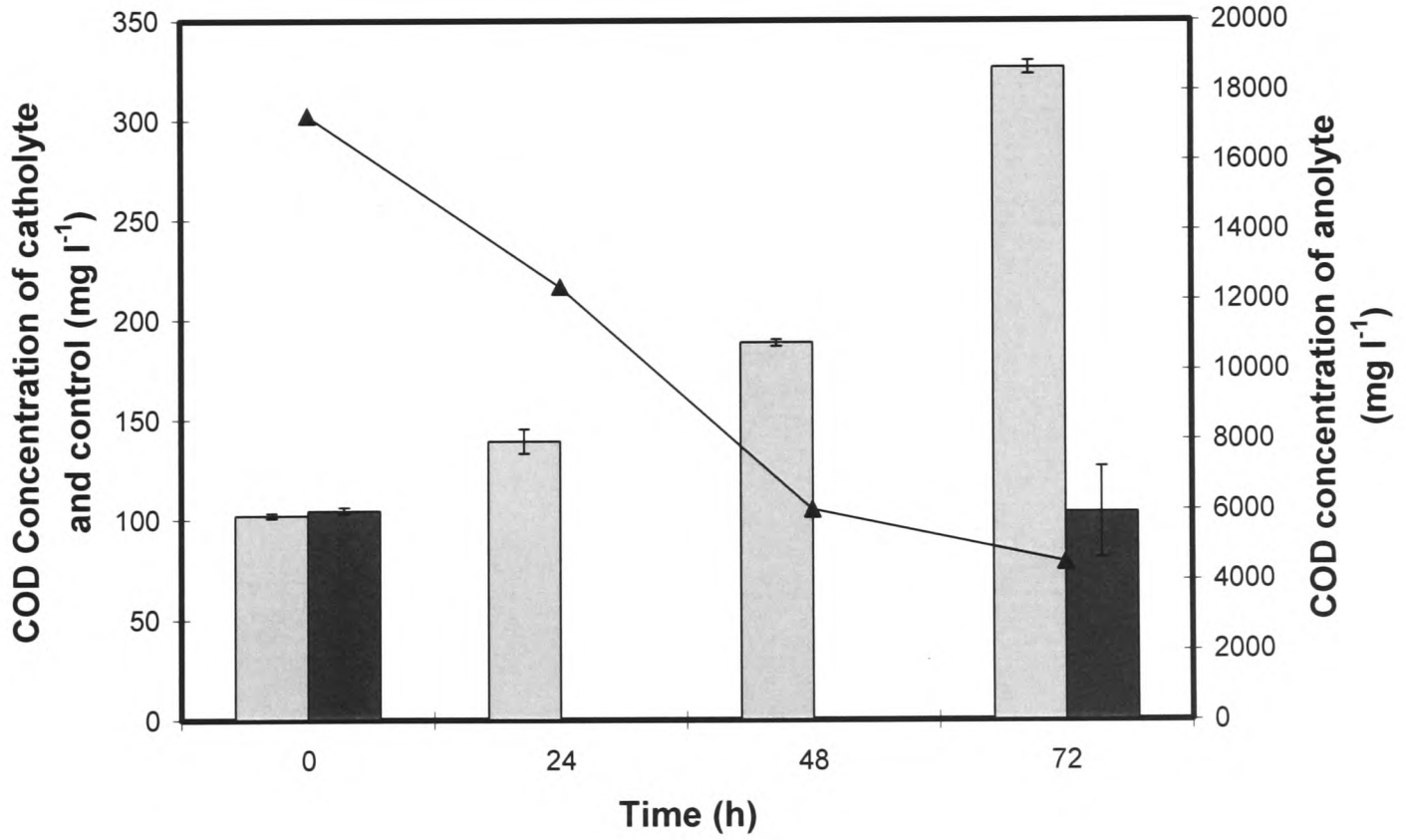


Figure 3.8 Changing concentration of Triton X-100 in the electrolytes on application of an electric field (60 V) after 0.4% Triton X-100 was added to the anode. Delivery of Triton X-100 via electroosmosis investigated over 72 h through a soil column containing Reading soil. COD (mg l^{-1}) of catholyte, ■ COD (mg l^{-1}) of control reservoir, and ▲ COD (mg l^{-1}) of anolyte (data are means ± SE, n = 3).

3.4.3 Mobilisation of contaminants with electrokinetics and surfactants

3.4.3.1 Ionic surfactant SDS

The use of SDS (0.4%) as a flushing solution to mobilise the target contaminant mix (DXT) was investigated using both Reading and Wytham soils. The control data detailing the distribution and concentration of the target contaminant mix (DXT) after 14 days of electrokinetics without the addition of surfactants in Reading and Wytham soil are detailed in Chapter 2; Figures 2.24 and 2.26, respectively.

Figure 3.9 (Reading soil) provides evidence that 0.4% SDS promoted the movement of dodecane and tetradecane towards the cathode compared to the electrokinetics chamber without surfactant addition (p = 0.02, 0.02, respectively, df = 10). After 14 days of treatment xylene isomers were below the level of GC-FID detection (< 1 mg kg^{-1}) in the electrokinetics chamber with SDS. There was a lower concentration of both dodecane and tetradecane in the electrokinetics chamber with SDS compared to the control (which used only mains water as a purging solution) (p = 0.02, 0.01, respectively, df = 10). The mass balance analysis data is recorded in Table 3.3.

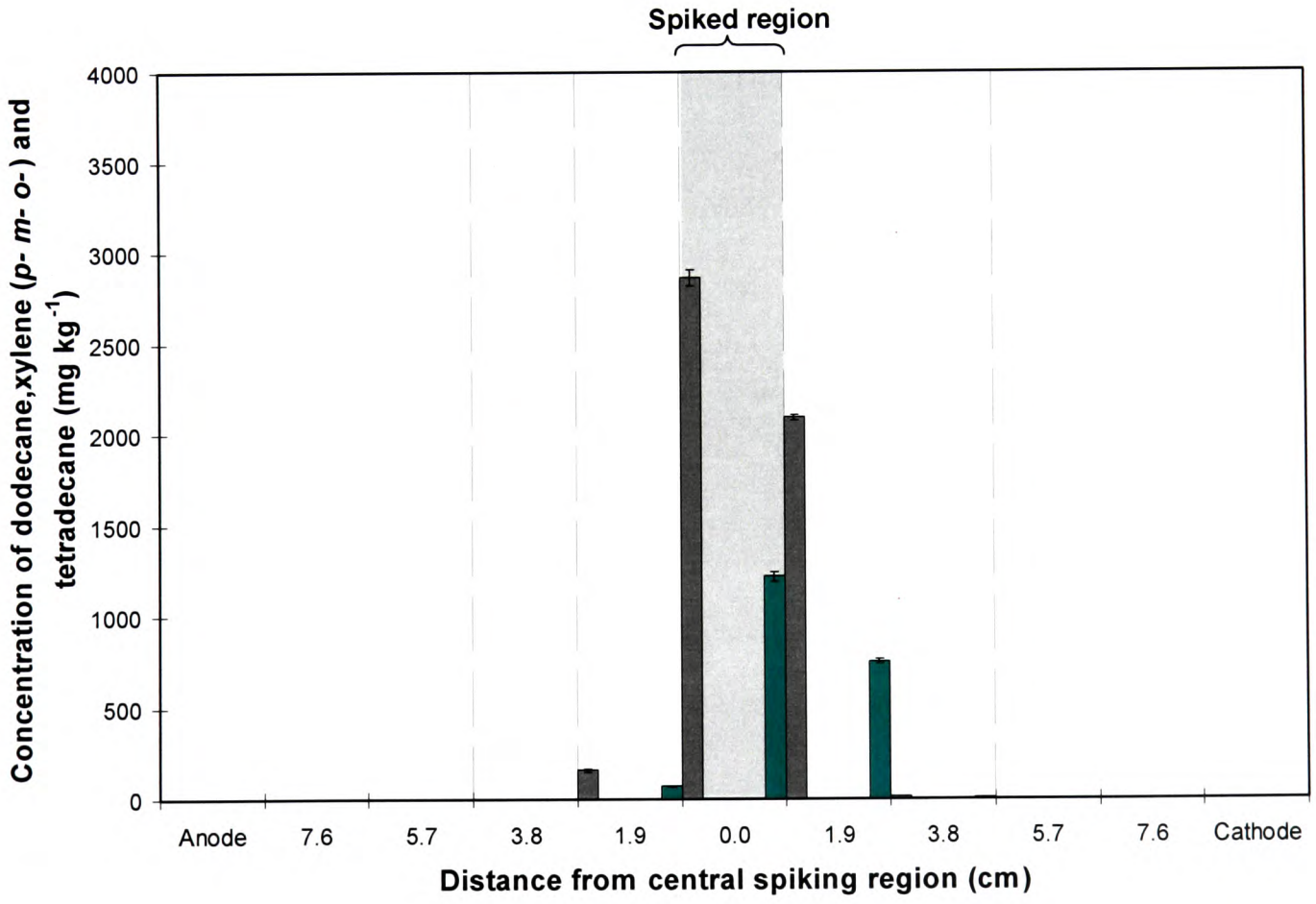


Figure 3.9 Electrokinetics chamber with Reading soil spiked with DXT mix and 0.4% SDS solution added to the cathode. Distribution in electrokinetics soil chamber of ■ dodecane (11 000 mg kg^{-1}); ■ *p*-xylene (1 900 mg kg^{-1}); ■ *m*-xylene (5 700 mg kg^{-1}); ■ *o*-xylene (1 500 mg kg^{-1}); ■ tetradecane (4 000 mg kg^{-1}), after 14 days with 60 V applied (~10 mA) (data are means ± SE; n=3). The concentrations in brackets denote the theoretical start concentrations of the contaminants.

In the electrokinetics chamber containing Wytham soil with 0.4% SDS added to the cathode (Figure 3.10) there was inconclusive evidence (not significant, $p = 0.06$, df = 10) that movement of dodecane had occurred in the direction of the cathode (1.9 cm), to a greater extent with SDS than without. Movement of tetradecane in the presence of SDS and electrokinetics from the spiked region was not significant ($p = 0.1$, df = 10). No movement of xylene was observed. The concentration of *p*-xylene and *o*-xylene remaining in the electrokinetics soil chamber with and without SDS after 14 days were not significantly different ($p = 0.09$, 0.1, respectively, df = 4). The disappearance of *m*-xylene from the soil was greater in the electrokinetics chamber with SDS compared to the control ($p = 0.05$, df = 4) whilst there was no significant difference between the final concentrations of either dodecane or tetradecane ($p = 0.4$, df = 10) (refer to mass balance analysis Table 3.4). Mobilisation of the target contaminants in the direction of the anode was not statistically significant ($p > 0.1$, df = 3).

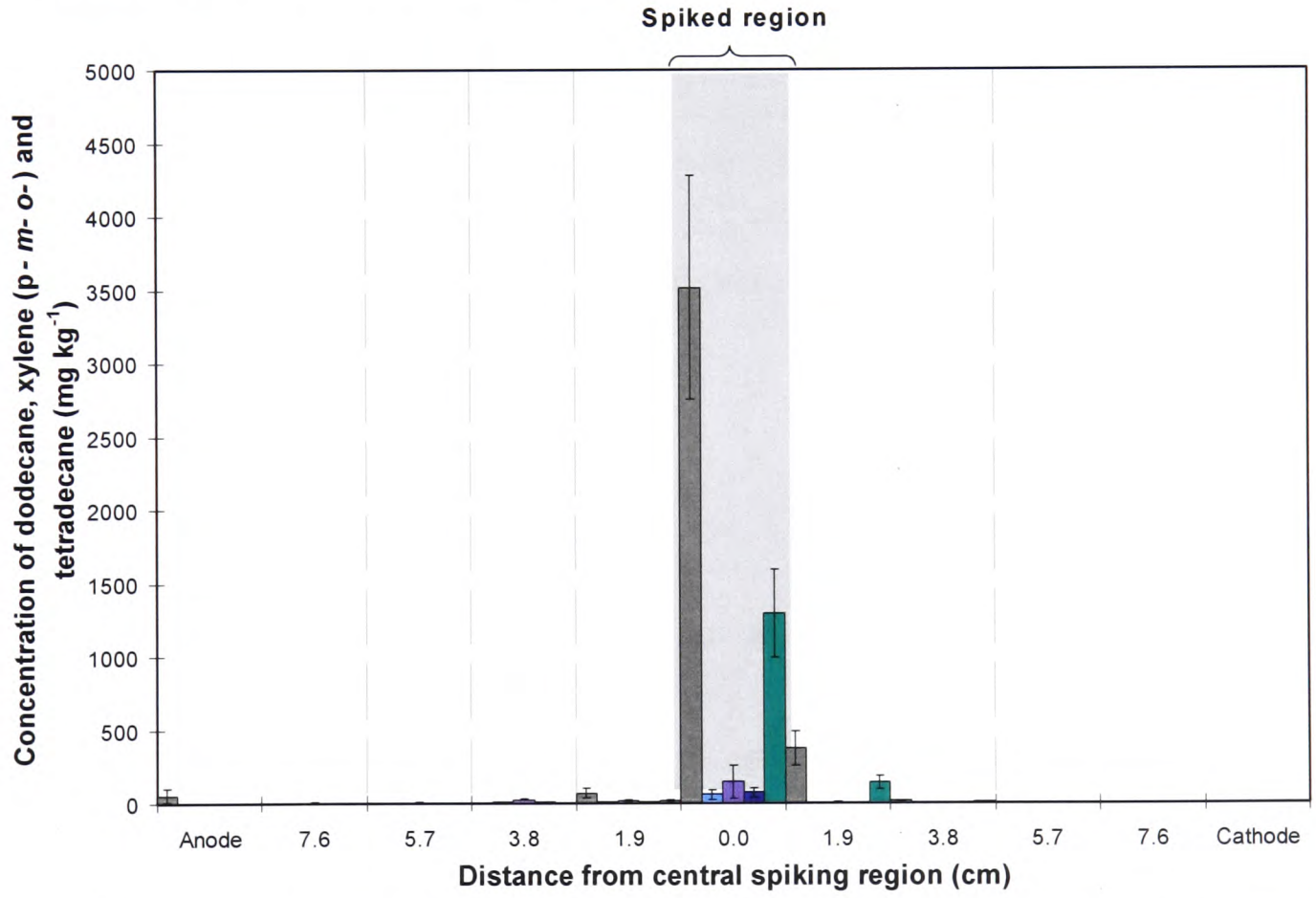


Figure 3.10 Electrokinetics chamber with Wytham soil spiked with DXT mix and 0.4% SDS solution added to the cathode. Distribution in electrokinetics soil chamber of ■ dodecane (11 000 mg kg^{-1}); ■ *p*-xylene (1 900 mg kg^{-1}); ■ *m*-xylene (5 700 mg kg^{-1}); ■ *o*-xylene (1 500 mg kg^{-1}); ■ tetradecane (4 000 mg $kg-^{1}$), after 14 days with 60 V applied (~10 mA) (data are means ± SE; n=3). The concentrations in brackets denote the theoretical start concentrations of the contaminants.

During the 14-day electrokinetics experiment with 0.4% SDS and Wytham soil, the soil structure was significantly disrupted. In a number of the experiments (but not all) the soil within the electrokinetics chamber swelled (Figure 3.11), a white precipitate formed on the

surface and in the electrolyte, and permeability of the soil to water appeared to have reduced resulting in regions of dryness and cracking (see also Appendix 8.7). Figure 3.12 shows the increase in electrical resistance observed across the electrokinetics soil chamber after 7 days of 60 V with 0.4% SDS added. With increased resistance the soil temperature increased above that of the non-electrokinetic control chamber. For example, on day 5 of the investigation the temperature of the soil in the electrokinetics chamber was 17.6 ± 1.6°C greater than the control soil chamber.

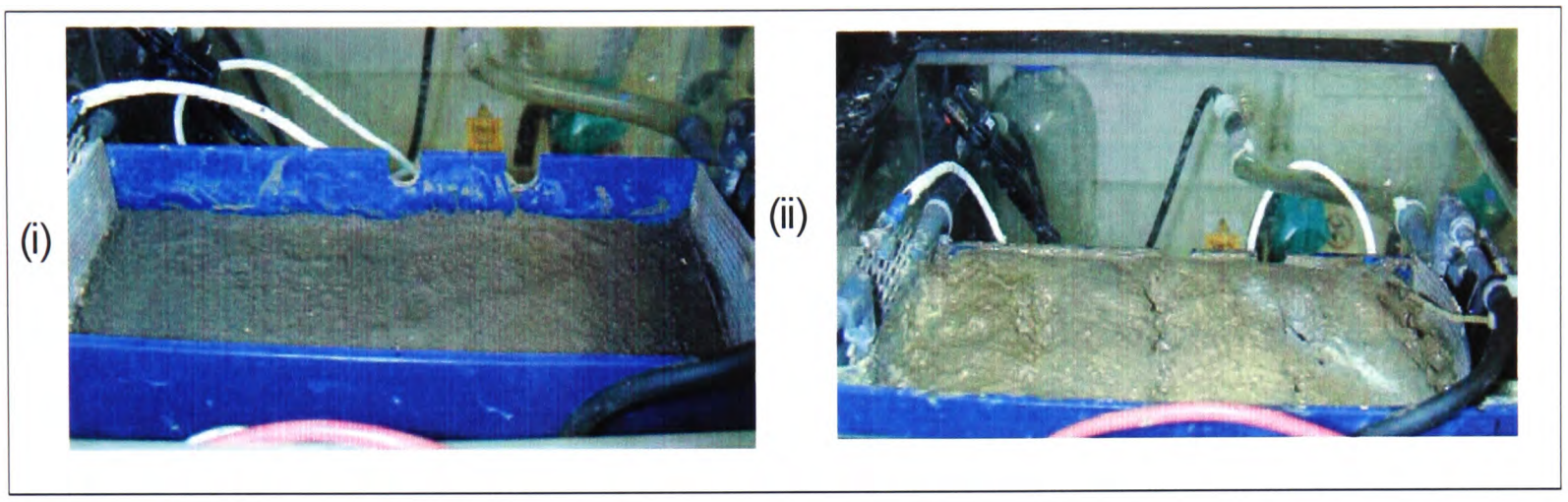


Figure 3.11 Swelling of Wytham soil within electrokinetics chamber on addition of surfactant. Compare electrokinetics chamber without surfactant (i) with electrokinetics chamber with 0.4% SDS (ii). Refer also to Appendix 8.7.

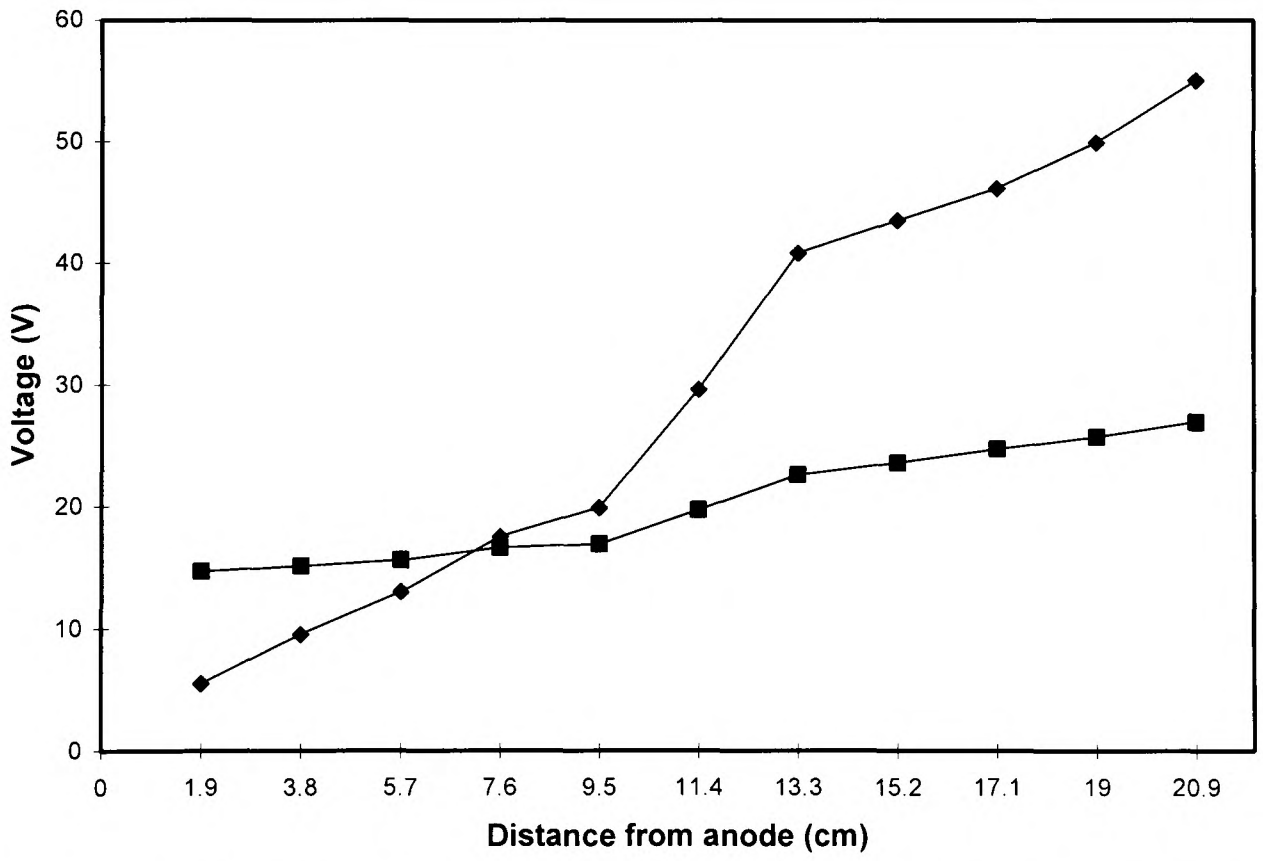


Figure 3.12 Electric potential distribution across the electrokinetics soil chamber, containing Wytham soil with SDS (0.4%) applied ◆ 1 day, and ■ 7 days, 60 volts applied (~ 10 mA) (data points are based on the results from one experiment).

3.4.3.2 Non-ionic surfactants Span 85 and Triton X-100

With 0.4% Span 85 added to the anolyte (and delivered through the soil via electroosmosis) mobilisation of dodecane and tetradecane towards the anode (1.9 cm from the central region) occurred in the electrokinetics chamber containing Reading soil (Figure 3.13). When compared to the distribution and concentration of dodecane and tetradecane in the electrokinetics soil chamber without the addition of Span 85 the movement observed was statistically significant ($p < 0.0001$, df = 10). There was no significant movement of either dodecane or tetradecane in the direction of the cathode ($p = 0.4$, 0.5, respectively, df = 10). Furthermore, there was no significant difference in the overall concentration of dodecane and tetradecane remaining in the electrokinetics soil chamber with and without the addition of Span 85 ($p = 0.2$, 0.3, respectively, df = 10). There was a significant reduction in the concentrations of the three xylene isomers after 14 days of applying electrokinetics with Span 85 compared to electrokinetics without surfactant ($p = 0.02$, 0.02, 0.01, df = 10).

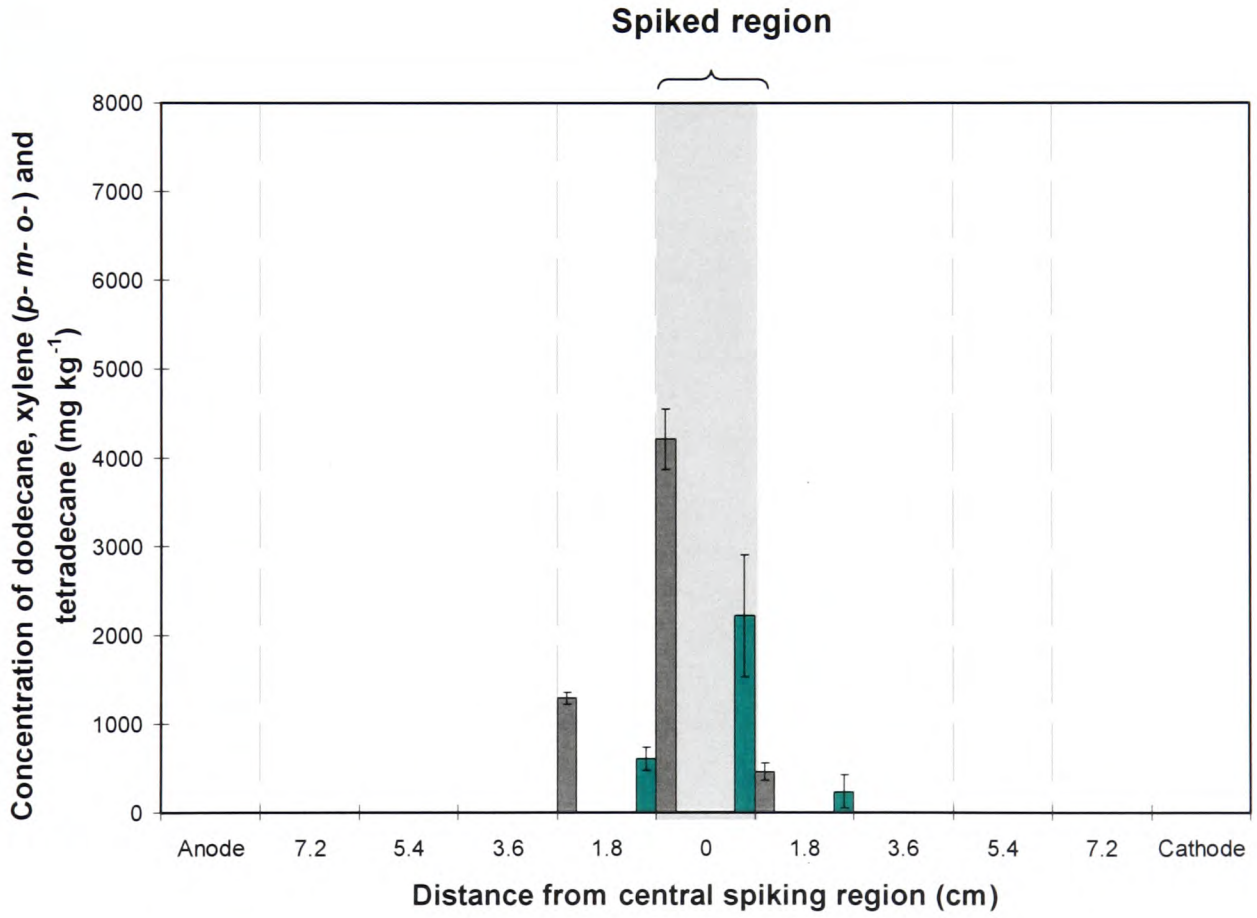


Figure 3.13 Electrokinetics chamber with Reading soil spiked with DXT mix and 0.4% Span 85 solution added to the anode. Distribution in electrokinetics soil chamber of ■ dodecane (11 000 mg kg^{-1}); ■ *p*-xylene (1 900 mg kg^{-1}); ■ *m*-xylene (5 700 mg kg^{-1}); ■ *o*-xylene (1 500 mg kg^{-1}); ■ tetradecane (4 000 mg kg^{-1}), after 14 days with 60 V applied (~ 10 mA) (data are means ± SE; n = 3). The concentrations in brackets denote the theoretical start concentrations of the contaminants.

It was evident that the non-ionic surfactant Triton X-100 also facilitated the movement of dodecane and tetradecane from the central spiked region towards the anode chamber (1.8 cm in the direction of the anode) (Figure 3.14) ($p = 0.001$, 0.0003, respectively,

df = 10). Furthermore, as observed with SDS, the concentration of dodecane remaining in the electrokinetics soil chamber with Triton X-100 additions was significantly lower than in the electrokinetics control soil chamber (p = 0.006, df = 10). However, there was no significant difference between the concentration of tetradecane within the electrokinetics soil chamber with or without Triton X-100 (p = 0.22, df = 10). All three xylene isomers were absent from the soil chamber after 14 days.

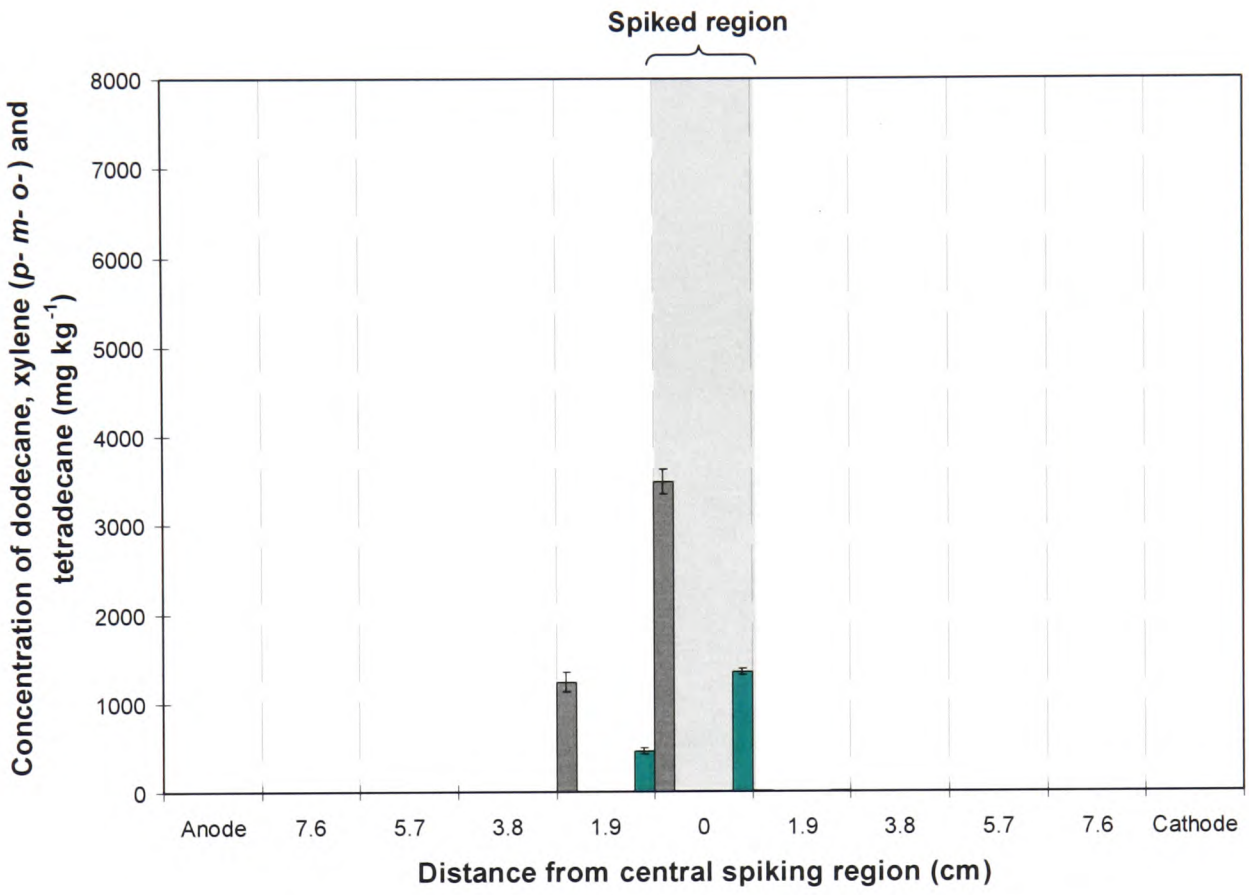


Figure 3.14 Electrokinetics chamber with Reading soil spiked with DXT mix and 0.4% Triton X-100 solution added to the cathode. Distribution in electrokinetics soil chamber of ■ dodecane (11 000 mg kg^{-1}); ■ *p*-xylene (1 900 mg kg^{-1}); ■ *m*-xylene (5 700 mg kg^{-1}); ■ *o*-xylene (1 500 mg kg^{-1}); ■ tetradecane (4 000 mg kg^{-1}), after 14 days with 60 V applied (~ 10 mA) (data are means ± SE; n= 3). The concentrations in brackets denote the theoretical start concentrations of the contaminants.

Table 3.3 Mass balance analysis for Reading soil. Analytical start concentration data are means ± SE, n = 11, for final concentration data are means ± SE, n = 6).

Test	Contam.	Theoretical start conc. (mg kg^{-1})	Analytical start conc. (mg kg^{-1})		Analytical final conc. (mg kg^{-1})		Difference %
Control	Dodecane	11235	6941.0	± 143.9	6690.0	± 1010.3	3.6
SDS	Dodecane	11235			5122.9	± 63.4	26.2
Span	Dodecane	11235			5959.3	± 720.7	14.1
Triton	Dodecane	11235			4736.8	± 160.1	31.8
Control	Tetradecane	3815	3769.5	± 101.5	3286.8	± 550.1	12.8
SDS	Tetradecane	3815			2044.2	± 38.9	45.8
Span	Tetradecane	3815			3049.3	± 429.8	19.1
Triton	Tetradecane	3815			3667.8	± 97.4	2.7
Control	*p*-xylene	2075	1054.0	± 101.0	217.4	± 101.0	79.4
SDS	*p*-xylene	2075			0.0	± 0.0	100
Span	*p*-xylene	2075			0.0	± 0.0	100
Triton	*p*-xylene	2075			0.0	± 0.0	100
Control	*m*-xylene	5624	3253.9	± 365.5	687.8	± 315.2	78.9
SDS	*m*-xylene	5624			0.0	± 0.0	100
Span	*m*-xylene	5624			0.0	± 0.0	100
Triton	*m*-xylene	5624			0.0	± 0.0	100
Control	*o*-xylene	1380	920.7	± 96.6	191.0	± 78.9	79.3
SDS	*o*-xylene	1380			0.0	± 0.0	100
Span	*o*-xylene	1380			0.0	± 0.0	100
Triton	*o*-xylene	1380			0.0	± 0.0	100

Control = Soil chamber with an electric field applied (60 V, ~10 mA)

Table 3.4 Mass balance analysis for Wytham soil (Data are means ± SE, n = 6).

Test	Contam.	Theoretical start conc. (mg kg^{-1})	Analytical start conc. (mg kg^{-1})		Analytical final conc. (mg kg^{-1})		Difference %
Control	Dodecane	11235	8229.4	± 88.7	1683.6	± 750.3	79.5
SDS	Dodecane	11235			1930.1	± 376.5	76.5
Control	Tetradecane	3815	4805.7	± 105.2	560.1	± 241.6	88.3
SDS	Tetradecane	3815			633.9	± 125.3	86.8
Control	*p*-xylene	2075	768.3	± 19.3	83.2	± 34.7	89.2
SDS	*p*-xylene	2075			31.6	± 5.3	95.9
Control	*m*-xylene	5624	2573.8	± 67.0	274.4	± 116.4	89.3
SDS	*m*-xylene	5624			48.6	± 13.3	98.1
Control	*o*-xylene	1380	727.7	± 19.7	85.6	± 32.5	88.2
SDS	*o*-xylene	1380			38.1	± 5.8	94.8

Control = Soil chamber with an electric field applied (60 V, ~10 mA)

3.5 Discussion

3.5.1 Delivery of surfactants into the soil using electrokinetics

The electrokinetic delivery of the anionic surfactant SDS and the non-ionic surfactants Triton X-100 and Span 85 was achieved through a column of soil (160 mm in length) (Figures 3.5 to 3.8). It was shown that SDS could be delivered via electromigration and/or electroosmosis. The delivery of the non-ionic surfactants (Triton X-100 and Span 85) was achieved using electroosmosis. The electroosmotic delivery of surfactants is dependent on the size of the electric field, the charge density, and permeability of the soil to the viscous surfactant solution. Conversely, electromigration is independent of the soil type and ion movement can take place even in the absence of independent water flow as long as pore fluid and an electrical potential exists (Alshawabkeh *et al.,* 2004). Generally electromigration, especially in ion rich media, is more effective than electroosmosis for delivery (West & Stewart, 2000). However, on occasions (for instance where the electric potential gradient is small) electroosmosis may exceed electromigration (Wada & Umegaki, 2001). In the case of SDS (and other anionic surfactants) addition to both the cathode and anode of the system could facilitate efficient delivery of the surfactant into the soil using a combination of electroosmosis and electromigration.

In all of the electrokinetic experiments, including the soil chamber experiments, movement of surfactants through the soil could be observed by the appearance of a surfactant emulsion in the electrolyte opposite in polarity to the electrolyte to which the surfactant was added to (*e.g.* a surfactant emulsion was observed in the catholyte after adding Span 85 to the anolyte and applying an electric field). To provide supporting evidence for surfactant movement in the electric field the concentration of COD in the electrolytes was measured, assuming that the change in COD concentration was representative of a change in surfactant concentration. Although simplistic the method provided an adequate means of investigating the electrokinetic delivery of surfactants through soil. The method of correlating COD concentration with the presence of surfactant can only be used when the concentration of surfactant is relatively high compared to the concentration of dissolved organics in the electrolyte solutions from the soil.

3.5.2 The use of surfactants to mobilise the target hydrocarbons

3.5.2.1 Anionic surfactant SDS in Reading and Wytham soil

The delivery of SDS through Reading soil with electrokinetics (via electromigration towards the anode) resulted in a reduction in the final concentration of the contaminant mix (DXT) compared to the concentration in the electrokinetics chamber without SDS. Furthermore, movement of the straight chain alkanes, dodecane and tetradecane, was observed 1.9 cm in the direction of the cathode in the presence of 0.4% SDS.

In Wytham soil movement of dodecane and tetradecane towards either the cathode or anode was not shown to be statistically significant. Furthermore, there was no significant overall reduction in final contaminant (DXT) concentration in the electrokinetics chamber with SDS compared to the soil chamber without surfactant addition ($p \geq 0.05$, df = 10).

3.5.2.2 Non-ionic surfactants Span 85 and Triton X-100 in Reading soil

The non-ionic surfactants were added to the anolyte and delivered into the soil, towards the cathode, via electroosmosis. Movement of dodecane and tetradecane towards the anode (1.9 cm) occurred after the delivery of the non-ionic surfactants to the central spiked region via electroosmosis. Additionally, as observed in the presence of SDS, xylene was completely depleted in the presence of the non-ionic surfactants, which was an improvement to the residual concentration of xylene isomers detected in the electrokinetics chamber without surfactants. Dodecane removal was enhanced in the presence of Triton X-100, but there was no significant difference in reduction in the presence of Span 85. The non-ionic surfactants did not appear to promote the disappearance of tetradecane from the soil. For logistical reasons there was no opportunity to evaluate the use of non-ionic surfactants in spiked Wytham soil.

3.5.2.3 Direction of movement of the target contaminants

Upon delivery of SDS to the central spiked region with the use of electromigration (from cathode to anode) dodecane and tetradecane were mobilised towards the cathode of the Reading soil system. Conversely, the delivery of Span 85 and Triton X-100 using electroosmosis (from anode to cathode) promoted the movement of dodecane and tetradecane towards the anode of the circuit in Reading soil. This effect is likely to be kinetic in origin: dissolution of the contaminants into the aqueous phase occurs first at the initial contact boundary of the spiked region, which is closest to the electrode from which

the surfactant originates. As the anionic surfactant approaches the spiked region from the direction of the cathode it contacts with the spiked region first on the cathode side, whereas the non-ionic surfactants make first contact with the anode side of the central spiked region.

An alternative explanation is that in the presence of the surfactant biodegradation of the target contaminants is suppressed with preferential utilisation of the surfactant as a carbon source. In particular the oleic acid side chain of Span 85 is structurally similar to tetradecane. With suppressed biodegradation a greater concentration would appear in the vicinity of the surfactant and could be perceived statistically as facilitated movement. However, the mass balance calculations (Tables 3.3 and 3.4) suggest that the presence of surfactants in the soil chambers has not suppressed biodegradation/disappearance of the spiked contaminants.

3.5.2.4 Disruption of the soil structure

As described (§ 3.4.3.1), swelling, consolidation, and subsequent cracking of Wytham and Reading soil were observed in some instances where SDS was supplied via electromigration. In the literature, soil cracking and swelling has been attributed to the precipitation of salts within the soil, resulting in reduced permeability as a consequence of dispersal of the soil particles and the pores becoming clogged (Acar & Alshawabkeh, 1993). In particular, anionic surfactants are reported to increase calcium precipitation in soils by competing for sorption sites on the soil surface. It is also reported that the addition of surfactants can result in reduced hydraulic conductivity (permeability) by increasing the viscosity of the pore fluid, through agglomeration of micelles (again clogging the soil pores) or by affecting the double diffuse layer (DDL) of the clay via sorption of ionic surfactants to the soil surface (Tumeo, 1997; Henry & Smith, 2003). Furthermore, the hydraulic capillary pressure of soil is a function of surface tension and as surfactants lower surface tension hydraulic conductivity can be reduced in this way (Bettahar *et al.*, 1998; Bettahar *et al.*, 1999; Khodadoust *et al.*, 2004).

It is postulated within this study that the addition of SDS to the soil may have also resulted in sodification of the soil chamber. Sodification of a soil describes the presence of excess sodium that can result in soil dispersion and clay platelet and aggregate swelling. Excess sodium in the soil, especially clay-rich soils, can give rise to the characteristic disruption seen during this study on addition of SDS (*e.g.* surface crusting, reduced infiltration of aqueous solutions and swelling) (Agassi *et al.*, 1981). Within the soil, salinity (*i.e.*

concentration of salt ions such as chlorides) can reduce the effects of sodification however under the electric field (§ 2.4.8) ions are stripped rapidly from the soil via electromigration and precipitation in regions of high pH (Figure 2.18). Alongside SDS sodium was introduced to the soil in the form of 0.1 M NaOH, which was used as a buffering solution in the anolyte (§ 2.3.5.3).

The work described in this chapter has highlighted the difficulties in investigating interactions with soil and re-emphasised the importance of carrying out site-specific feasibility studies before undertaking *in situ* field trials based on limited data. For instance, if SDS had only been investigated with Reading soil it may have been presumed that SDS could be delivered into a soil system via electroosmosis or electromigration to increase the bioavailability of organic contaminants. However, the results from the electrokinetics using Wytham soil and SDS yielded very different results with significant disruption of the soil structure. As a result of the complex nature and unknown consequences of surfactant–soil, surfactant–pore fluid, and surfactant–contaminant interactions, laboratory experiments are required to assess the influence of surfactants with a given set of parameters (Bettahar *et al.*, 1998, 1999; Jain & Demond, 2002; Henry & Smith, 2003). There are several papers that describe the unexpected behaviour of surfactants in soil systems highlighting the difficulty in predicting the outcome of an experiment and interpreting the results. For example, it has been reported that the non-ionic surfactant Triton X-100 may become charged by cations from the soil binding to the polyoxethylene side chain (Hägerstrand *et al.*, 2001).

3.6 Interpretive summary

1. The delivery of non-ionic surfactants, Triton X-100 and Span 85, was achieved using electroosmosis from the anode to cathode of the electrokinetics system. Mobilisation of the anionic surfactant SDS was achieved by both electromigration and electroosmosis (§ 1.7, Objective 2).

2. Dodecane and tetradecane were mobilised in the direction of the cathode (1.9 cm) in Reading soil, but significant movement was not observed in Wytham soil on introduction of SDS to the electrokinetics chamber.

3. Movement of dodecane and tetradecane in the direction of the anode (1.9 cm) in Reading soil was achieved using the non-ionic surfactants Triton X-100 and Span 85.

4. The evidence presented indicates that as surfactant is delivered to the central 'spiked' region dissolution of the contaminant occurs. The mobilisation observed in the opposite direction of surfactant movement may therefore be attributable to enhanced dissolution rather than direct electrokinetic movement of the target contaminants.

5. In Reading soil after 14 days of applying an electric field (60 V, ~ 10 mA) and introducing either SDS, Span 85 or Triton X-100, all of the spiked xylene (~ 9 100 mg kg^{-1}) was removed from the soil.

6. In Reading soil there was evidence that SDS and Triton X-100 enhanced the removal of dodecane from the electrokinetics soil chamber. The disappearance of tetradecane was enhanced by SDS but the non-ionic surfactants did not significantly increase the removal efficiency.

7. Upon electrokinetic injection of SDS into Wytham soil and, to a lesser extent, Reading soil, the soil structure was disrupted leading to reduced hydraulic conductivity, surface crusting, swelling and increased electrical resistance resulting in a rise in the soil temperature. It is likely that soil structure was affected by a combination of sodification and via direct interactions between the surfactant and soil leading to precipitation of minerals and modifications to the double diffuse layer.

8. The studies have provided evidence that electrokinetics can be used to deliver non-ionic and anionic surfactants into the soil and to promote mobilisation and disappearance of the target contaminants. The effectiveness of surfactants to promote mobilisation and degradation from the soil appeared to be influenced by soil type (§ 1.7, Objective 2).

4 The feasibility of using electrokinetics to promote the degradation of hydrocarbons in the sub-surface

4.1 Introduction

The use of electrokinetics to mobilise organic hydrocarbons from the soil has not proven to be effective for n-alkanes with a low water solubility and high octanol-water partition coefficient, such as dodecane and tetradecane (Chapter 2). The use of electrokinetics to deliver surfactants into the 'spiked' soil chambers promoted the local dissolution of dodecane and tetradecane into the aqueous phase but did not facilitate significant mobilisation through the soil (Chapter 3). The main focus of the work undertaken within this chapter was to ascertain whether electrokinetics could be used to enhance the degradation of the target contaminant(s) in the sub-surface, either by optimising conditions for biodegradation or by the introduction of chemical oxidants into the soil to facilitate chemical degradation.

4.2 The enhancement of degradation by stimulating microbial degradation

4.2.1 Capabilities of soil bacteria to degrade hydrocarbon contaminants

"It is inappropriate to assume that a compound biodegraded in one environment is *ipso facto* going to be transformed in another" (Alexander, 1994).

Preparing a site to facilitate biodegradation relies on two major requirements, namely the presence of microorganisms with the relevant metabolic capabilities (§ 1.4.2), and a sufficiently permeable sub-surface to allow the introduction of biodegradative enhancing agents, such as the macronutrients N and P (Thomas & Ward, 1989). A technique of stimulating sub-surface microorganisms to degrade hydrocarbon pollutants was patented by R.L. Raymond in the 1970s (Raymond *et al.*, 1975 cited in Thomas & Ward, 1989), identifying the need to supply nutrients and electron acceptors to stimulate microorganisms indigenous to a contaminated soil to biodegrade the target contaminants.

4.2.2 Factors influencing bacterial degradation

In addition to the metabolic capabilities of microorganisms in the sub-surface, environmental conditions have a significant influence in determining if, and at what rate, biodegradation of the contaminants can take place. For biodegradation to occur, microorganisms require the following: a carbon source (electron donor); an energy source; terminal electron acceptor (*e.g.* O_2, SO_4^{2-} and NO_3^-) (§ 1.4.2.1); macronutrients (*e.g.* N as nitrate [NO_3^-] and P as phosphate [PO_4^{3-}]); micronutrients for enzyme function (*e.g.* trace metals, Fe, S and Ca); an appropriate pH, temperature and moisture content; absence or control of toxic substances, such as other contaminants or toxic metabolites; and adequate contact between the microorganisms and the target contaminant (bioavailability) (Parkin, 1996; Yerushalmi *et al.*, 2003; Niqui-Arroyo *et al.*, 2004). Within the sub-surface it is rare for all of these requirements to be fulfilled to facilitate efficient biodegradation without amendments being made. Furthermore, even with the supplements to aid *in situ* biodegradation it is a challenge to maintain an optimum environment for microbial degradation; with nutrients and electron acceptors typically becoming limiting more rapidly than the rate at which they can be reintroduced to the sub-surface.

4.2.3 Delivery of nutrients and electron acceptors into the sub-surface

4.2.3.1 Conventional methods

The delivery of nutrients and electron acceptors into the sub-surface presents a significant engineering challenge. Conventionally, the introduction of enhancing agents (such as fertiliser mixes containing N and P) is achieved by direct application to the soil surface relying on gravity, pressure, and/or diffusion, with the use of injection-recovery wells or via window infiltration galleries (Durant *et al.*, 1995; Electorowicz & Boeva, 1996; Parkin, 1996). The introduction of nutrients in this way is only successful when the hydraulic conductivity of the soil is $\geq 10^{-4}$ cm s^{-1}, otherwise delivery of the nutrients to the target zone cannot be achieved effectively (Thomas & Ward, 1989). Recently, pneumatic fracturing has been used in an attempt to increase hydraulic conductivity by breaking up aquifer material prior to injection, but there remains a requirement for novel technologies to enhance the efficiency of nutrient delivery into the sub-surface with minimal disruption to surrounding soils and services.

Conventionally, O_2 is delivered to the sub-surface using the technique of air sparging (by directly injecting oxygen into the groundwater) or may be added in the form of low concentrations of H_2O_2 or commercially available oxygen release compounds (ORC®,

Regenesis, San Clemente, CA). The efficiency of O_2 transfer through the unsaturated sub-surface is dependent on the permeability of the soil matrix and the potential for gaseous exchange, which is low where buildings and hard standing are present on the surface. The introduction of O_2 to the sub-surface is an inefficient process and tends to be technically challenging and expensive because of its low aqueous solubility (~9 mg l^{-1}) (Lovley, 2001). In addition, O_2 added to the sub-surface may be consumed readily in abiotic reactions, such as the oxidation of SOM and metals *e.g.* ferrous iron.

As discussed previously (§ 1.4.2), alternative electron acceptors may be used for the biodegradation of hydrocarbons from the sub-surface. Within this study, NO_3^- was the alternative electron acceptor of interest (§ 1.4.2.1). Nitrate is an anionic nutrient with a high aqueous solubility and a low tendency to sorb to the soil. It is therefore feasible to use electromigration for the delivery of nitrate into the soil from the cathode, which may be utilised as an electron acceptor in the absence of O_2, and/or as a source of N.

4.2.3.2 The use of electrokinetics to deliver of nutrients and electron acceptors

In Chapter 2 it was demonstrated that after 14 days of applying an electric field (60 V, ~10 mA) to soil (without recirculation of the electrolyte) the inorganic nutrients NO_3^-, PO_4^{3-}, SO_4^{2-}, and Cl^- were mobilised from the soil (§ 2.4.8). Furthermore, when the electrolyte was not recirculated the inorganic nutrients were quickly depleted, which would have reduced the potential for biodegradation of the target contaminants to occur in the soil. However, with appropriate engineering controls in place (*e.g.* the recirculation of the electrolyte) the mobilisation of soluble ionic nutrients and electron acceptors could be beneficial in stimulating and maintaining biodegradation in the sub-surface.

The movement of charged ions through a soil as a result of electromigration is influenced by the strength of the electric field, soil porosity, the tortuosity of the soil, the charge and ion valence/ionic mobility of the nutrient and its effective diffusion coefficient (as described by the Nernst-Townsend-Einstein relationship [Acar and Alshawabkeh, 1993]). Mitchell (1991) reported that the ionic mobility of abundant ions (such as NO_3^-) through a soil system is between 3×10^{-9} to 1×10^{-8} $m^2 (V \cdot s)^{-1}$. However, there is limited research on the movement of more than one ion under the influence of an electric field. As reviewed briefly by Eid *et al.* (2000) each ionic species will compete for the current. For example Ca^{2+} may compete with cadmium (Cd^{2+}) for the electric current. Acar and Alshawabkeh (1993) calculated that the movement rate of H^+ and OH^- through a kaolinte soil (clay) as a result of electrokinetics would be 7.60×10^{-4} and 4.32×10^{-4} cm^2 $V \cdot s^{-1}$,

respectively. Therefore, in an electrokinetic soil system without neutralisation, mobilisation of the electrolysis products (H^+ and OH^-) may result in them carrying more of the current and therefore limit the delivery of inorganic nutrients into the soil (Eid *et al.*, 2000; Alshawabkeh *et al.*, 2004).

The potential to use electrokinetics to control the movement of nutrients in soil has been previously investigated. Principally, electrokinetics was examined as a technique for controlling the concentration and movement of NO_3^- in soils that were being (or had been) intensively farmed (*e.g.* Cairo *et al.*, 1996; Chew & Zhang, 1998; Luo *et al.*, 2004; Jia *et al.*, 2005; Manokararajah & Ranjan, 2005). The work of Manokararajah & Ranjan (2005) identified that, in a silty loam, electromigration could be used to negate the movement of NO_3^- through the soil under the influence of an hydraulic gradient of 1.25. Animal wastes and agricultural fertilisers are both major sources of NO_3^- in soils and water (both surface and groundwater). In the UK, the maximum concentration of NO_3^- within the groundwater is restricted to 50 mg l^{-1} and lower concentrations are required in sites designated as NO_3^- vulnerable zones, as described in the 91/676/EEC Directive (Beeson & Cook, 2004). Both NO_3^- and nitrite (NO_2^-) are toxic and if ingested can cause the life threatening condition methemoglobinemia, where haemoglobin is converted to methemoglobin that is unable to carry oxygen within the blood (Chow & Hong, 2002). This condition is mostly found in children and is referred to as 'blue baby syndrome'.

Investigations have previously been carried out to assess the potential for electrokinetics to be used to deliver nutrients into the soil to facilitate biodegradation. For instance Segall and Bruell (1992) examined the use of electroosmosis to deliver NO_3^- and PO_4^{3-} as sources of N and P respectively, from the anode towards the cathode. They reported the successful electroosmotic movement of NO_3^- into the soil but concluded that the delivery of P was limited by its sorption to the soil and tendency to precipitate out of the aqueous phase as PO_4^{3-} in association with one or more of Ca^{2+}, Fe^{3+}, and Al^{3+}. Their studies also highlighted the tendency of NO_3^- to become reduced at the cathode to ammonium ions (NH_4^+) and nitrogen gas (N_2) (Equation 4.1).

$$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O,\ E^0 = +0.84\ V$$
$$NO_2^- + 8H^+ + 6e^- \longrightarrow NH_4^+ + 2H_2O,\ E^0 = +0.89\ V$$
$$2NO_3^- + 12H^+ + 10e^- \longrightarrow N_2 + 6H_2O,\ E^0 = +1.24\ V$$

Equation 4.1 Reduction of NO_3^- at the cathode at different electrical potentials.

The introduction of NH_4^+ into the soil could benefit biodegradation as an energy-rich source of N; NH_4^+ is used in place of NO_3^- as its presence inhibits the assimilatory nitrate reduction pathway. However, in a system where the anolyte and catholyte are not recirculated, the migration of NH_4^+ to the cathode via electromigration and electroosmotic movement may result in a rapid depletion of the inorganic nutrient from the soil. Budhu *et al.* (1997) and Thevanayagam and Rishindran (1998) studied the use of electromigration to deliver NO_3^- into the soil, successfully demonstrating that NO_3^- could be delivered (non-uniformly) from the catholyte through the soil towards the anode.

Kim and Han (2003) investigated the potential to uniformly deliver SO_4^{2-} and NH_4^+ ions into, and through, the soil by the addition of H_2SO_4 and ammonium hydroxide (NH_4OH) into the anolyte and catholyte, which were recirculated together, by reversing the polarity of the electric field at day 8 of a 17-day experiment. From their results it appeared that a more uniform distribution of NH_4^+ and SO_4^{2-} was achieved without reversing the polarity, although they concluded that by reversing the polarity lower concentrations of the ions were observed to accumulate in the vicinity of the electrodes, which may reduce the occurrence of biofouling as a result of eutrophication.

Electorowicz and Boeva (1996) investigated the use of electrokinetics to deliver commercial fertiliser as a source of ammonium nitrate (NH_4NO_3). PO_4^{3-} was distributed through the soil by both electroosmosis and electromigration, which was found to distribute PO_4^{3-} more rapidly and uniformly (Alshawabkeh *et al.*, 2004). Recently published work of Godschalk & Lageman (2005) reported the use of an Electrokinetic Biofence (EBF) to enhance the remediation of VOCs in groundwater. The field trial demonstrated chlorinated hydrocarbon degradation upon addition of Na^+, Ca^+, N, PO_4^{3-} and NH_4^+ compounds. Nutrient filled trenches were installed upstream of the electrodes and then electrodes were placed (at a distance of 5 m apart) to disperse the nutrients in the groundwater. Furthermore, the 'Lasagna' process was designed to use electrokinetics to enhance *in situ* biodegradation (as described, § 1.6.6) (Murdoch & Chen, 1997; Lageman, 1993).

4.2.3.3 The use of electrokinetics to control the temperature and pH of the soil

The optimum temperature for the biodegradation of hydrocarbons by bacteria in temperate soils (mesophilic bacteria) has generally been found to be in the range of 20 to 30°C (Atlas & Bartha, 1992). Although, in the presence of sufficient macronutrients, effective biodegradation has been observed to occur at lower temperatures such as 10°C and the presence of psychrophiles may facilitate biodegradation at temperatures below freezing in cold climate soils (Schlegel, 1992; Margesin & Schinner, 1997). With regard to the pH of the soil, soil microorganisms generally prefer a pH around neutral, with a tendency to prefer slightly alkali conditions (pH 8) (Schlegel, 1992). There is some evidence to suggest that acidic conditions increase the toxicity of organic acids to degrading bacteria (Alexander *et al.* 1987). For the electrokinetics experiments reported here (both laboratory and field-scale), the pH of the soil was maintained at around neutral; the study does not therefore examine the effect of pH on the rate of biodegradation.

4.2.3.4 The use of electrokinetics to remove toxic metabolites

In addition to insufficient concentrations of nutrients and electron acceptors, and a rapid depletion of supplied nutrients, toxic metabolites may accumulate preventing further degradation of the 'parent' compound. Of the contaminants targeted within this study, inhibition through the production of toxic metabolites has only been reported in the literature to affect the degradation of PCBs. For example, Stratford *et al.* (1996), Vrana *et al.* (1996), and Sondossi *et al.* (1992) describe the inhibitory effect of the secondary metabolite chlorobenzoic acid (CBA) on further degradation of the parent compound by disrupting the oxygenases involved in the degradative pathway of both biphenyl and chlorobiphenyl. Microbial oxidation of long chain hydrocarbons (such as dodecane and octadecylbenzene) usually proceeds completely without the accumulation of intermediate products, and where accumulation does occur, the intermediates are frequently non-toxic (Schelgel, 1992).

4.3 Chemical oxidation

4.3.1 *In situ* chemical oxidation (ISCO)

Chemical oxidation may be used to oxidise (degrade) hydrocarbon contaminants where the time-frame for remediation is too short for biodegradation to be employed, and it is logistically challenging to physically remove the contaminants. There are four principal oxidants currently used for the ISCO of contaminated soils: hydrogen peroxide (H_2O_2),

ozone (O_3), permanganate (MnO_4^-), and persulphate ($S_2O_8^{2-}$) (*e.g.*, Yang and Long 1999; Qiang *et al.*, 2002). Oxidants remove electrons from the organic contaminants resulting in their subsequent chemical degradation. Information regarding the oxidation of alkenes (potential constituents of kerosene) is limited but it is thought oxidation results in the formation of a cyclic hypomanganate ion, and then further oxidation occurs to carboxylic acids and CO_2, respectively (Stewart, 1973 cited in Hood *et al.*, 2000). There are a number of constraints to the effective use of chemical oxidation *in situ*, including: delivery of the oxidant to the contaminant, the pH of the soil, and the potential for the oxidants to react with and subsequently affect the integrity of structures and/or underground services.

4.3.2 Methods for the delivery of chemical oxidants

Conventional delivery mechanisms of oxidants include relying on passive diffusion under the pressure of an hydraulic head, by low-pressure injection or application as a solid. Therefore, the delivery of chemical oxidants in low-permeability soils to the contaminant is inefficient. Reddy and Madhusudhana (2006) investigated the use of electrokinetics to deliver the chemical oxidant H_2O_2 via electroosmosis into a soil chamber spiked with nickel and phenanthrene. Their study showed that phenanthrene was oxidised by H_2O_2, but the delivery of the oxidant via electroosmosis was limited. For the remediation of nickel an acidic pH was required, which has the effect of reducing the efficiency of electroosmotic flow.

4.4 Chapter objectives

The focus of this chapter was to investigate electrokinetics as a potential tool for promoting the degradation of hydrocarbons in the sub-surface, in the instance that the contaminant cannot be effectively mobilised (§ 1.7, Objective 3 and 4). The potential to enhance biodegradation *in situ* was investigated through experiments to deliver nutrients and alternative electron acceptors into soil systems (Figure 4.1). In addition, the possibility of maintaining biodegradation even with the development of toxic metabolites was investigated, using electromigration to mobilise charged metabolites away from the spiked region. Furthermore the use of electrokinetics to deliver chemical oxidants into the sub-surface was investigated using $NaMnO_4$ as a model oxidant.

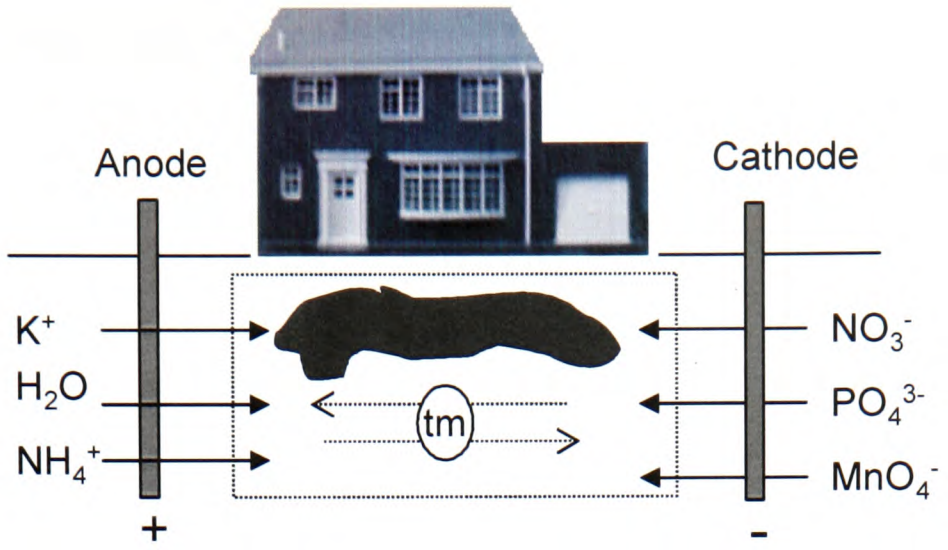


Figure 4.1 Schematic showing the utilisation of electrokinetics to deliver nutrients to the sub-surface.

4.5 Materials and Methods

4.5.1 The feasibility of using electrokinetics to enhance microbial degradation

4.5.1.1 Development of an enrichment culture from Reading soil

Bacteria were enriched from soil by adding 1 g wet weight (w/wt) of Reading soil to 50 ml M9 Minimal Media (M9) (42.27 mM Na_2HPO_4; 22.06 mM KH_2PO_4; 8.62 mM NaCl; 18.6 mM NH_4Cl; 0.1 M $MgSO_4.7H_2O$; 0.01 M $CaCl_2.6H_2O$, final pH ~7.2 [Miller, 1972]) with 500 µl kerosene in a 250-ml conical flask. The conical flasks were sealed with serum caps and incubated at 25°C in a shaker incubator (150 rpm). Every 7 days, 5 ml of the enrichment culture was transferred into 45 ml fresh M9 containing 500 µl kerosene for a total of 64 days (8 weeks). A control of sterile M9 medium containing 500 µl kerosene was also incubated alongside the enrichment culture.

Samples of the enrichment cultures were visualised using an Eclipse E600 microscope (Nikon, Japan). Approximately 5 µl of cell suspension was pipetted onto a microscope slide and covered with a coverslip. An oil immersion lens was used with a x 100 objective giving a total magnification of x 1 000. The images were photographed using a Digital Pixel FISH capture programme (Digital Pixel Ltd., Brighton, UK) (Figure 3.2).

The enrichment cultures were grown up on solid media to facilitate identification of the bacteria, achieved by noting differences in morphology and through subsequent DNA extractions (§ 4.5.1.2). Cell suspensions (40 µl) were spread onto minimal media agar plates; M9 medium was solidified with 1% A1 Agar (Oxoid, Hampshire, UK). Kerosene was added by soaking discs of sterilised filter paper (40 mm^2) with 100 µl of kerosene, and

then placing the discs within the lidded agar plate. Inoculated plates were placed into a sealed glass desiccator. In addition, an open vial of kerosene was placed inside the desiccator to supplement the kerosene-soaked filter paper. Colonies were selected by morphological difference and streak-plated to produce pure cultures. The cells were further characterised genotypically using 16S rDNA (ribosomal deoxyribonucleic acid) polymerase chain reaction (PCR) amplifications.

4.5.1.2 Identification of bacteria using 16S rDNA sequencing

The highly conserved 16S rRNA sequences were used as a method for characterising bacteria by extracting DNA and amplifying the variable region of the 16S rDNA gene. The conserved regions of the 16S rRNA are flanked by variable regions and it is these regions that provide a means of ranking bacteria within lower taxonomic groups (Stackebrandt *et al.*, 1991).

DNA extraction

Approximately 100 mg of cells were placed into a multimix tube (Bio-101, Anachem, UK), combined with equal volumes of a phosphate buffer (240 mM, pH 8.0), and phenol: chloroform: isoamyl alcohol (25:24:1) and shaken (5 m s^{-1}) in a Fastprep bead-beater (FP120, Anachem, UK) for 30 sec, before being centrifuged (9 000 x *g*, 5 min). The aqueous (top) layer was removed and combined with an equal volume of chloroform: isoamyl alcohol (24:1), before being centrifuged (9 000 x *g*, 10 min). The top layer was again removed and the nucleic acids precipitated with 0.6 volumes of ice-cold isopropanol. The solution was mixed by gently inverting, and left on ice for 10 min before being centrifuged (9 000 x *g*, 5 min). The pellet was washed with 200 µl ice-cold ethanol and resuspended in 200 µl *Tris*-EDTA (TE) with RNAse (10 units per µl of DNA suspension). Approximately 75-100 µg DNA was obtained (quantified using a Genequant RNA/DNA calculator, Biochrom Ltd., Cambridge, UK). The presence of DNA was confirmed by gel electrophoresis using a 1% agarose gel containing 0.01 µg ml^{-1} ethidium bromide (90 V for 40 min). The gel was visualised under UV light using a Synegene gel imager (Synegene, Cambridge, UK).

Polymerase Chain Reaction (PCR)

The variable region (nucleotide region 158 through 203) of 16S rDNA was amplified using PCR. Primers BF1 and 530R were used specifically to target the variable region, as described by Lane (1991). DNA (1 µl) was added to a standard reaction mixture containing 0.2 µl primer BF1 (100 pmol μl^{-1}) (5′ GACGGTGTATGTGAT-

TTGTCTGAGAGA 3′) and 0.2 µl primer 530R (100 pmol μl^{-1}) (5′ GTATTAC-CGCGGCTGCTG 3′) (MWG-Biotech), 0.2 µl dNTPs (25 mM of each) (Bioline), 0.2 µl Taq DNA polymerase (5 U μl^{-1}) (Sigma-Aldrich), 2 µl 10 x PCR buffer (Sigma-Aldrich), 0.2 µl Bovine Serum albumin (BSA) (1 mg ml^{-1}) (New England Biolabs) and 16 µl dH_2O. All plastic ware (*e.g.* microcentrifuge tubes) was treated with UV for 2 min and molecular grade sterilised water was reautoclaved prior to use (Kwok & Higuchi, 1989; Kitchin et al., 1990) to reduce the risk of cross contamination with nucleic acids. The following PCR programme was followed using a Peltier Thermo Cycler (PTC-225; MJ Research Inc., Watertown, USA):

1. 95°C for 2 min
2. 95°C for 1 min ←
3. 60°C for 45 s — **Repeat 34 times**
4. 72°C for 1 min 30 s →

PCR products (10 µl) were run on a 1% agarose gel containing 0.01 µg ml^{-1} ethidium bromide (90 V for 40 min). The PCR products were cleaned prior to sequencing using a QIAGEN PCR clean-up kit. DNA bands were visualized using a SynGene UV Viewer and photographed using Genesnap 3.00.09 (Syngene, Synoptics Ltd., Cambridge, UK).

Sequencing of the PCR products

Sequencing reactions were carried out using the Applied Biosystems Big Dye terminators version 3.1 and run on an Applied Biosystems 3730xl DNA Analyser according to the manufacturer's instructions. The BF1 and 530R primers were added to a final concentration of 0.32 µM for sequencing. Sequencing was carried out at The Department of Biochemistry Sequencing Service, University of Oxford.

A BLAST (basic local alignment search tool) search was carried out on the National Center for Biotechnology Information (NCBI) website using an expect value of 10 (default) and the default filter (http://www.ncbi.nlm.nih.gov/) (Altschul et al., 1997).

4.5.1.3 Biodegradation of kerosene in microcosms

Reading soil (30 g d/w) was re-wetted to 70 % WHC with M9 solution. The soil was spiked with kerosene to a final concentration of approximately 30 000 mg kg^{-1} (assuming a kerosene density of 810 mg ml^{-1}) and incubated at ambient temperature (~25°C) for 30 days. At the end of the experiment the soil was extracted in hexane and analysed using

GC-FID (§ 2.3.7.3) and the chromatograms were compared qualitatively (there was no resource available to quantify the individual kerosene constituents).

4.5.1.4 'Sterile' microcosms

To provide further evidence that the loss of the target contaminant mix in the soil chambers (with and without the presence of an electric field) was as a consequence of biodegradation and not as a result of extraction efficiency and/or volatilisation, sterile soil microcosms were set up. Reading soil was sterilised (§ 2.3.2.1) and then spiked with the target contaminant mix (DXT) at concentrations of approximately 11 000, 9 100 and 4 000 mg kg^{-1}, respectively. In triplicate, 1 g of the sterilised 'spiked' soil was placed into 7-ml glass vials with PTFE lids and extracted in hexane immediately or incubated at 25°C for either 7 or 14 days. At the end of the incubation period, the vials were vented for 10 min in a laboratory fume hood before being destructively extracted in hexane and analysed using GC-FID (as described in general method § 2.3.7.3).

4.5.1.5 Biodegradation of target contaminants in isolation or as a mixture

Microcosm studies were used to identify whether there was a difference in the degradation of the target contaminants (DXT) in isolation when compared to being 'spiked' into the soil as a mixture. One g d/w (normalised to 3 d.p.) of Reading soil was placed into 7-ml glass vials with PTFE lids. The soil was moistened to 70% of its WHC with mains water and was then spiked with the target contaminants at the standard concentrations (§ 2.3.7.1) of ~11 000, 9 100 and 4 000 mg kg^{-1} as either single contaminants or as a mix. The microcosms were stored at 28°C and triplicate samples were destructively analysed at days 1, 3, 5, 7, 11, and 14 (as described by the general method § 2.3.6.3).

4.5.1.6 The effect of temperature on the biodegradation of the target contaminants

To investigate the effect of temperature on the biodegradation of the target contaminant mix (DXT), 30 g d/w Reading soil was rewetted to 70% WHC with filtered (< 0.2 μm) mains water, and spiked with the standard contaminant mix (DXT) at concentrations of 11 000, 9 100, and 4 000 mg kg^{-1} respectively, in 100-ml wide screw-necked glass jars (Fisher Scientific, Loughborough, UK). For each time point: 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 14 days, triplicate samples were held at each of the four temperatures, 4, 18, 28 and 38°C. Abiotic controls for each temperature were prepared by autoclaving the soil three times in a 48 h period; inoculation of the soil onto PCA was used to verify the sterility of the soil. CO_2 traps (15 ml NaOH in 25-ml glass Bijou bottles [Fisher Scientific,

Loughborough, UK]) were added to the microcosms to measure microbial respiration using standard methods (Chase & Gray, 1957; Bartha & Pramer, 1965; Pramer & Bartha, 1972). To create an airtight seal the screw thread of each jar was coated with vacuum grease before the PTFE lids were tightly secured. Extra controls, in triplicate, were set up including: CO_2 traps within empty glass jars; and CO_2 traps within jars containing 30 g rewetted Reading soil without contaminants, to account for the source of CO_2 from the soil that may be released upon rewetting (Sharabi & Bartha, 1993).

To determine the volume of trapped CO_2 at 24 h intervals, the NaOH was titrated with 0.1 M HCl following the addition of 5 drops of phenolphthalein solution (1% in 60% ethanol) as an indicator, and 2 ml of 0.5 M barium chloride ($BaCl_2$). The amount of CO_2 was determined using the following equation (Stotzky, 1965; cited in Zibilske, 1994):

$$\text{mg } CO_2 \text{ per gram dry soil} = \frac{(c - s) \times (NE)}{w}$$

Equation 4.2 Equation to determine the amount of CO_2 trapped in the NaOH traps in the form of $NaCO_3$. Where *c* is the volume (ml) of HCl used for the titration of controls (to assess the O_2 and CO_2 exchange in an undisturbed soil system with no addition of contaminants), *s* is the volume (ml) of HCl used for the titration of soil samples, N is the normality of the acid used (0.1), in milliequivalents ml^{-1}, E is the equivalent weight of C in CO_2 (E = 22 if the data is expressed in terms of mg CO_2). *w* was the quantity of Reading soil used in the experiments (30 g d/w).

At the end of the predetermined incubation period all microcosms were stored at -80°C prior to being destructively sampled and analysed by GC-FID following extraction in hexane (§ 2.3.7.3).

4.5.1.7 The effect of nutrient amendments on the biodegradation of the target contaminants

One g d/w (normalised to 3 d.p.) of Reading soil was placed into 7-ml glass vials with PTFE lids. The soil was moistened to 70% of its WHC with either MilliQ water, M9 or M9 without a source of N. The soil was spiked with the contaminant mix (DXT) at concentrations of 225, 263 and 229 mg kg^{-1}, respectively. The soil microcosms were incubated in the dark at ambient temperature (~25°C) for 0, 6, 12, 24, 48, 72 and 168 h. Three independent soil microcosms were set up for each incubation period. At the end of the experiment, samples were destructively sampled and analysed using GC-FID (general method § 2.3.7.3).

4.5.1.8 The use of NO_3^- as a nutrient and/or electron acceptor

Experiments were performed to determine whether NO_3^- was being used as an electron acceptor and/or a nutrient in the degradation of hydrocarbons. Microcosms containing 6 g Reading soil were spiked with the standard contaminant mix (DXT) at concentrations of ~11 000, 9 100, and 4 000 mg kg^{-1}, respectively. The soil was then rewetted to 70% WHC (§ 2.3.2) with either 5 mM NH_4^+ (as NH_4Cl), 5 mM NO_3^- (as $NaNO_3$), or a mix containing 5 mM of NH_4^+ and 5 mM NO_3^-. The microcosms were prepared in triplicate in 20-ml glass vials with a PTFE septum lid. At time point 0 h, each microcosm was homogenised by vortexing for 30 s and was then flushed with He for 3 min to displace the O_2. The microcosms were incubated at 28°C for 12, 24, 48, or 96 h. At the end of the incubation period the soil was homogenised and then weighed into fresh vials for extraction and analysis using IC and GC-FID (§ 4.5.19 and § 2.3.6.3, respectively).

4.5.1.9 IC Dionex system – analysis of NO_3^- and NO_2^-

Towards the end of the study a new IC system was made available for the analysis of anions. Soil samples for IC analysis were prepared as previously described (§ 2.3.6.4). IC was carried out using a Dionex AS50 Chromatograph, with an EG50 eluent generator, an ED50 electrochemcial detector and a Dionex AS11-HC anion column (4 mm) (Buckinghamshire, UK), with deionised water as the mobile phase, which was degassed for 10 min with He prior to use. The flow rate was 1.5 ml min^{-1}. The solutions were analysed for NO_2^- and NO_3^-.

4.5.2 Using electrokinetics to mobilise nutrients in the sub-surface

4.5.2.1 Movement of NO_3^- through the soil from a 'spiked' region

The electrokinetics chamber was set up as described in Chapter two (§ 2.3.5), with mains water as the electrolyte. The only difference in experimental set up was that the flow rate of the acid and alkali buffering solutions, neutralising the electrolyte, were operated at 68 ml min^{-1} and the electrolyte was mixed using a magnetic plate stirrer and follower, in attempt to improve pH control.

A 1 cm wide section of soil was removed from the chamber (~100 g) and spiked in a glass beaker with NH_4NO_3 (160 mg kg^{-1}) before being repacked into the soil chamber. The spiked region was positioned 3.5 cm from the cathode of the electrokinetics chamber. The electrokinetics chamber was operated for 24 h with 60 V applied. Soil sampling was

performed (§ 2.3.5.6, Figure 2.5) at time points 1, 6, 12 and 24 h. Each soil sample was analysed for NO_3^- (§ 2.3.6.4) and moisture content (§ 2.3.6.1) in triplicate.

4.5.2.2 Movement of NO_3^- into the soil from the cathode reservoir

The electrokinetics chamber was set up as described in chapter two (§ 2.3.5) with Reading soil. In the first set of experiments a 0.1 M (8 000 mg l^{-1}) NH_4NO_3 solution was added to the cathode reservoir (250 ml) and mains water to the anode reservoir (250 ml). The electrokinetics chamber was set up with Reading soil and was operated for 24 h with 60 V applied. Soil samples were collected along the length of the soil chamber at time points 1, 6, 12 and 24 h (§ 2.3.5.6, Figure 2.5). Triplicate soil samples were collected and analysed for the concentration of NO_3^- (§ 2.3.6.4) and for moisture content (§ 2.3.6.1).

4.5.2.3 Movement of NO_3^- into the soil from the recirculated anolyte and catholyte

The electrokinetics soil chamber (§ 2.3.5) packed with Wytham soil was operated for 24 h with 60 V applied (~10 mA) and the catholyte and anolyte reservoirs were recirculated together. At time point zero (0 h), 0.1 M (8 000 mg l^{-1}) NH_4NO_3 was added to the electrolyte solution. Soil samples were collected from along the length of the soil chamber at time points 0 (after the addition of NH_4NO_3) and 24 h (§ 2.3.5.6, Figure 2.5). Soil samples were collected in triplicate and analysed for the concentration of NO_3^- (§ 2.3.6.4) and for moisture content (§ 2.3.6.1). The initial concentration of NO_3^- detected in Wytham soil was analysed as 115.4 ± 16.6 mg kg^{-1} (§ 2.3.2, Table 2.2).

In a second series of experiments the electrokinetics chamber was packed with Reading soil (§ 2.3.5) and was spiked with the standard DXT contaminant mix at a concentration of ~11 000, 9 100, and 4 000 mg kg^{-1}, respectively. Approximately 12 000 mg l^{-1} NH_4NO_3 was added to the combined anolyte and catholyte reservoir at 0 h. After 14 days of applying an electric field (60 V, ~10 mA) the soil chamber was destructively sampled (§ 2.3.5.5, Figure 2.4). Soil samples were collected in triplicate and the concentration of NO_3^- (§ 2.3.6.4), the concentration of the target contaminants (DXT) (§ 2.3.6.3) and moisture content (§ 2.3.6.1) were determined.

4.5.2.4 Addition of nutrients and/or electron acceptors coupled with pH control

It was proposed that in addition to pH control, a buffer solution could be selected to act as a source of nutrients to aid biodegradation while simultaneously neutralising the electrolyte. A 10:1 mix of nitric (HNO_3) and phosphoric acid (H_3PO_4) was used as a

buffer solution for the catholyte. The chamber was operated in the same way as described elsewhere (§ 2.3.5), but acid additions were monitored using a 50-ml glass measuring cylinder with 1-ml graduations and the anolyte and catholyte were recirculated together. Soil sampling was undertaken at the start and after 7 days (§ 2.3.5.6, Figure 2.5). Triplicate soil samples were analysed for the anions NO_3^- and PO_4^- (§ 2.3.6.4) and for moisture content (§ 2.3.6.1).

4.5.3 Using electrokinetics to mobilise toxic metabolites in the sub-surface

4.5.3.1 Movement of 3-chlorobenzoate (3-CBA) from the catholyte

The electrokinetics soil chamber was set up as described previously (§ 2.3.5). Mains water was used as the anolyte and a 25 mg l^{-1} 3-CBA solution (a metabolite of PCB) was added to the catholyte reservoir (the catholyte and anolyte solutions were not recirculated together). 60 V (~10 mA) was applied for 12 h and 11 samples were collected along the length of the soil chamber at time points 2, 4, 6, 8, and 12 h (§ 2.3.5.6, Figure 2.5). Each soil sample was analysed in triplicate for moisture content (§ 2.3.6.1) and the concentration of 3-CBA was determined using high performance liquid chromatography (HPLC) (§ 4.5.3.2).

4.5.3.2 HPLC – analysis of 3-CBA

Soil samples, (1 g; samples weighed and normalised to 3 d.p.), were placed into 2-ml plastic microcentrifuge tubes, and 1.5 ml of dH_2O was added. The soil slurries were then mixed using a bench-top whirlimixer for 30 s before being centrifuged at 7200 x *g* for 10 min using a Labnet Microcentrifuge. The solutions were analysed by HPLC using a Ascentis C_{18} column (581324-U: dimensions: 15 cm x 4.6 mm x 5 µm; Supelco, USA) equipped with a pre-filter. A 40% acetonitrile and 60% 50 mM NaH_2PO_4 (~pH 5) mobile phase was used. Peaks were measured using a Dionex HPLC analyser (UVD170S; Dionex, UK) and analysed at a wavelength of 206 nm using Chromeleon v6.10 software (Dionex, UK). 40 µl samples were injected for analysis at a flow rate of 1 ml min^{-1}.

4.5.3.3 Movement of 3-CBA through the soil from a spiked region

The electrokinetics soil chamber was set up, as described previously (§ 2.3.5) with mains water as the electrolyte. A 1 cm wide section was removed from the chamber (~100 g) and spiked in a glass beaker with 3-CBA (~12 mg kg^{-1}) before being repacked into the soil chamber. The spiked region was positioned 3.5 cm from the cathode of the electrokinetics chamber. Eleven soil samples were collected along the length of the soil chamber (as

described § 2.3.5.6, Figure 2.5) at time points 1, 6, 12 and 24 h and sampled for moisture content (§ 2.3.6.1) and 3-CBA concentration (§ 4.5.3.2).

4.5.4 The electrokinetic delivery of the chemical oxidant $NaMnO_4$

In two separate experiments, a 1% $NaMnO_4$ solution was added to either the cathode or anode reservoir and 60 V applied for 7 days using the standard electrokinetics apparatus (§ 2.3.5) to investigate delivery of permanganate via electromigration and electroosmosis, respectively. The influence of the electric-field on the movement of $NaMnO_4$ was assessed by determining the concentration and distribution profile for sodium (Na^+) and manganese (Mn[VII]) throughout the soil chamber at the end of the experiment. Soil samples were collected from the soil chamber in triplicate using metal borers (refer to § 2.3.5.6, Figure 2.5) and duplicate soil samples were analysed by a private laboratory (I_2, Middlesex, UK) using inductively-coupled plasma mass spectrometry (ICP-MS).

4.6 Results

4.6.1 Microbial studies

4.6.1.1 Identification of hydrocarbon degraders from Reading soil

A sample of Reading soil was enriched with kerosene and then subsequently the culturable bacteria were cultured and sequenced using standard methods. *Rhodococcus erythropolis, Stenotrophomonas maltophilia* T3-c and two species of *Pseudomonas* (genus not defined) were sequenced from the enrichments.

4.6.1.2 Degradation of kerosene from a microcosm

Reading soil was spiked with kerosene at a concentration of approximately 30 000 mg kg^{-1}. After 30 days of incubation with M9 nutrient solution, a marked reduction in the peak areas was observed in the majority of the kerosene fractions compared with neat kerosene (Figure 4.2). The experiment was used for indicative purposes to show the potential for degradation of kerosene to occur in the model soil and was not used to assess the efficiency of biodegradation.

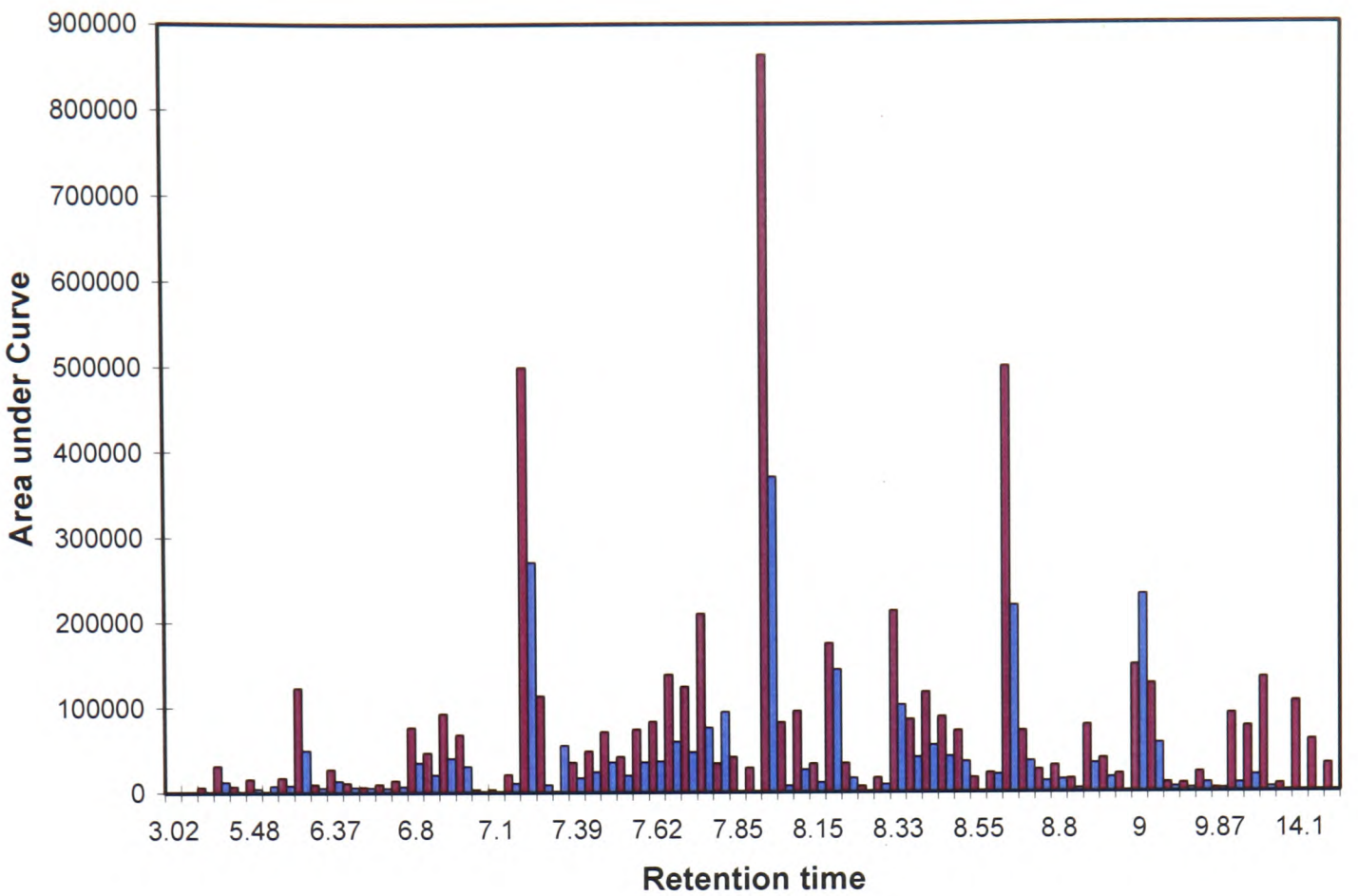


Figure 4.2 Comparison between the GC-FID profile of ■ kerosene spiked into Reading soil (rewetted to 70% water holding capacity with M9) and extracted immediately and ■ kerosene spiked into Reading soil (rewetted to 70% water holding capacity with M9) and incubated at ~25°C for 30 days.

4.6.2 Microcosm studies for the biodegradation of the target contaminants

4.6.2.1 'Sterile' microcosms

There was no significant difference ($p > 0.05$, df = 4) between the start and final concentrations of the target contaminant mixture (DXT) spiked onto autoclaved Reading soil following incubation at 25°C for 14 days (Figure 4.3). Before extracting the microcosms in hexane, the vials were vented for 10 min to enable volatilised contaminants to escape from the microcosm and not be included in the final calculation of concentration, to be consistent with the set up in the soil chamber experiments.

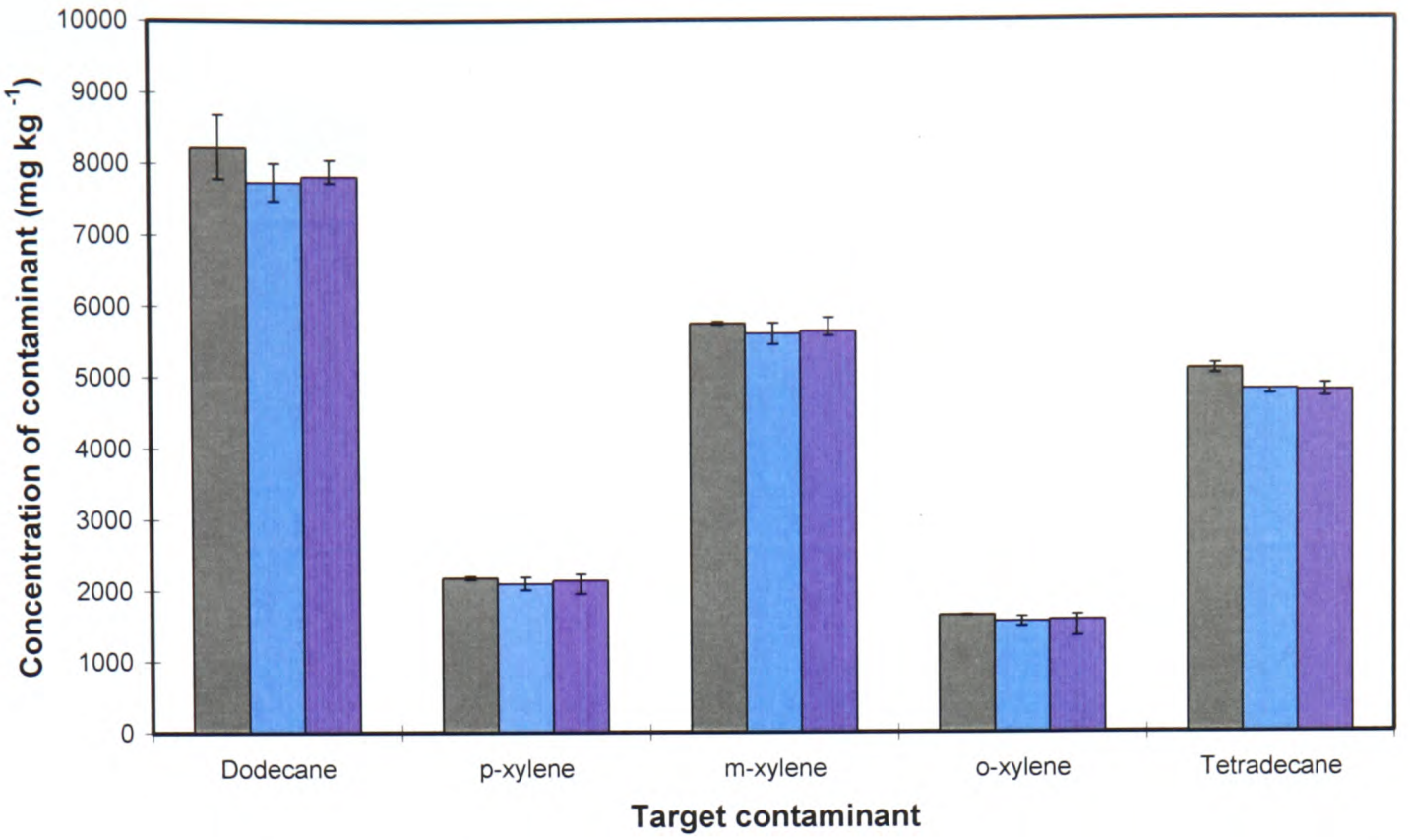


Figure 4.3 Concentration of the target contaminant mix (DXT) in autoclaved Reading soil following incubation at 28°C for ■ 0 days, ■ 7 days, and ■ 14 days (data are means ± SE; n = 3). Initial concentration (~) of the target contaminants was as follows: dodecane 11 000 mg kg^{-1}, *p*-xylene 1 900 mg kg^{-1}, *m*-xylene 5 700 mg kg^{-1}, *o*-xylene 1 500 mg kg^{-1}, and tetradecane 4 000 mg kg^{-1}.

4.6.2.2 Biodegradation of contaminants in isolation or mixtures

The biodegradation/removal of dodecane from the soil microcosms was significantly higher when it was added as a contaminant mixture with xylene and tetradecane compared to microcosms where it was the only contaminant ($p = 0.03$, df = 4) (compare Figures 4.4 and 4.5). There was no significant difference between the disappearance of the other target contaminants (xylene isomers and tetradecane) from the microcosms as either mixtures or as single contaminants ($p > 0.05$, df = 4).

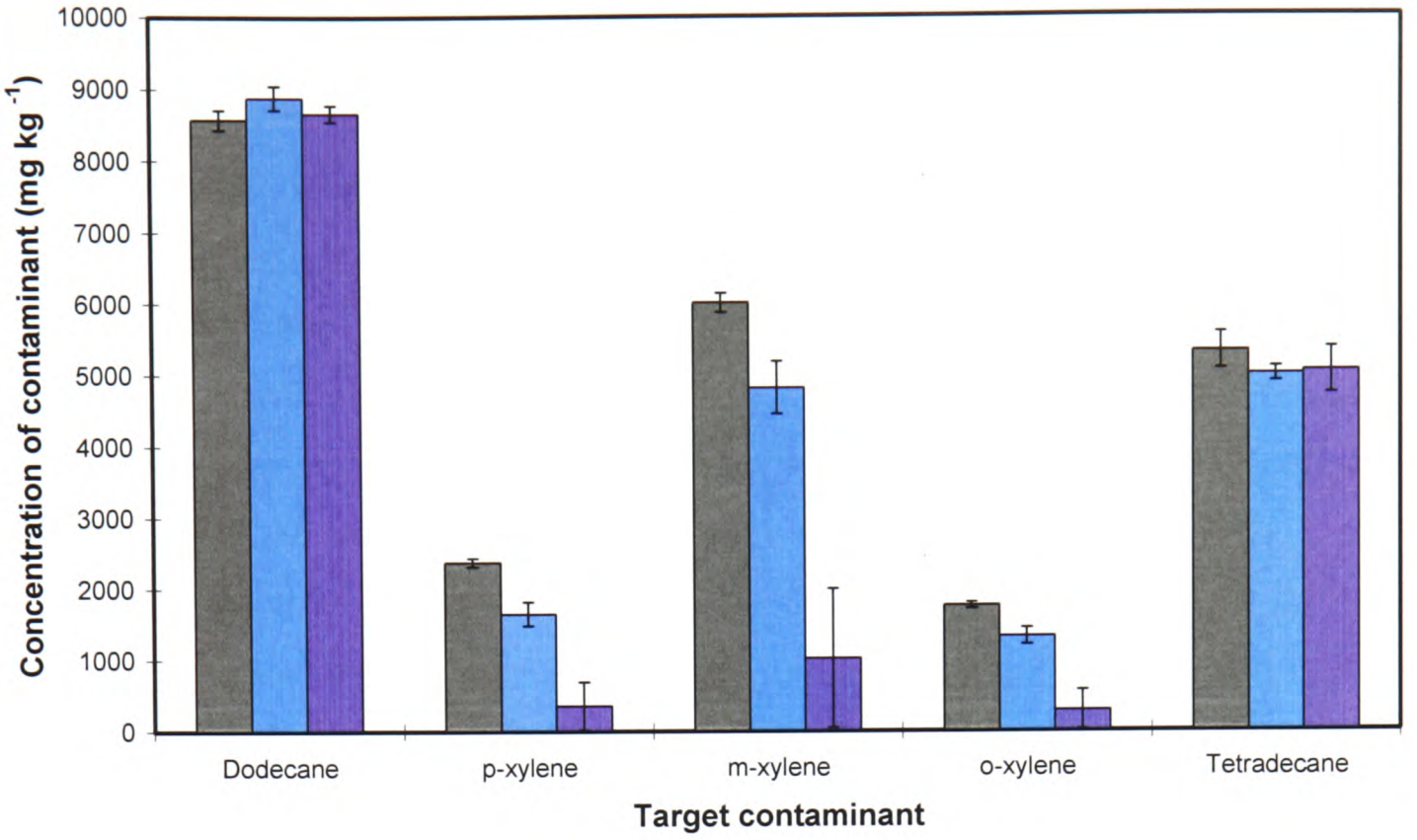


Figure 4.4 The concentration of the target contaminants after being spiked in isolation in individual microcosms containing 1 g Reading soil and then incubated at 28°C for ■ 0 days , ■ 7 days , and ■ 14 days (data are means ± SE; n = 3). Initial concentration (~) of the target contaminants was as follows: 11 000 mg kg^{-1} dodecane, *p*-xylene 1 900 mg kg^{-1}, *m*-xylene 5 700 mg kg^{-1}, *o*-xylene 1 500 mg kg^{-1}, and tetradecane 4 000 mg kg^{-1}.

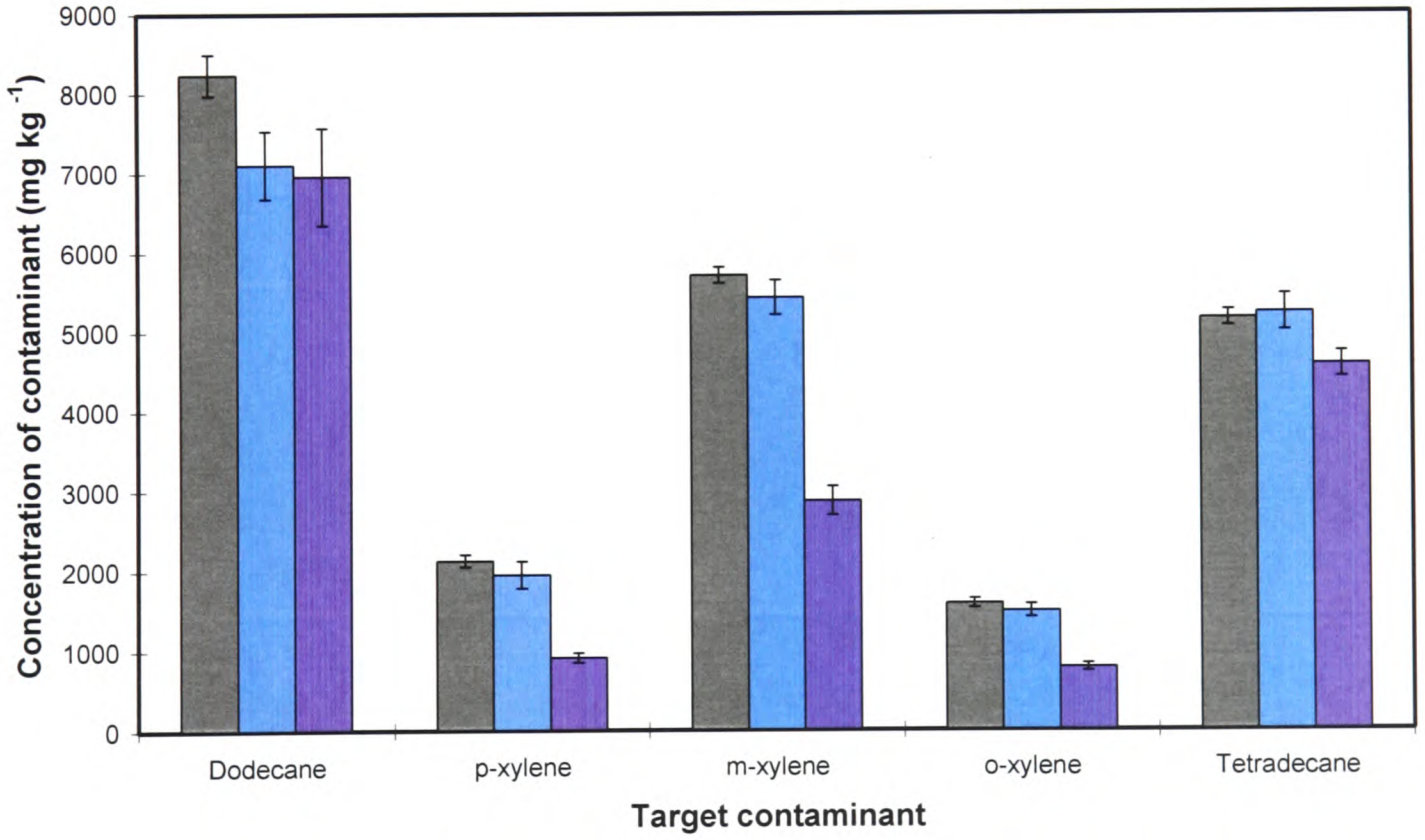


Figure 4.5 Concentration of the target contaminant mix (DXT) in Reading soil following incubation at 28°C for ■ 0 days, ■ 7 days, and ■ 14 days (data are means ± SE; n = 3). Initial concentration (~) of the target contaminants was as follows: 11 000 mg kg^{-1} dodecane, *p*-xylene 1 900 mg kg^{-1}, *m*-xylene 5 700 mg kg^{-1}, *o*-xylene 1 500 mg kg^{-1}, and tetradecane 4 000 mg kg^{-1}.

4.6.2.3 The effect of temperature on the biodegradation of the target contaminants

Soil microcosm studies were set up to investigate the influence of temperature on the degradation of the target contaminant mix (DXT) (§ 4.5.1.6). It was evident that the removal of the target contaminants was enhanced with an increase in temperature (Figure 4.6). The concentration of *p*- and *m*-xylene, dodecane and tetradecane in soil incubated at 4°C was significantly lower than the concentration remaining in the microcosms incubated at 0°C after 7 days (p = <0.0001, 0.0002, 0.004, 0.02, respectively, df = 10). There was no signficant reduction of *o*-xylene at 4°C compared with 0°C (p = 0.06, df = 10). The statistical signficance of the variations in degradation at different temperatures was calculated using un-paired t-tests. Total contaminant removal within 7 days was compared between 0 - 4°C, 4 - 18°C; 18 - 28°C; and 28 - 38°C (Table 4.1).

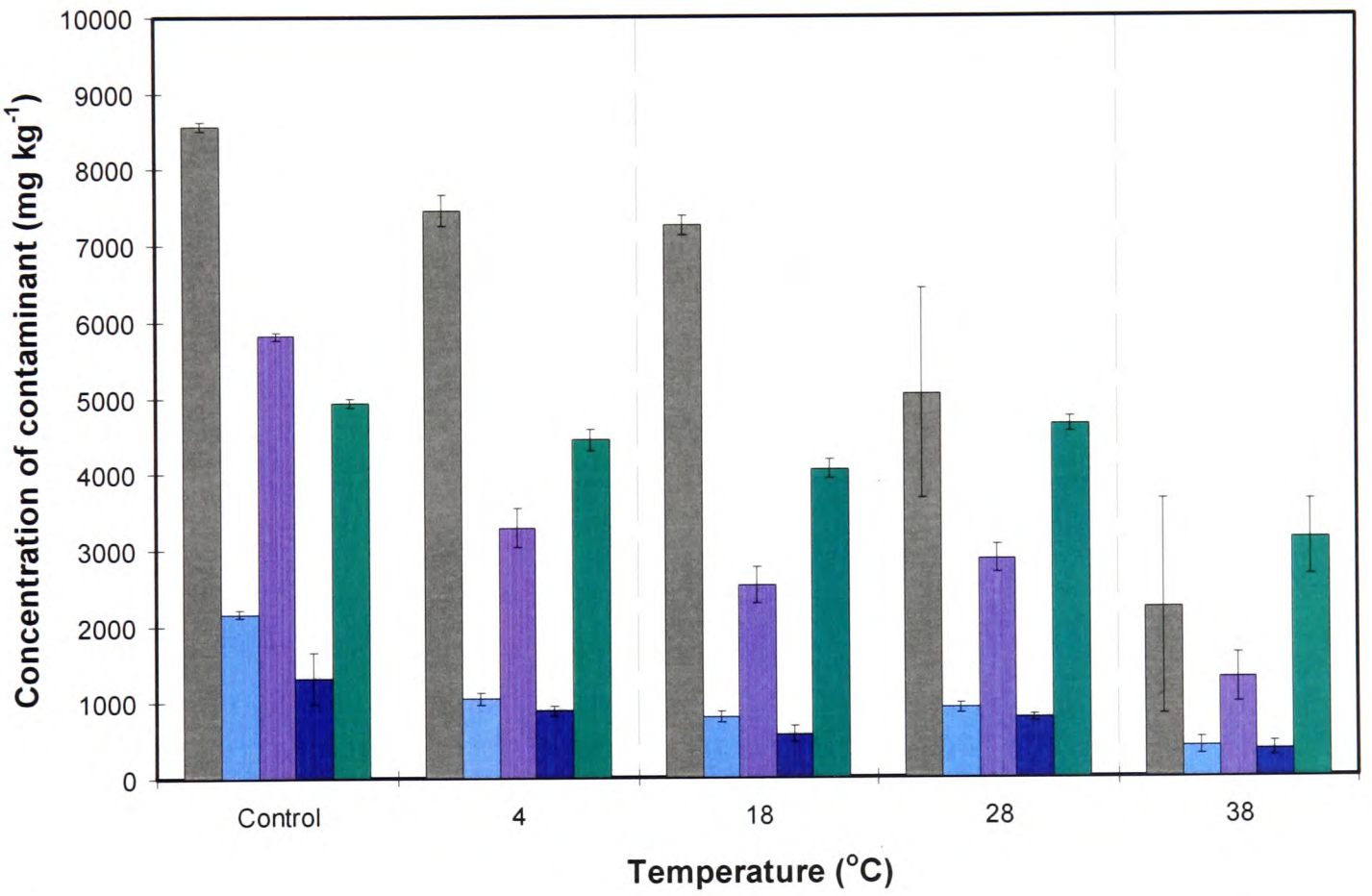


Figure 4.6 Remaining concentrations of the target contaminant mix (DXT) at four different incubation temperatures compared to the contol (0°C) after 7 days. ■ dodecane (11 000 mg kg^{-1}), ■ *p*-xylene (1 900 mg kg^{-1}), ■ *m*-xylene (5 700 mg kg^{-1}), ■ *o*-xylene (1 500 mg kg^{-1}), ■ tetradecane (4 000 mg kg^{-1}) (data are means ± SE; n=6).

Table 4.1 A comparison between the decrease in contaminant concentration at two different temperatures. Unpaired t-test (two-sample, equal variance) was used to determine the statistical significance. Significant differences exist where $p \leq 0.05$ (degrees of freedom = 10).

Temperatures compared (°C)	*p*-Xylene	*m*-Xylene	*o*-Xylene	Dodecane	Tetradecane	
0-4	0.00002	0.0002	0.06	0.004	0.02	*p* Value
4-18	0.03	0.03	0.02	0.23	0.03	
18-28	0.15	0.14	0.05	0.02	0.003	
28-38	0.001	0.0009	0.0009	0.03	0.007	

■ shaded cells indicate that there was a significant difference between the data sets, $p \leq 0.05$

The respiration rate within the soil microcosms increased with an increase in temperature, providing further evidence of enhanced degradation upon increased soil temperature (Figure 4.7). A control microcosm was held at each incubation temperature to account for an increased release of CO_2 known to occur with an increase in soil temperature. This provides further evidence that the increased loss in concentration of contaminant was a result of enhanced degradation rather than an increase in volatilisation from the soil.

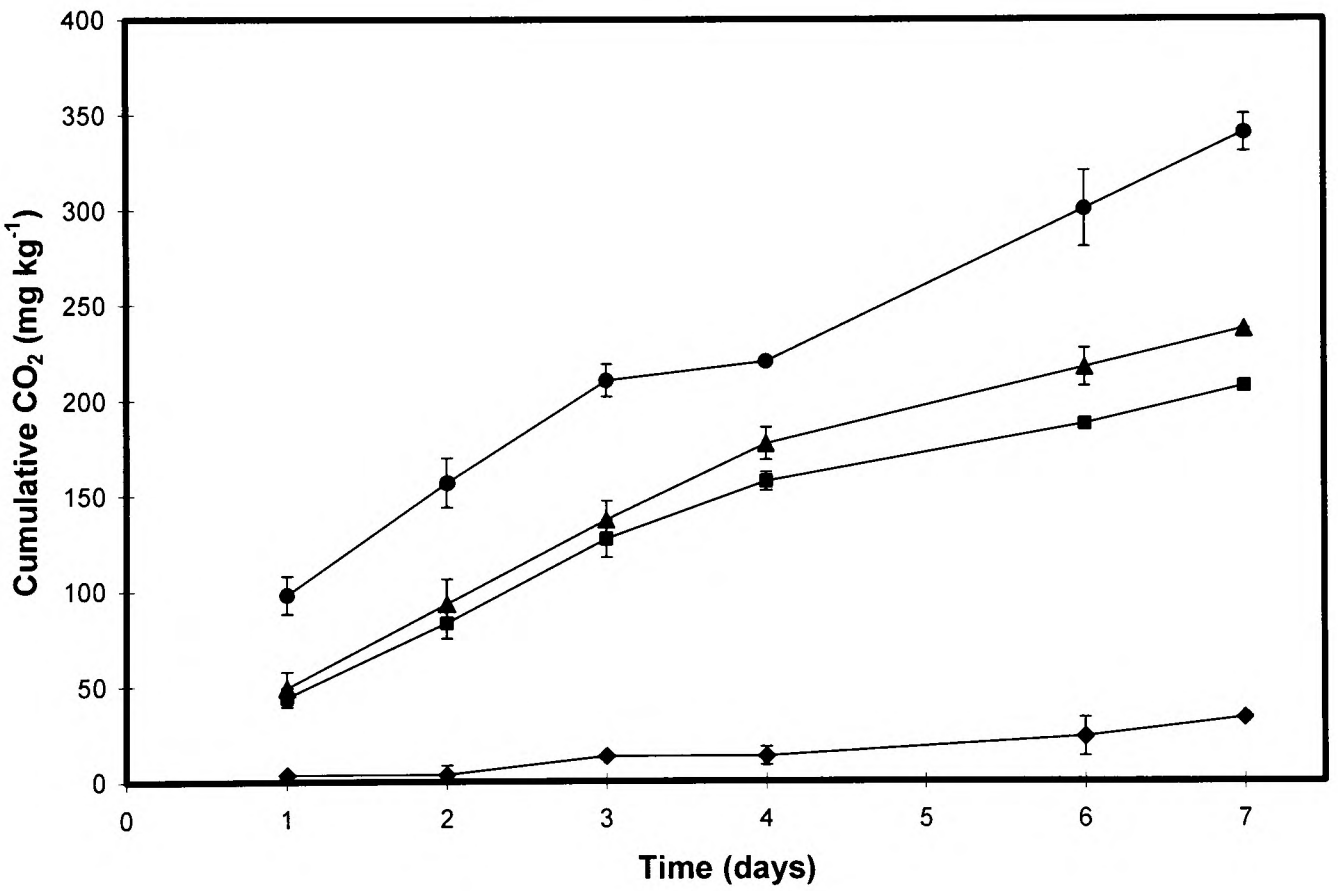


Figure 4.7 A comparison between the respiration rate of Reading soil spiked with the target contaminant mix (dodecane, xylene and tetradecane) at different air temperatures. ◆ 4°C; ■ 18°C; ▲ 28°C; ● 38°C, (data are means ± SE, n = 3).

4.6.2.4 The effect of nutrient amendments on the biodegradation of the target contaminants

The degradation of the target contaminant mix (DXT) with and without nutrient amendments was investigated using 7-day (168 h) microcosm studies. Degradation of each of the contaminants was observed (Figures 4.8 to 4.12). There was no significant difference between the degradation achieved in the presence of sterile water, M9 nutrient solution or M9 (less nitrogen) nutrient solution over 7 days ($p \geq 0.3$, df = 2 (ANOVA, single factor) (Figures 4.8 to 4.12).

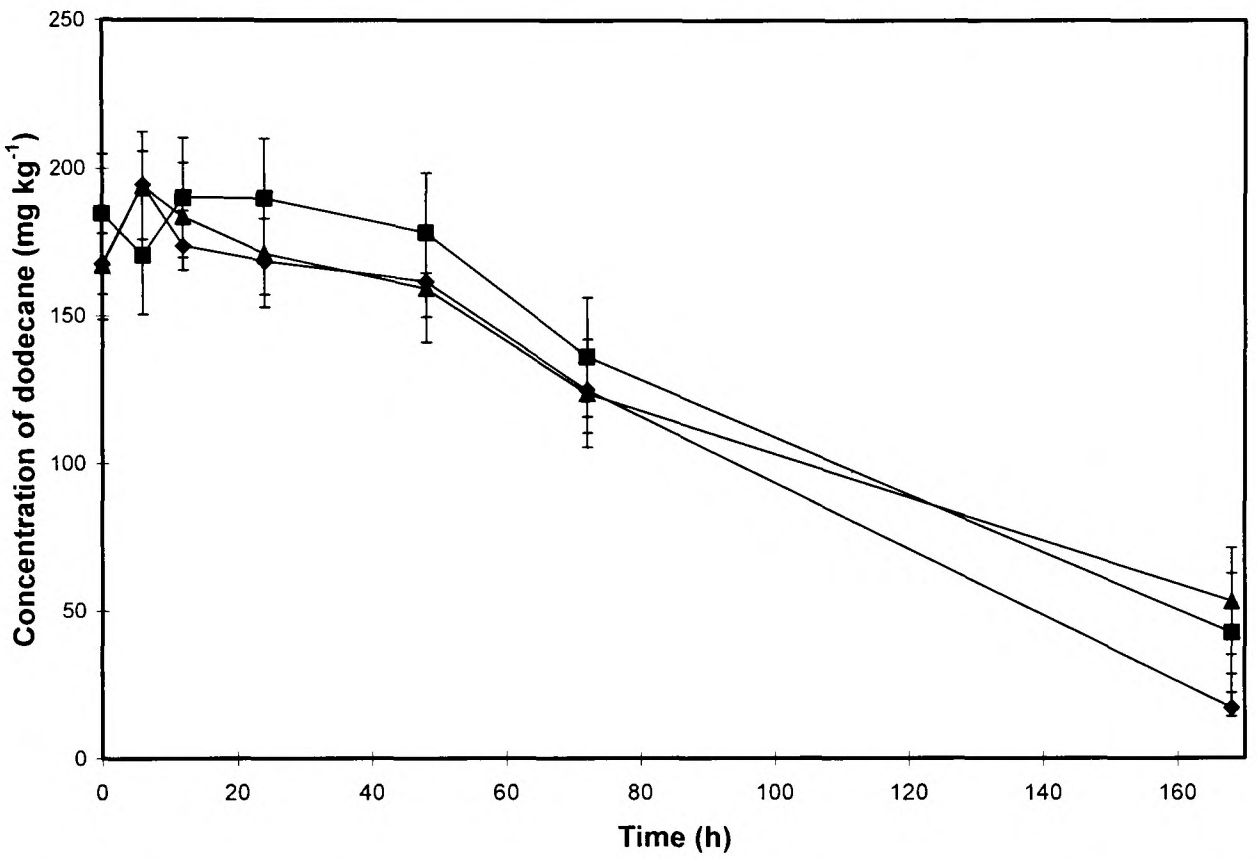


Figure 4.8 Comparison between the degradation rate of dodecane (~225 mg kg^{-1}) from soil microcosms containing 1 g of Reading soil amended with either: ◆ sterile water; ■ M9 nutrient solution; ▲ M9 nutrient solution without source of N (data are means ± SE; n=3).

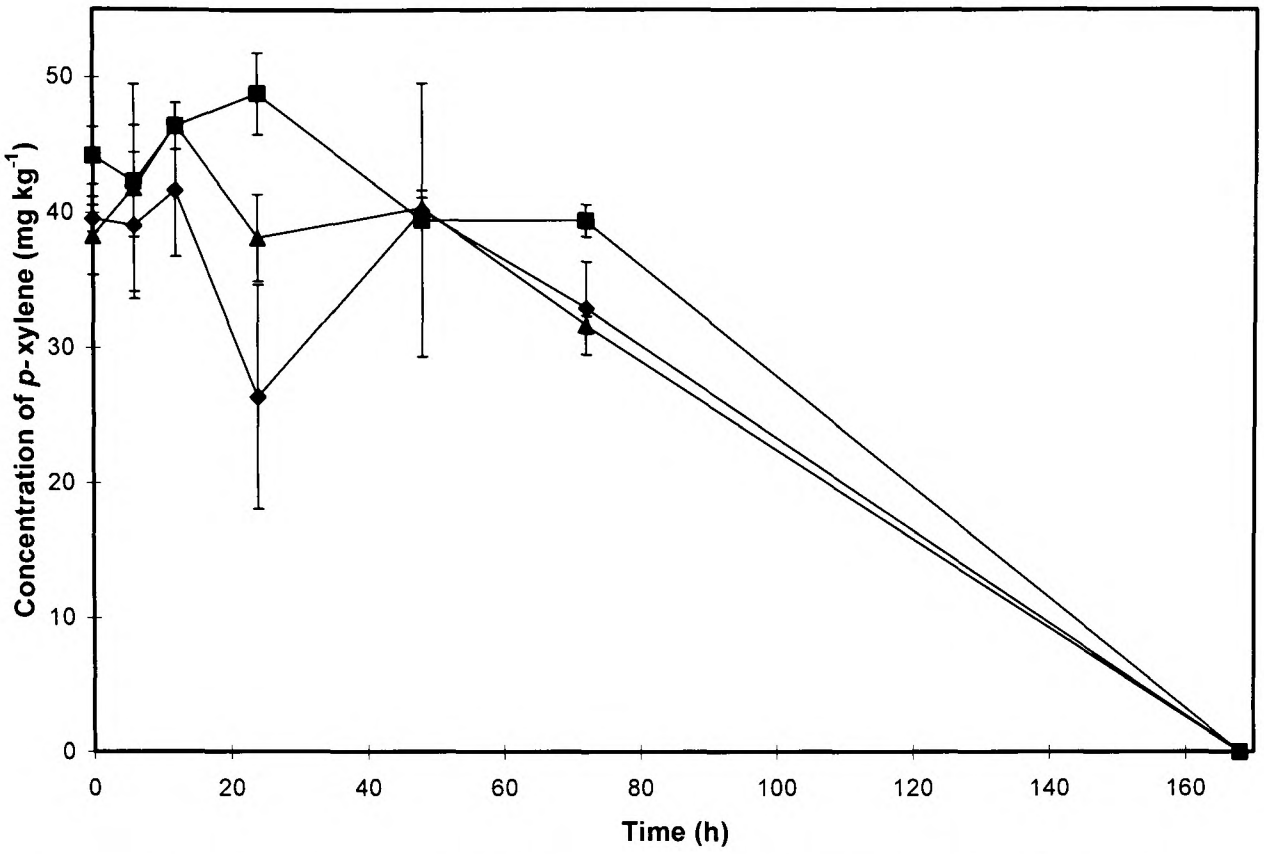


Figure 4.9 Comparison between the degradation rate of *p*-xylene (~56 mg kg^{-1}) from soil microcosms containing 1 g of Reading soil amended with either: ◆ sterile water; ■ M9 nutrient solution; ▲ M9 nutrient solution without source of N (data are means ± SE; n = 3).

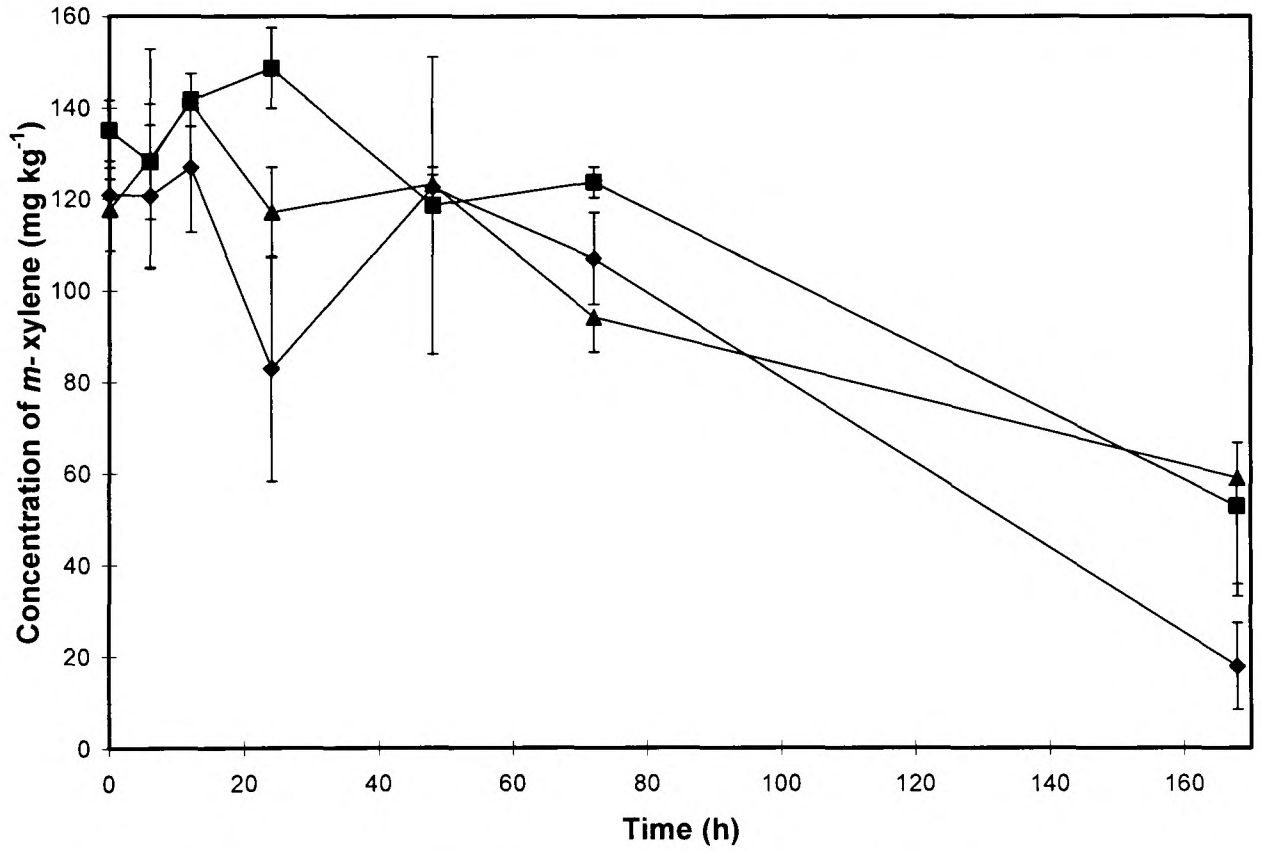


Figure 4.10 Comparison between the degradation rate of *m*-xylene (~165 mg kg^{-1}) from soil microcosms containing 1 g of Reading soil amended with either: ◆ sterile water; ■ M9 nutrient solution; ▲ M9 nutrient solution without source of N (data are means ± SE; n = 3).

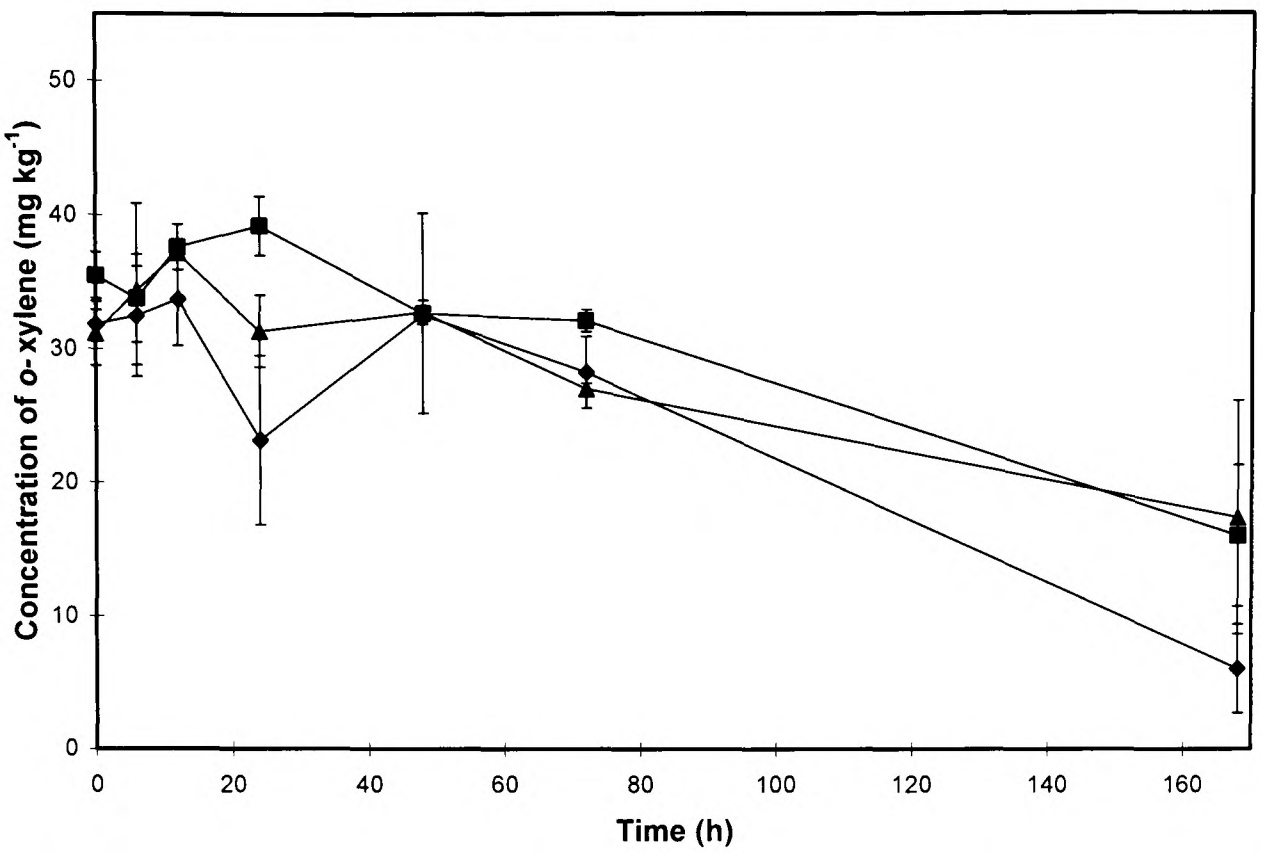


Figure 4.11 Comparison between the degradation rate of *o*-xylene (~42 mg kg^{-1}) from soil microcosms containing 1 g of Reading soil amended with either: ◆ sterile water; ■ M9 nutrient solution; ▲ M9 nutrient solution without source of N (data are means ± SE; n = 3).

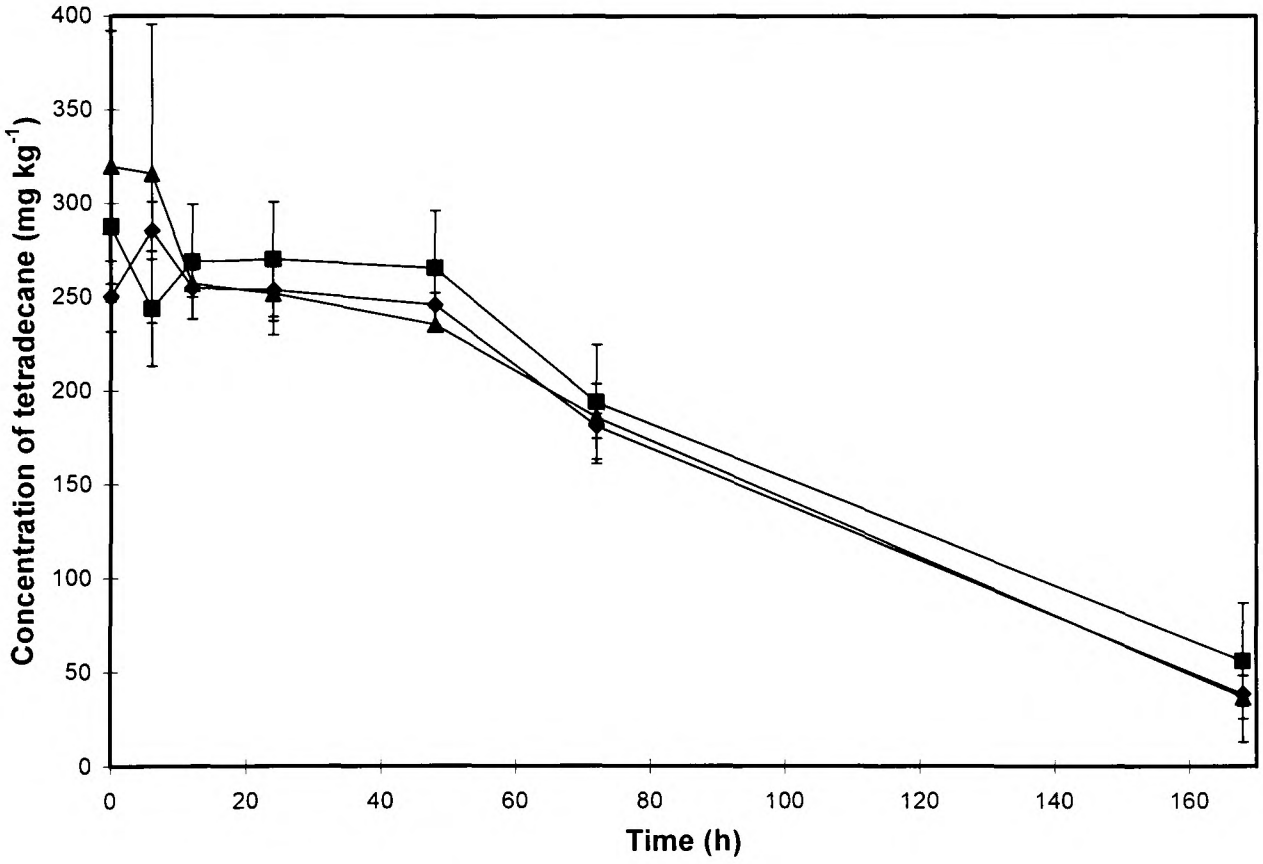


Figure 4.12 Comparison between the degradation rate of tetradecane (~229 mg kg^{-1}) from soil microcosms containing 1 g of Reading soil amended with either: ◆ sterile water; ■ M9 nutrient solution; ▲ M9 nutrient solution without source of N (data are means ± SE; n = 3).

4.6.2.5 The utilisation of NO_3^- for the degradation of the target contaminants

Figures 4.13 and 4.14 show the depletion of NO_3^- from the central spiked region of the control soil chamber (without an electric field applied), compared with the non-contaminated neighbouring regions after 14 days. As reported in Chapter 2 (§ 2.4.8), after 14 days of applying an electric field across the soil chamber, without recirculation of the electrolyte, NO_3^- had been stripped from the soil via electromigration into the anolyte.

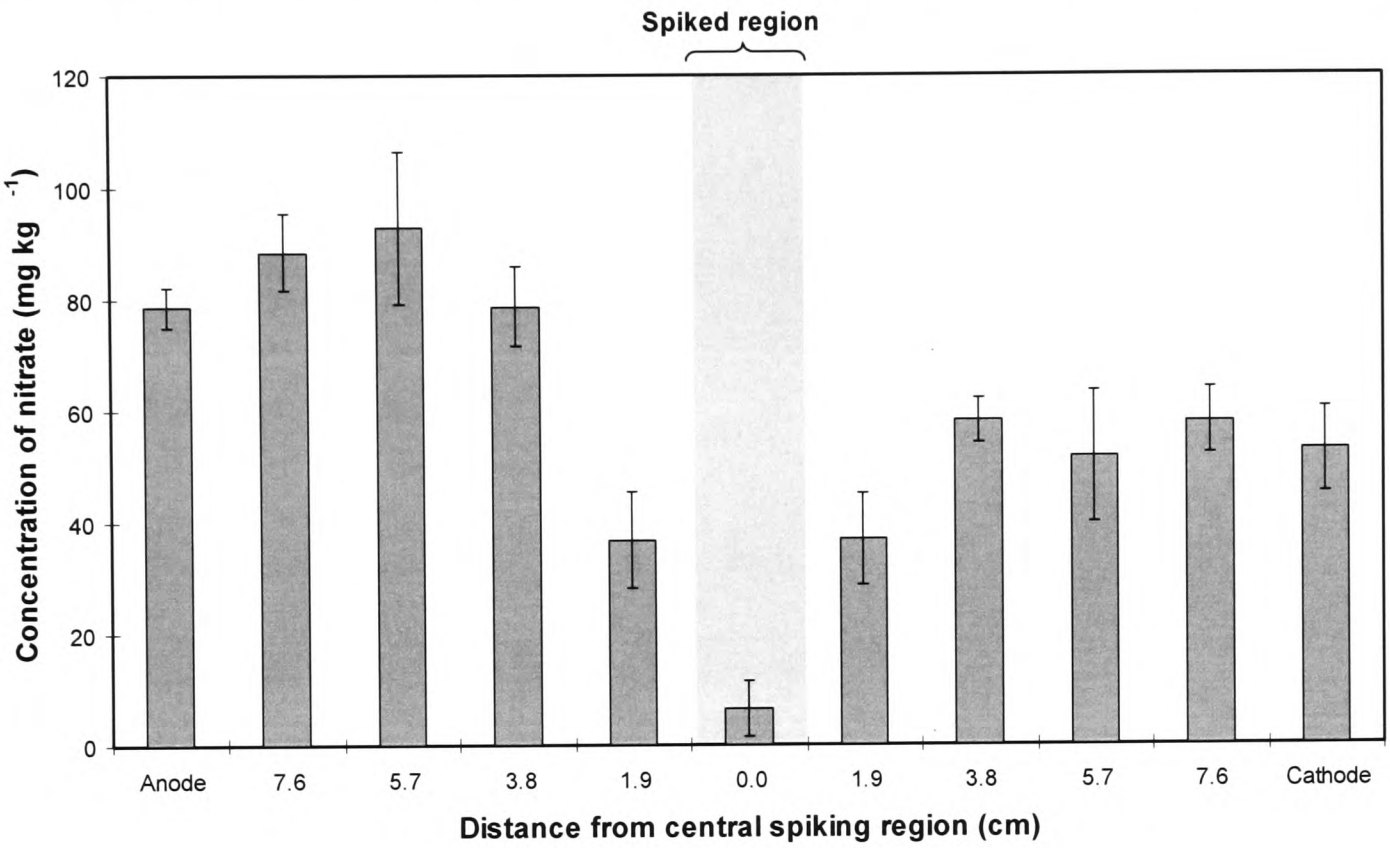


Figure 4.13 NO_3^- distribution in soil chamber containing Reading soil after 14 days without a current (data are means ± SE; n=3).

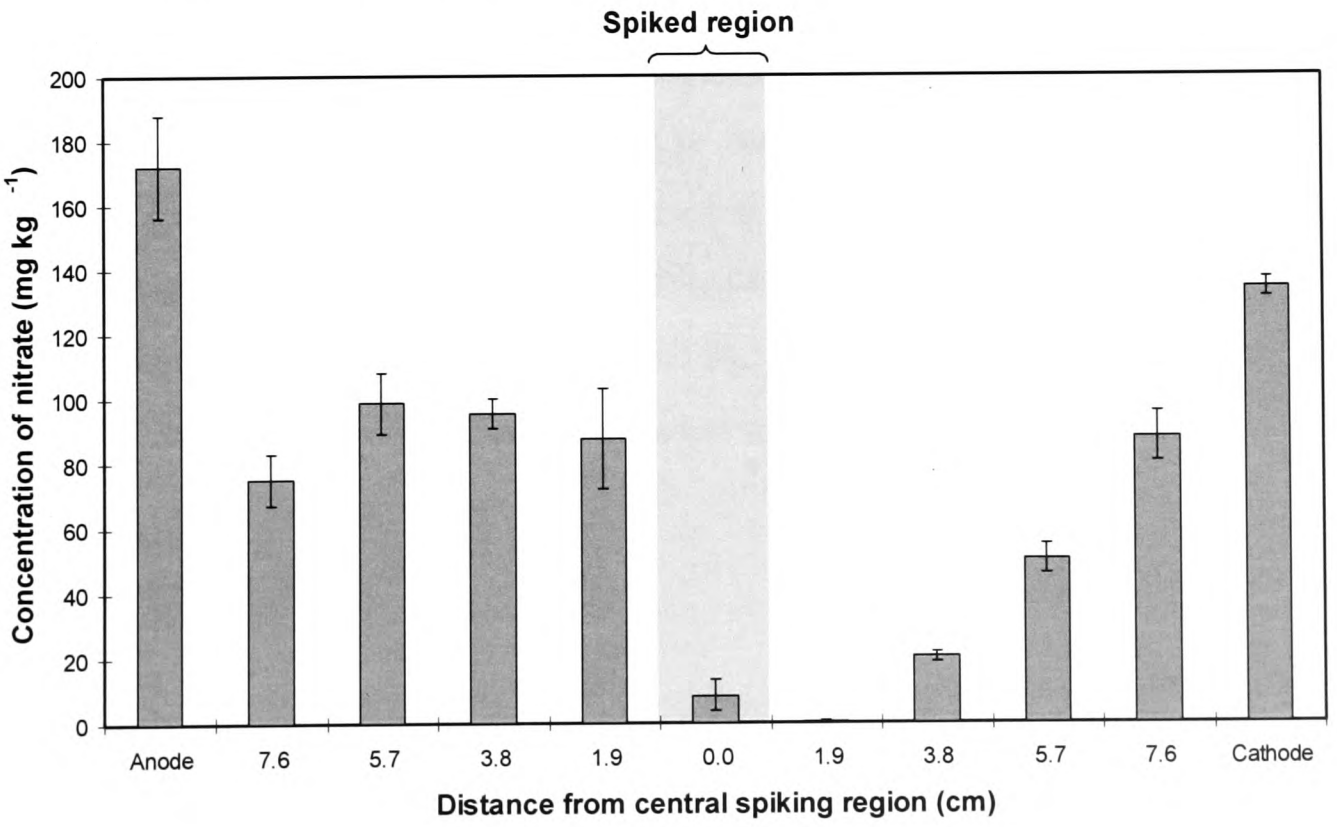


Figure 4.14 NO_3^- distribution in soil chamber containing Wytham soil after 14 days without a current (data are means ± SE; n=3).

4.6.3 The use of NO_3^- as a nutrient and/or electron acceptor

Microcosms (6 g Reading soil) were used to investigate the utilisation of NO_3^- during the degradation of the target contaminants from the soil (§ 4.5.1.8), to determine whether NO_3^- was used as a source of N and/or as an electron acceptor. After an incubation period of 96 h, degradation of the target contaminant mix (DXT) from the microcosms had not occurred. Consequently, depletion of NO_3^- with and without the addition of NH_4^+ did not provide statistically significant evidence of its role in degradation. The concentration of NO_3^- and NO_2^- observed in the soil samples between 0 and 96 h are included in Appendix 8.8 for completeness. In the presence of NO_3^- (without NH_4^+) after 12 h of incubation of the 'spiked' microcosms NO_2^- was detected in the soil. The formation of NO_2^- is concurrent with both the dissimilatory and assimilatory nitrate reduction pathways and therefore a distinction cannot be made between whether NO_3^- was used as an electron acceptor or as a source of N (§ 1.4.2.1).

4.6.4 Using electrokinetics to mobilise nutrients into the sub-surface

4.6.4.1 Movement of NO_3^- through the soil from a 'spiked' region

Figure 4.15 shows the potential to direct NO_3^- through the soil from a spiked region in the soil using electromigration (§ 4.5.2.1). After 6 h of applying an electric field (60 V, ~10 mA) NO_3^- had migrated through the soil and passed into the anode reservoir (anolyte) (Figure 4.16). The anode and cathode reservoirs were independent (as opposed to being mixed) throughout the experiments so there was no replenishment of NO_3^- into the soil once it had reached the anolyte via electromigration. Consequently, after 24 h of applying an electric field the concentration of NO_3^- throughout the soil chamber was less than 10 mg kg^{-1}, as detected at 11 locations sampled along the length of the soil chamber. The initial concentration of NO_3^- detected in Reading soil was analysed as 66.3 ± 2.2 mg kg^{-1} (§ 2.3.2, Table 2.2). The concentration of NO_3^- in the catholyte remained constant at a concentration consistent with that in mains tap water (~11 mg l^{-1}).

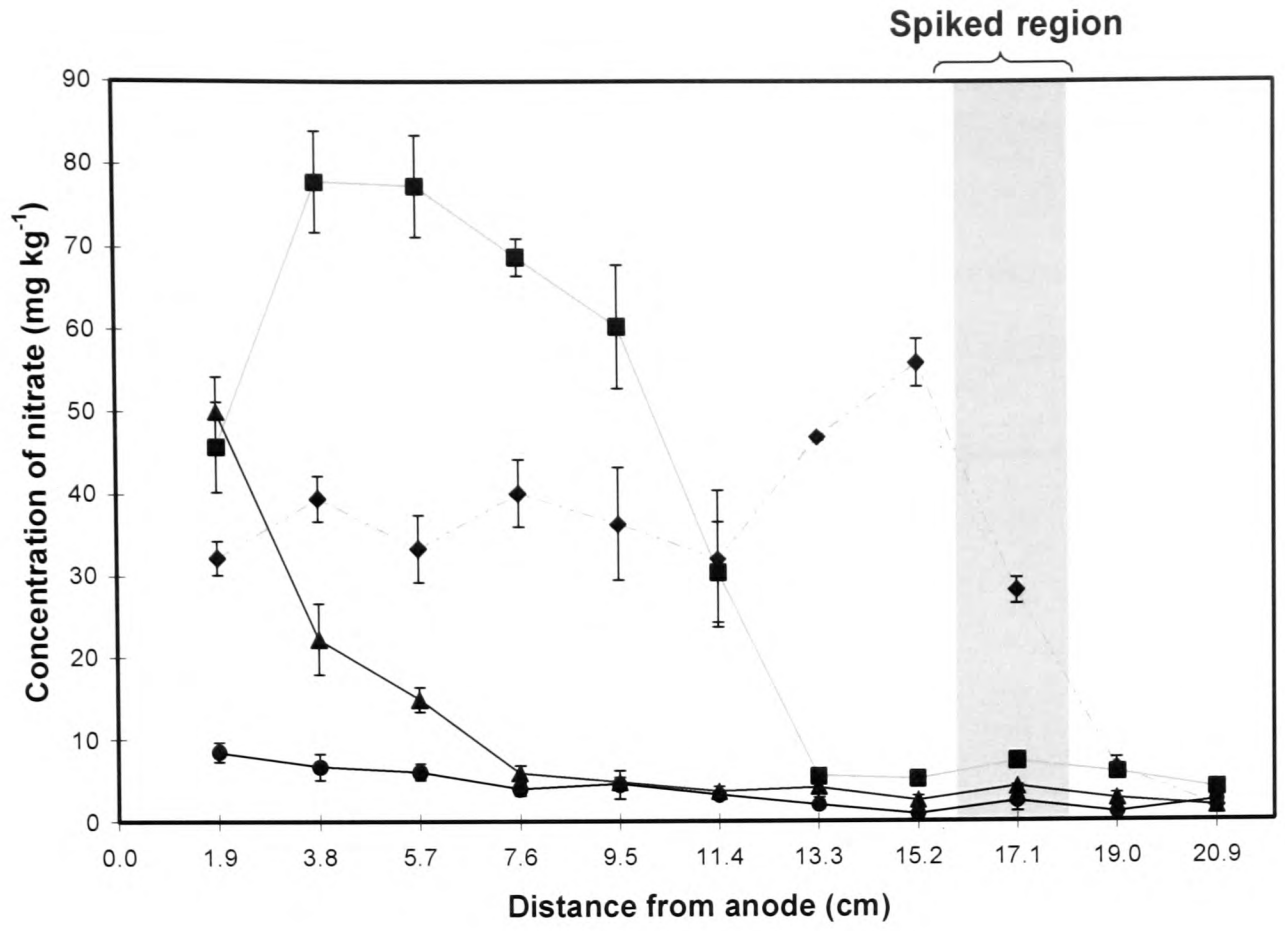


Figure 4.15 Directed movement of NO_3^- through electrokinetics chamber (Reading soil) with 10 mA applied. NH_4NO_3 (160 mg kg–1) spiked into the soil chamber 17.4 cm from the anode electrode. Samples taken at ◆ 1 h; ■ 6 h; ▲ 12 h; ● 24 h. (Data are means ± SE; n = 3).

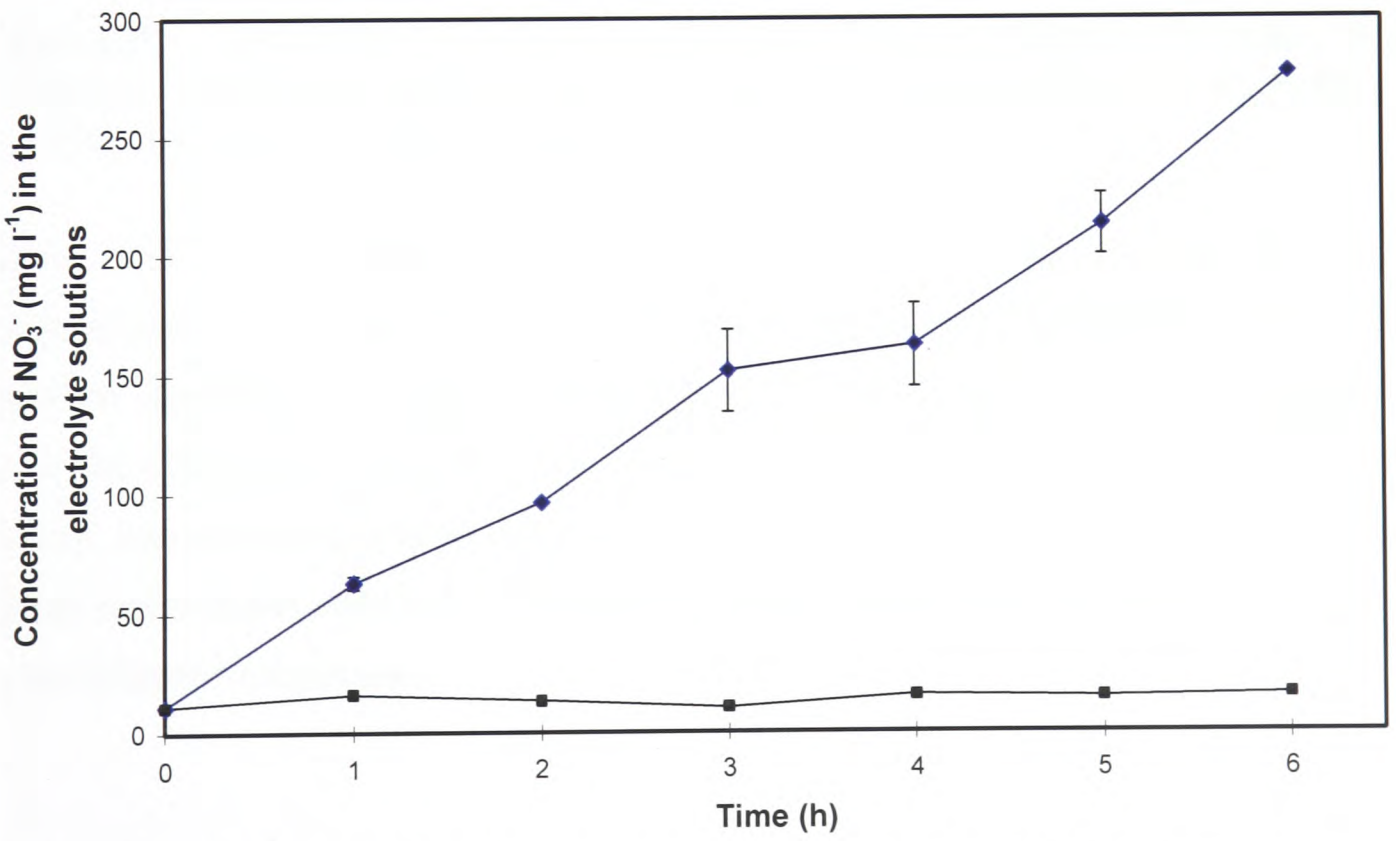


Figure 4.16 Concentration of NO_3^- in the ♦ anolyte and ■ catholyte after electrokinetics (60 V, ~10 mA) applied to the soil chamber spiked with NO_3^- (160 mg kg^{-1}) 17.4 cm from the anode chamber (data are means ± SE; n = 3, for the catholyte data the SE = ≤ 1).

4.6.4.2 Movement of NO_3^- into the soil from the cathode reservoir

A 0.1 M (8 000 mg l^{-1}) NH_4NO_3 solution was added to the catholyte at time point 0 h and was moved into, and through, the soil under the influence of the electric field (60 V, ~10 mA) (§ 4.5.2.2). Figure 4.17 shows the progressive movement of NO_3^- through the soil from the catholyte in the direction of the anode as a result of electromigration.

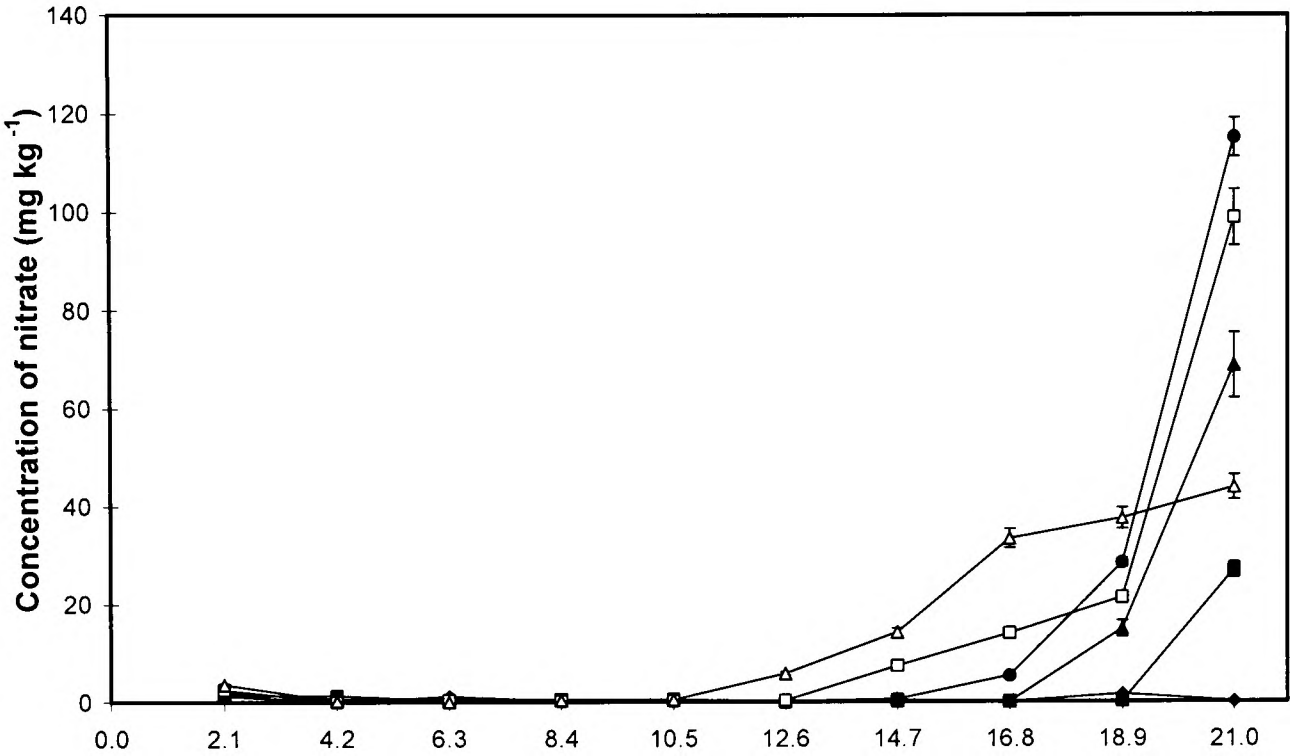


Figure 4.17 Delivery of NO_3^- into the soil chamber using electrokinetics (~10 mA). NH_4NO_3 (8 000 mg l^{-1}) added to the catholyte reservoir at time 0 h. Samples collected at ◆ 0 h; ■ 1 h; ▲2 h; ● 3 h; □ 4 h; and Δ 7 h. (Data are means ± SE; n = 2).

Figure 4.18 gives further evidence of electromigration with an increase in NO_3^- concentration in the anolyte while simultaneously the concentration of NO_3^- in the catholyte decreased. Overall it appears that the delivery rate of NO_3^- through the soil under the influence of an electric field (60 V, ~10 mA) is greatest when the NO_3^- is spiked directly into soil compared to when NO_3^- is supplied in the anolyte. It is likely that the slower rate is attributable to the resistance exerted on the movement of the electrolyte fluid by the Daramic membrane.

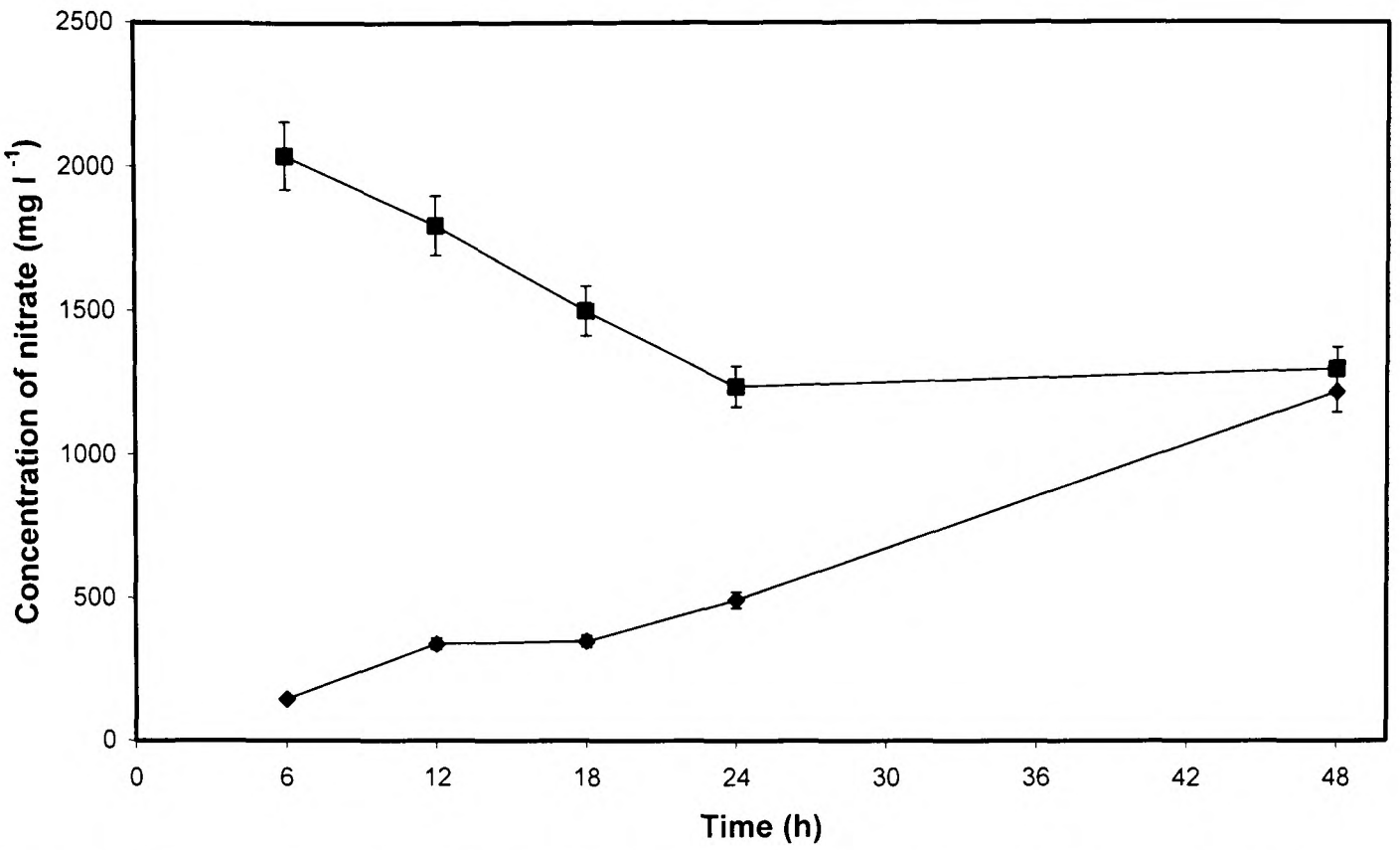


Figure 4.18 Concentration of NO_3^- in the ♦ anolyte and ■ catholyte after electrokinetics (60 V, ~10 mA) applied to a soil chamber. NH_4NO_3 (8 000 mg l^{-1}) added to the catholyte reservoir at time 0 h. (data are means ± SE; n = 3).

4.6.4.3 Movement of NO_3^- into the soil from the recirculated anolyte and catholyte

Reading soil

The target contaminant mix (DXT) was spiked into the central region of the soil chamber (containing Reading soil). After 14 days of applying an electric field (60 V, ~10 mA) and recirculating the anolyte and catholyte together, NO_3^- was distributed throughout the soil chamber (Figure 4.19). Degradation of the contaminant mix (DXT) from the soil was not observed after 14 days of delivering NO_3^- into the soil using electrokinetics. The concentrations and distribution of the contaminants in the electrokinetics soil chamber after 14 days are shown in Figure 2.30 (page 74).

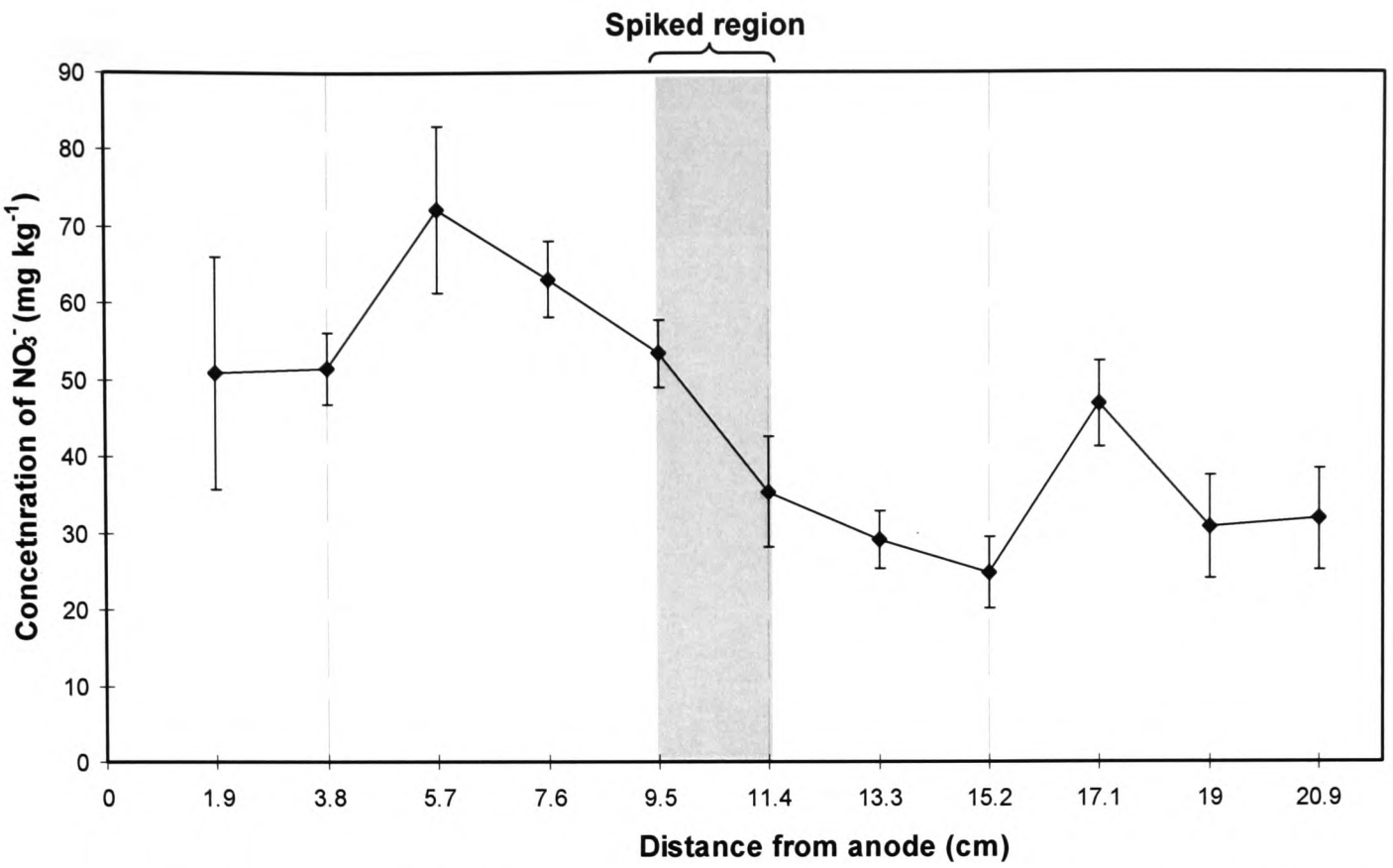


Figure 4.19 Delivery of NO_3^- into the Reading soil chamber using electrokinetics (60 V, ~10 mA). NH_4NO_3 (~12 000 mg l^{-1}) added to the catholyte reservoir at time 0 h. Samples taken at ◆ 14 days (data are means ± SE; n = 3). Anolyte and catholyte reservoirs were recirculated together. NO_3^- starting concentration 65.6 ± 16.2 (n = 9).

Wytham soil

The limited permeability of Wytham soil restricted the movement of water into the soil once the pore space was saturated, as highlighted in the desoprtion experiments undertaken in Chapter 2 (§ 2.3.3.2). Increasing the hydraulic head was insufficient to enable delivery of an aqueous solution through a column of Wytham soil. Figure 4.20 provides evidence that electrokinetics can be used to deliver NO_3^- into soils in which limited permeability prevents delivery of nutrients to the sub-surface by gravity alone. By recirculating the anolyte and catholyte together it was also evident that a more uniform distribution of NO_3^- through the soil could be achieved. Both electromigration and electroosmosis may have an influence on the movement of NO_3^- through the soil, and by recirculating the electrolyte the ions can be recycled instead of becoming depleted from the system.

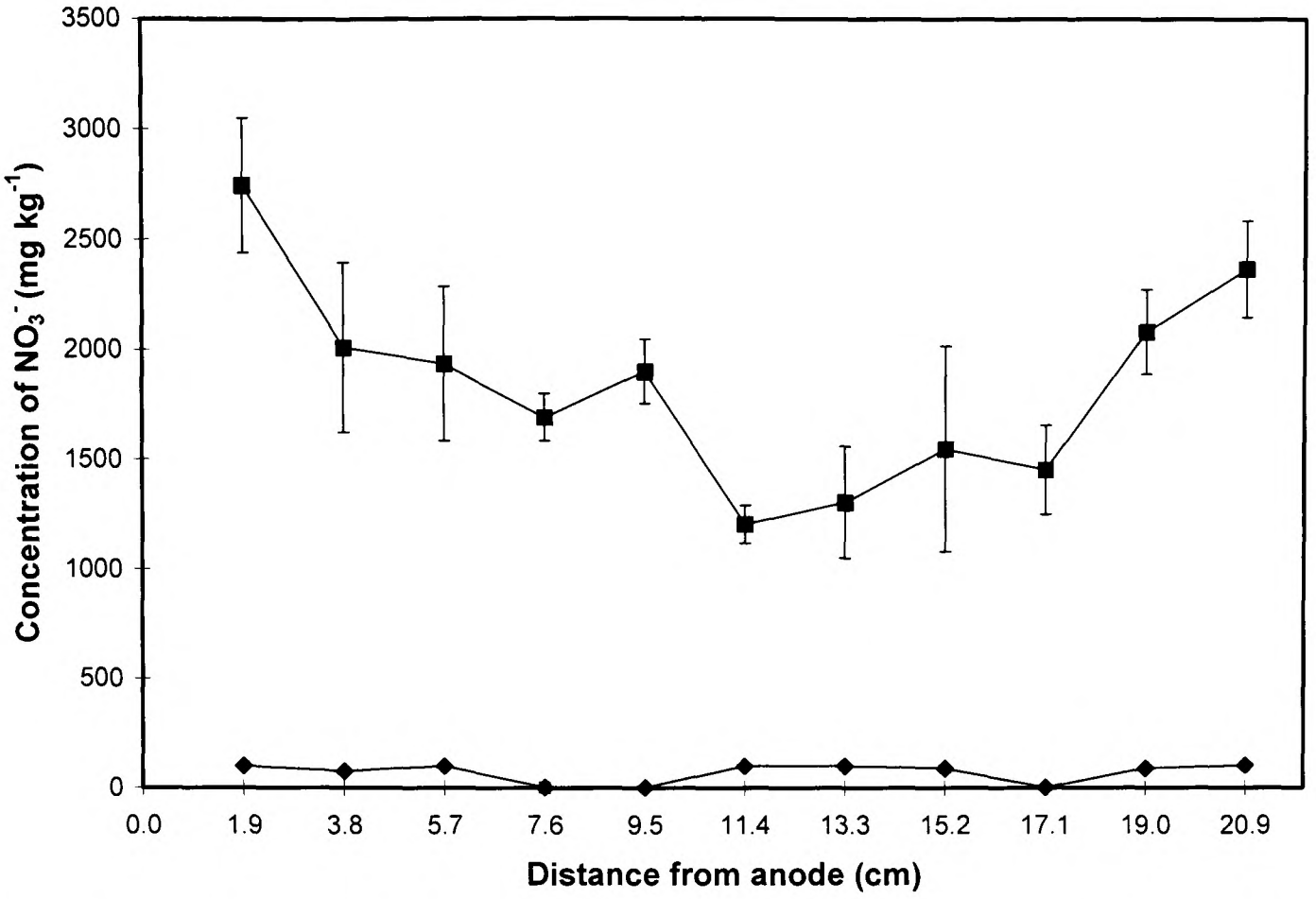


Figure 4.20 Delivery of NO_3^- into the Wytham soil chamber using electrokinetics (~10 mA). NH_4NO_3 (8 000 mg l^{-1}) added to the catholyte reservoir at time 0 h. Samples taken at ◆ 0 h; ■ 24 h (data are means ± SE; n = 2). Anolyte and catholyte reservoirs were recirculated together.

4.6.4.4 Addition of nutrients and/or electron acceptors coupled with pH control

Both NO_3^- and PO_4^{3-} were delivered into, and through, the soil (Figure 4.21) after addition of a 10:1 HNO_3:H_3PO_4 mix to the electrolyte as a buffer, and a near neutral soil pH was maintained over a period of 7 days (Figure 4.22). As the anolyte and catholyte were recirculated together a distinction between movement induced by electroosmosis and electromigration could not be made.

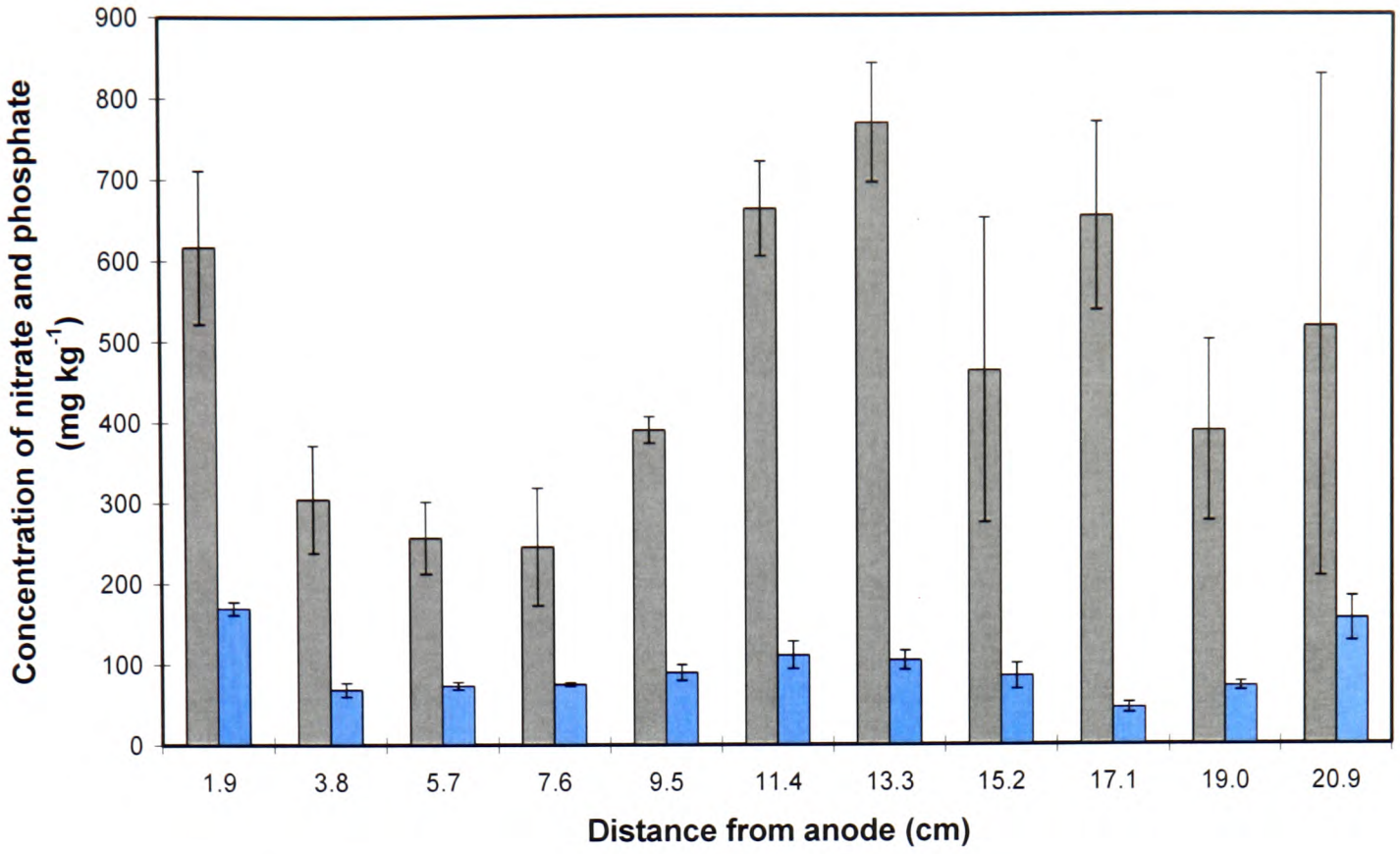


Figure 4.21 Distribution of ■ NO_3^- and ■ PO_4^{3-} across the soil chamber (Wytham soil) after 7 days of applying an electric field (60 V, ~10 mA). Anolyte and catholyte were recirculated together and a nitric and phosphoric acid mix (10: 1) was used to buffer the electrolyte. (Data are means ± SE; n = 3).

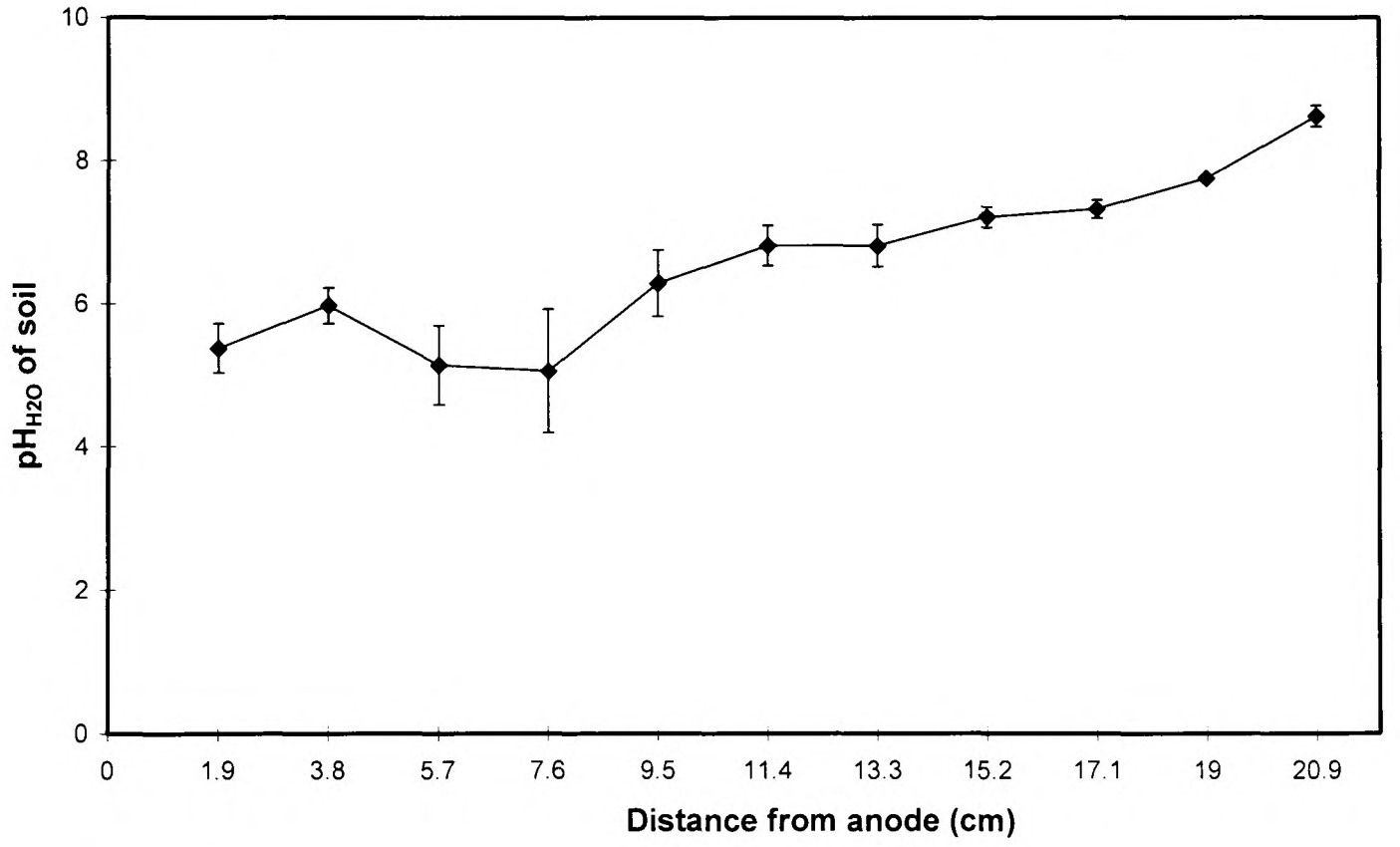


Figure 4.22 pH profile across the soil chamber (Wytham) after 7 days of applying an electric field (60 V, ~10 mA). Anolyte and catholyte were recirculated together. (Data are means ± SE; n = 3).

4.6.5 Using electrokinetics to mobilise toxic metabolites in the sub-surface

It was demonstrated that 3-CBA (a potentially toxic metabolite of the degradation of polychlorinated biphenyl) could be mobilised through the soil from a spiked region towards the anode under the influence of an electric field (60 V, ~10 mA) (Figure 4.23). Furthermore, 3-CBA migrated via electromigration into, and through, the soil after being added to the catholyte at time point 0 h (Figure 4.24).

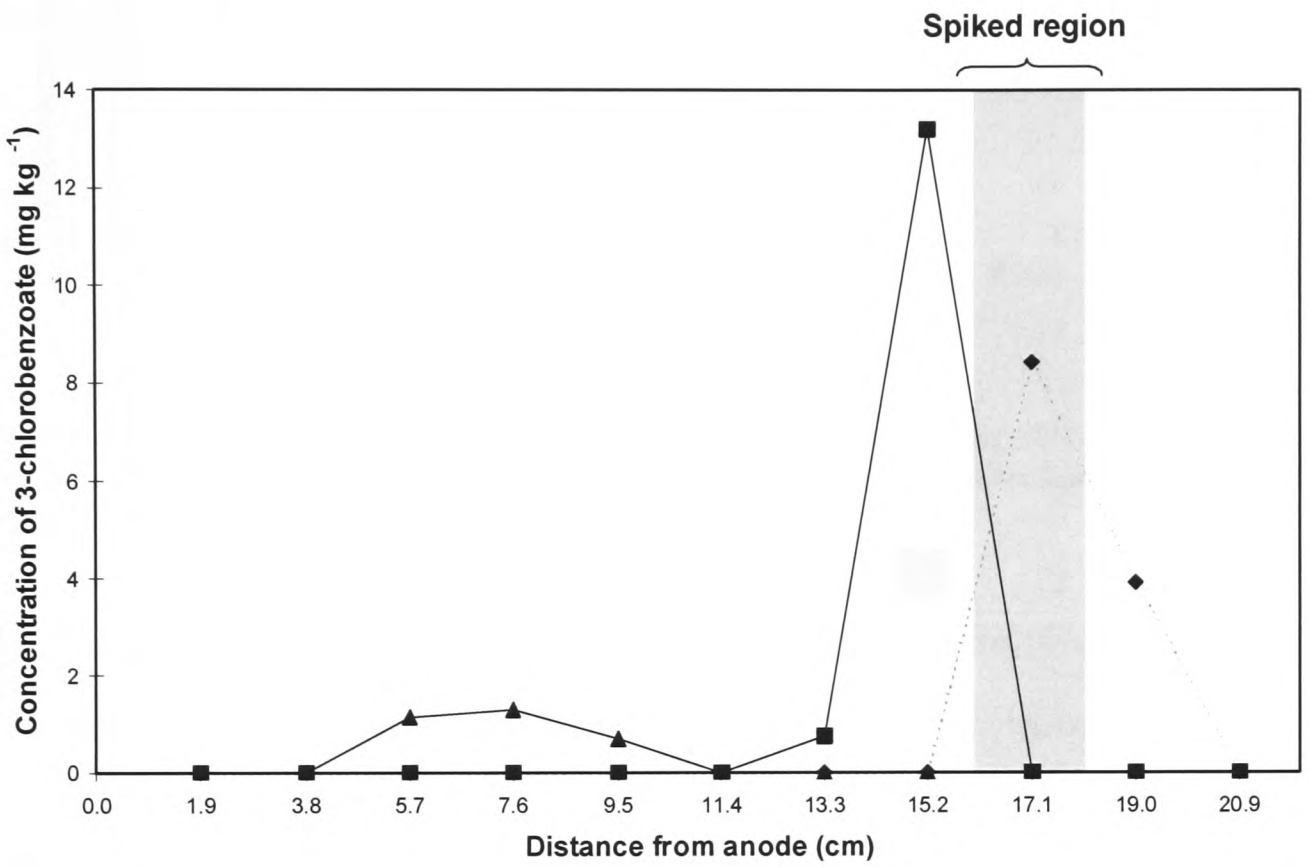


Figure 4.23 Movement of 3-chlorobenzoate (~12 mg kg^{-1}) through the soil chamber (Reading soil) from a 'spiked' region, 17.4 cm from the anode chamber. 60 V (~10 mA) was applied across the soil chamber between two electrodes for 12 h. ◆ 0 h; ■ 6 h; ▲ 12 h. (Data are based on one experiment).

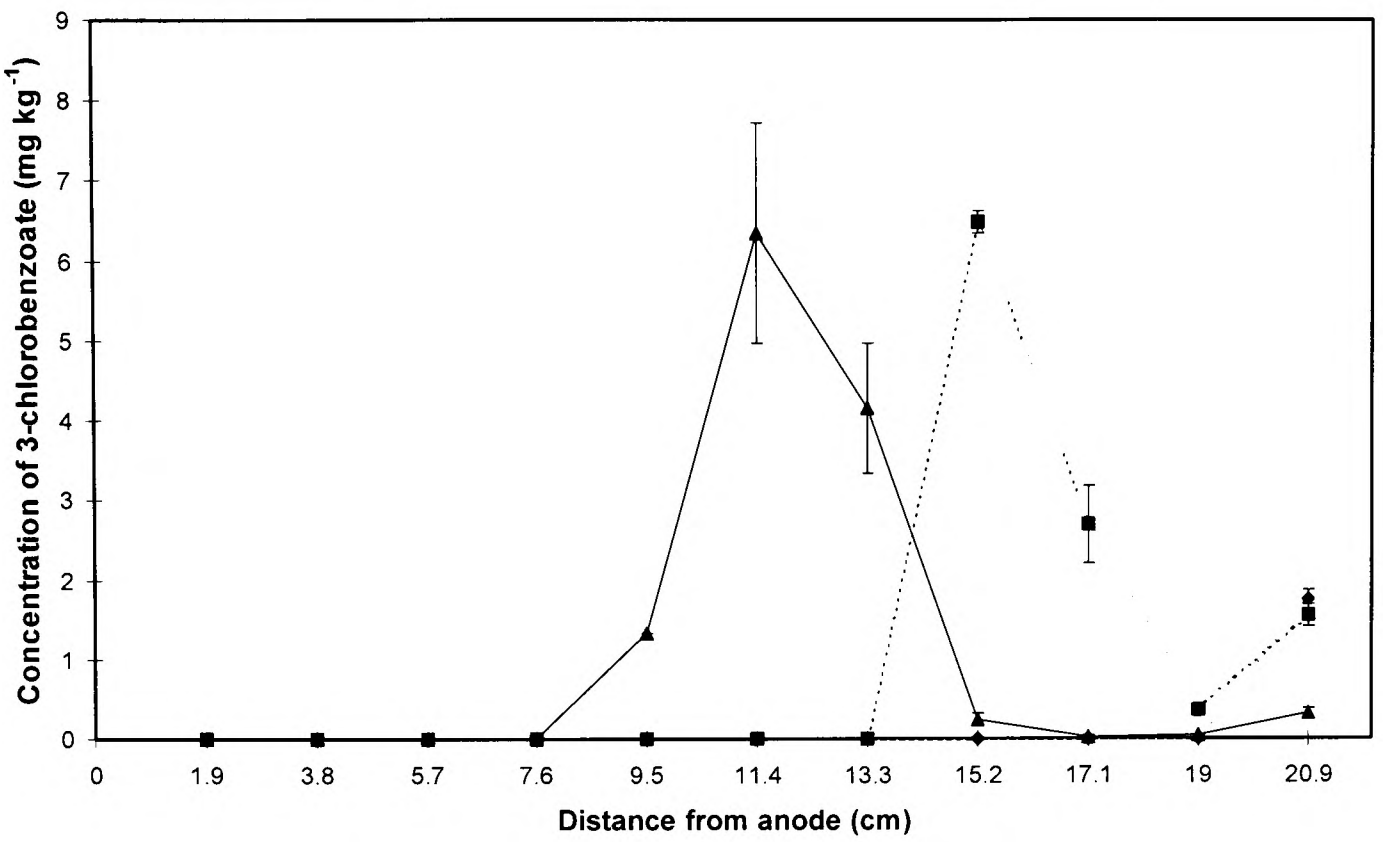


Figure 4.24 Movement of 3-chlorobenzoate into and through the soil chamber (Reading soil) from the catholyte. 60 V (~10 mA) was applied across the soil chamber for 12 h. Concentration of 3-CBA at ◆ 2 h; ■ 8 h; ▲ 12 h. (Data are means ± SE; n = 3).

4.6.6 The electrokinetic delivery of the chemical oxidant $NaMnO_4$

To investigate the potential to use electrokinetics to deliver the chemical oxidant $NaMnO_4$, a 1% (w/v) aqueous solution was added to the catholyte and anolyte in two separate experiments. It was assumed that upon entry into the soil the $NaMnO_4$ would exist as Na^+ and MnO_4^-. After addition of $NaMnO_4$ to the anolyte reservoir and applying an electric field (60 V, ~10 mA) for 7 days, no movement of permanganate ions into the soil via electroosmosis was observed (Figure 4.25). When $NaMnO_4$ was added to the catholyte there was evidence of mobilisation via electromigration towards the anolyte (Figure 4.26).

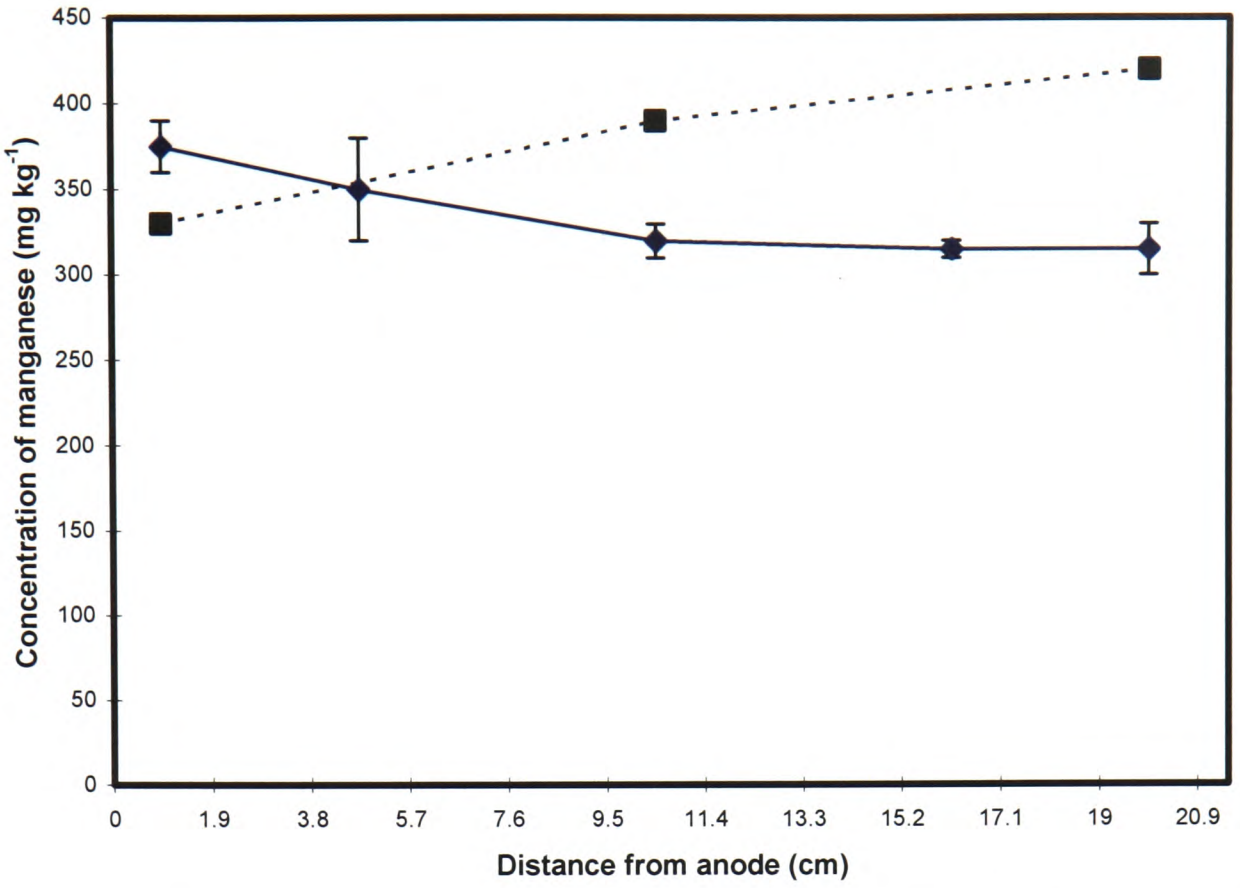


Figure 4.25 Movement of 1% $NaMnO_4$ through the soil from the anode reservoir. 60 V (~10 mA) was applied across the soil chamber between two electrodes for 7 days. ◆ MnO_4^- (as Mn) distribution in the electrokinetics soil chamber (data are means ± SE, n = 2), ■ MnO_4^- (as Mn) distribution in the control chamber (data points are based on analysis from one sample).

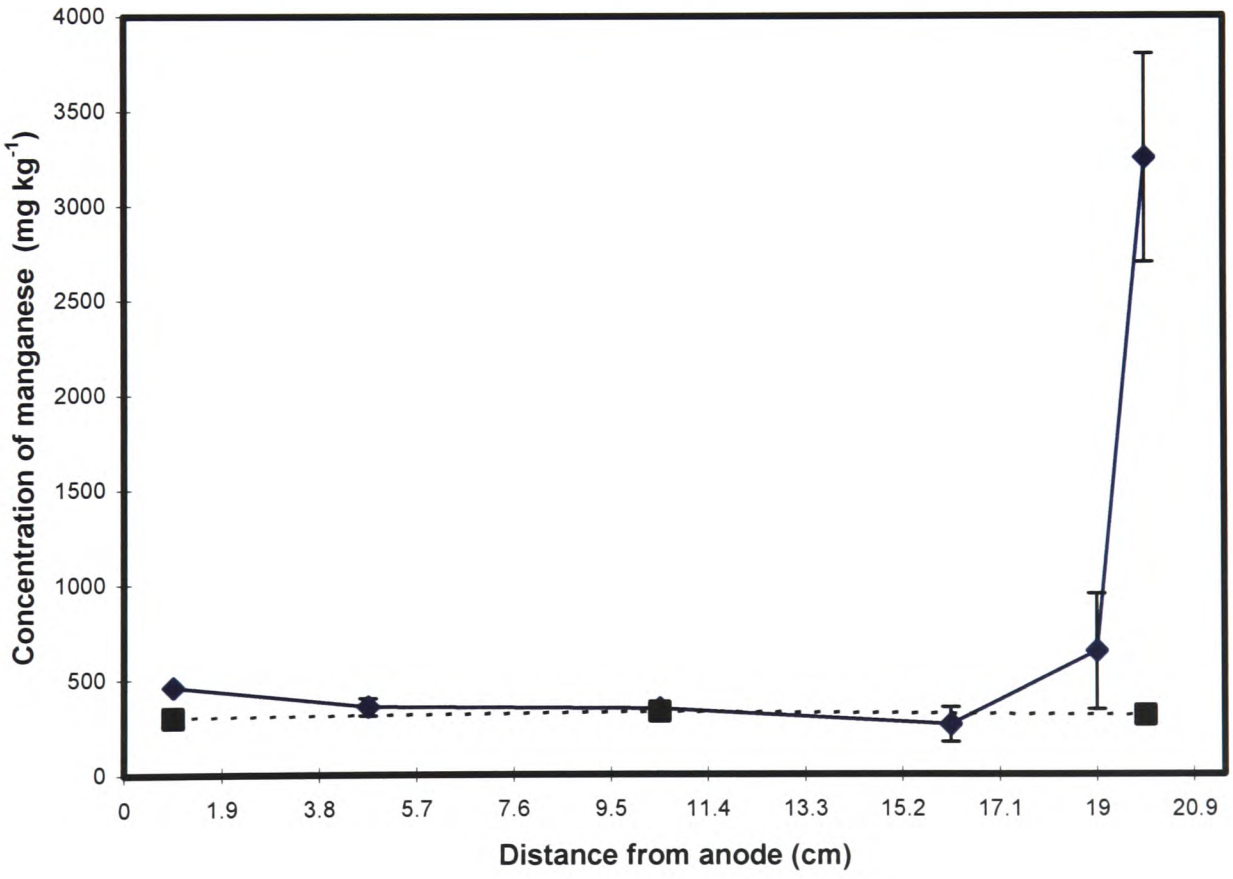


Figure 4.26 Movement of 1% $NaMnO_4$ through the soil from the cathode reservoir. 60 V (~10 mA) was applied across the soil chamber between two electrodes for 7 days. ◆ MnO_4^- (as Mn) distribution in the electrokinetics soil chamber (data are means ± SE, n = 2). ■ MnO_4^- (as Mn) distribution in the control chamber (data points are based on analysis from one sample).

4.7 Discussion

4.7.1 Microbial studies

4.7.1.1 Isolation and identification of culturable bacteria from kerosene enrichments

In total, four bacterial species were cultured and identified from a sample of Reading soil enriched on kerosene. There was evidence to support that each species was capable of degrading hydrocarbons in the literature. *R. erythropolis* is noted for its capabilities to degrade hydrocarbons, in particular n-alkanes (Christofi & Ivshina, 2002; Alvarez, 2003; Carvalho & Fonseca, 2005) and also have the ability to produce biosurfactants to enhance bioavailability (Bognolo, 1999). It is reported that *S. maltophilia* is capable of utilising pyrene and other high molecular weight PAHs as a sole carbon source (Boonchan *et al.*, 2000) and of particular relevance to this study was identified as being capable of degrading BTEX compounds in laboratory-based studies (Lee *et al.*, 2002). Thus, the enrichment studies highlighted the feasibility of enriching hydrocarbon degrading microorganisms from soil with no known historic exposure to synthetic hydrocarbon compounds. However, it cannot be assured that a bacterium with hydrocarbon degrading capabilities enriched in the laboratory will play an active role in the biodegradation of hydrocarbons *in situ*.

4.7.2 Microcosm studies for the biodegradation of the target contaminants

The microcosms were set up to create a homogenous contaminant-spiked soil to investigate biodegradation. There are significant differences between degradation taking place in soil slurries and that occurring in the soil *in situ* (Allard & Neilson, 1997). For instance, ageing of contaminants has a significant role in bioavailability and was not investigated within this study, as experiments were carried out using freshly spiked soils. In aged soils, contaminants will have become more strongly associated with the soil matrix and may no longer be accessible to bacteria (Reid *et al.*, 2000). However, ageing of the contaminant would have a limited influence if remediation were undertaken shortly after the spill, which frequently occurs following the release of oil under residential properties

4.7.2.1 Biodegradation of contaminants in isolation or mixtures

Using soil microcosm studies, the target contaminant mix of dodecane, xylene, and tetradecane was not degraded to any greater or lesser extent compared to when they were added as single contaminants. It was reported (§ 4.6.2.2) that a higher concentration of dodecane was degraded in the presence of xylene and tetradecane compared to when

dodecane was spiked into the soil microcosms in isolation. In the electrokinetic experiments, described in Chapters 2 and 3, the degradation of xylene was the most prevalent of the three target contaminants (DXT). One possible reason for the increased degradation of dodecane could be that the presence of xylene may have stimulated an increase in biomass and/or metabolic activity, promoting the degradation of dodecane. The proliferation of bacteria capable of degrading xylene may, for instance, benefit the development of those bacteria capable of degrading dodecane. Alexander (1994) reported that little research has been undertaken on the apparent stimulation of biodegradation in the presence of more than one contaminant, and that research of the inhibitory effects of contaminant mixtures is more prevalent. A variable between the microcosm studies was the carbon loading. The concentration of contaminant was not increased when it was used in isolation to match the concentration of carbon in the microcosms containing the target contaminant mix (DXT), and therefore differences in degradation may also be attributed to differences in the contaminant concentrations.

4.7.2.2 The effect of temperature on biodegradation

An increase in incubation temperature was observed to enhance biodegradation of the target contaminants from the soil microcosms. Of significant relevance to this study, it was shown that by increasing the temperature from 0°C to 4°C, biodegradation of the target contaminant mix (except *o*-xylene) was significantly increased (refer to Figure 4.6 and Table 4.1). Electrokinetics, via ohmic heating, can result in increased soil temperatures. In the experiments carried out in Chapter 2, a maximum temperature increase of 2.2 ± 0.4°C was observed in an open system (Table 2.8), and a temperature increase of 17.6 ± 0.5°C in a closed system (with Wytham soil) (Figure 2.19). Therefore, there is potential to use electrokinetics to enhance biodegradation of the target contaminants by an increase in soil temperature. The optimum temperature for bacterial degradation is species specific, but for degradation in soils it is widely accepted that the optimum temperature is in the 20-30°C range, a temperature infrequently reached in soils of the British Isles (Leahy and Colwell, 1990; Eriksson *et al.*, 2001). In the longer term, one of the few positive effects of projected global warming levels may be enhanced bacterial degradation of contaminants *in situ* in the UK.

4.7.2.3 The effect of nutrient amendments on biodegradation

Soil microcosms were used to identify the effect of nutrient amendments on the degradation of the target contaminant mix from the soil. After 7 days of incubation at

~25°C there was no statistically significant difference between the degradation of the three target contaminants (DXT) with and without nutrient amendments. It is likely that the failure to identify a difference in degradation was due to the fact that the concentration of inorganic nutrients already available in Reading soil was sufficient to support degradation of the target contaminants during the short incubation period (the concentration of N and P in Reading soil are detailed in Table 2.2).

These experiments highlight the difference between microcosm studies and larger scale experiments. In the control electrokinetic experiments (without an applied electric field), the level of degradation was not comparable to the level of degradation observed in the microcosms. The starting concentration of the target contaminant mix was significantly lower in the microcosms compared to the concentration added to the soil chambers. Therefore increased biodegradation in the microcosms may be attributed to a lower toxicity of the target contaminants on the microbial hydrocarbon-degrading community. Another possible explanation is that increased degradation of the contaminants from the microcosms may have occurred with greater aeration and bioavailability, compared to the conditions in the soil chamber.

4.7.2.4 The use of NO_3^- as a nutrient and/or electron acceptor

Experiments designed to determine whether NO_3^- was being utilised as a nutrient and/or electron acceptor for the degradation of the target contaminant mix (DXT) were unsuccessful: degradation did not occur in the 96 h incubation period. Microorganisms preferentially use NH_4^+ over NO_3^- as a source of N, and furthermore NH_4^+ can inhibit the assimilatory nitrate reduction pathway. Therefore, were NO_3^- to be utilised in the presence of NH_4^+, it is likely that the NO_3^- would be utilised as a terminal electron acceptor and not as a source of N. There was no facility to analyse for NH_4^+ in the soil samples alongside NO_3^- and NO_2^-.

4.7.3 Using electrokinetics to mobilise nutrients in the sub-surface

In this investigation the focus was primarily on supplying sufficient nutrient levels to the soil to ensure biodegradation could occur at an optimal rate. It is evident that electrokinetics is an effective mechanism to introduce nutrients into soils. This is of particular significance in low permeability soils. Wytham soil has a low permeability (§2.3.2) and consequently the delivery of nutrient solutions via application to the surface of the soil is ineffective and unlikely to maintain biodegradation in the sub-surface. The

progressive movement of NO_3^- through the soil chamber (Figures 4.15, 4.17, 4.19 and 4.20) under the influence of an electric field was achievable in both Reading and Wytham soil. In the experiments the effect of an opposing hydraulic gradient has not been considered. To apply the technique *in situ* it would be necessary to ensure that electromigration could exceed any opposing flow induced by the hydraulic gradient.

Further investigations are required to determine the rate of nutrient uptake, in order to establish an injection programme that enhances biodegradation but does not lead to the accumulation of excess nutrients in the soil. Rabbi *et al.* (2000) discuss the potential for nutrient and/or cometabolite addition through electrokinetics to be counterproductive, if for instance the concentrations added lead to biofouling and subsequent plugging of the soil pores. If the hydraulic conductivity of the soil is reduced, further delivery of nutrients into the subsurface to enhance biodegradation would be limited. However, the role of biofouling on the non-uniform distribution of nutrients under the influence of the electric field may be overstated. For example, Kim and Han (2003) infer that biofouling plays a key role in influencing ion migration without considering that high concentrations of nutrients may indeed be inhibitory and the heterogeneous nature of the soil that readily results in differences in soil tortuosity.

As discussed (§ 2.4.2.1), an increased number of free ions within the soil decreases the resistance when an electric field is applied. Thus with the introduction of NO_3^- and PO_4^{3-} the concentration of free ions was increased and resistance was lowered. The effect of the magnitude of the current on the movement rate of NO_3^- was not investigated because the current achieved in the electrokinetics experiments was in all cases less than 10 mA, which was the minimum current measurable using the experimental apparatus. It should also be noted that the rate of movement of the ions into the soil can be influenced by factors such as the concentration of kerosene within the soil. For example, Benazon *et al.* (1995) demonstrated that the transportation of NO_3^- through a soil column was faster in the presence of kerosene than in 'clean soil'. The difference in transportation rates was attributed to a decrease in the volume of pore water in soil contaminated with hydrophobic contaminants compared with 'clean' soil.

4.7.3.1 Addition of nutrients and/or electron acceptors coupled with pH control

By recirculating the anolyte with the catholyte, charged ions that had been stripped from the soil by electromigration and electroosmosis could be reintroduced to the soil. If mobilisation of the target contaminants is achieved across a significant distance a separator

system would have to be incorporated (*e.g.* granular activated carbon [GAC]) to strip the contaminants from the electrolyte before recirculation would reintroduce the contaminants into the soil. Circulating the anolyte and catholyte together to buffer the electrolysis products (OH^- and H^+) has now been reported in the literature (Kim & Han, 2003).

4.7.4 Using electrokinetics to mobilise toxic metabolites in the sub-surface

The PCB metabolite 3-CBA was successfully mobilised in the soil chamber using electromigration. This has significant possibilities with regard to enhancing bioremediation through removal of toxic by-products from their metabolism. It is known that the degradation of PCBs is prone to inhibition, whereby the metabolites formed from the degradation of the parent compound inhibit further metabolism (Sondossi *et al.,* 1992). There is some debate regarding the exact identity of the metabolite of PCB degradation that acts as the inhibitor to further degradation. Some studies indicate that chlorobenzoate is the inhibitor (*e.g.* Sondossi *et al.,* 1992) while others identify chlorocatechols (breakdown product of chlorobenzoate) as being responsible (*e.g.* Hickey *et al.,* 1993). Nevertheless, it is likely that the removal of chlorobenzoate from the central plume could be advantageous to enhancing the efficiency of biodegradation. Another product of PCB degradation that can cause toxicity and is a groundwater contaminant is Cl^-, which as demonstrated (§ 2.1.1), can be mobilised effectively under the influence of the electric field. In this instance a Cl^- removal system would have to be implemented between the anolyte and the anode to prevent the formation of Cl_2 at the anode.

For electromigration to be used as a method of mobilisation, the charged contaminant of interest must not preferentially sorb to the soil surface in comparison to exchangeable cations (Na^+, K^+, Ca^{2+} and Mg^{2+}); therefore the contaminant must exceed the soil CEC (cation exchange capacity) (West & Stewart, 2000). The potential to use electromigration to direct toxic, or repressive, metabolites from the soil will depend on the electrochemical activity of the target metabolite (Cairo *et al.,* 1996). Once removed from the central region, amendments could be made to promote further degradation of the charged metabolites, or they may be removed from the soil at the electrode for disposal at an appropriately licensed site.

There is also the potential to use electrokinetics to introduce inducers to a soil system to stimulate biodegradation. For instance, it is known that salicylate can induce/enhance the biodegradation of naphthalene (Ogunseitan & Olson, 1993). Rabbi *et al.* (2000) investigated the use of both electroosmosis and electromigration to distribute cometabolites

for the stimulation of TCE degradation, and demonstrated the electrokinetic delivery of benzoate ion (via electromigration) and benzoic acid (via electroosmosis).

4.7.5 The electrokinetic delivery of the chemical oxidant $NaMnO_4$

The investigation into the use of electromigration to deliver permanganate into low permeability soils produced promising results. Determining the efficiency of $NaMnO_4$ to oxidise the residual target contaminants and its effect on biodegradation was outside the scope of this study. With regard to a combination of chemical oxidation and bioremediation, chemical oxidants such as potassium permanganate and Fenton's Reagent are known to have a negative effect on microbial communities (Waddell & Mayer, 2003). The number of experiments and replicate samples that could be analysed was restricted by limited resources as there were no facilities within the research department to perform ICP-MS for the analysis of manganese.

Subsequent, and in addition, to the work undertaken in this study Athmer and Woody (2003) and Roach and Reddy (2004) have reported the use of electrokinetics for the delivery of permanganate in low permeability soils. (Both of the studies are published as conference proceedings that were not accessible). From the abstract of Athmer and Woody (2003), the authors report the successful delivery of permanganate to 17 cm through soil from cathode to anode, which promoted the degradation of TCE in the soil.

A brief study was undertaken to investigate the potential for electrokinetics to be used to acidify soil prior to the addition of Fenton's reagent (Appendix 8.9). The influx of protons, through electrolysis, was insufficient to cause a significant reduction in pH across the soil chamber. The buffering capacity of Reading soil has a significant impact on the efficiency of the process, which is dependent on the readiness of the media to exchange cations with hydrogen ions.

4.8 Summary of findings

1. Hydrocarbon degrading bacteria were enriched from Reading soil including *Rhodococcus erythropolis, Stenotrophomonas maltophilia* T3-c and an undefined *Brevibacterium*.

2. An increase in soil temperature increased the rate of degradation of the target contaminant mix (DXT). An increase from $0^{o}C$ to $4^{o}C$ showed significantly improved degradation rates.

3. Within soil microcosms incubated for 7 days at ~25°C, the degradation of the contaminant mix (DXT) was not significantly different with and without nutrient amendments. It appeared that the concentrations of N and P found in the model soil were sufficient to support hydrocarbon degradation for an incubation period of 7 days.

4. There was a notable increase in the degradation of dodecane when it was present in the soil as a mixture, with xylene and tetradecane, compared to when it was spiked in isolation. The degradation of xylene and tetradecane did not appear to be influenced by the presence of the contaminant mixture.

5. In the soil chamber experiments described in Chapters 2 and 3, NO_3^- was depleted from the central 'spiked' region. Experiments undertaken to identify if NO_3^- was used as an electron acceptor and/or source of N were not conclusive and warrant further investigation.

6. Whilst NO_3^-, SO_4^{2-}, and PO_4^{3-} were mobilised from Wytham and Reading soil under the influence of the electric field (Chapter 2 and 3), it was identified that recirculation of the electrolyte was effective at providing a continual supply of the nutrients N and P into the soil. In addition, it was shown that nutrient addition and pH control could be coupled to increase the efficiency of electrokinetics.

7. The PCB metabolite 3-CBA was mobilised through the soil chamber, providing evidence for the use of electrokinetics to remove potentially toxic metabolites from the site of biodegradation and facilitate sustained biodegradation.

5 Application of electrokinetics in the field

5.1 Introduction

The principal aim of this Industrial CASE Studentship was to develop and test a field-scale electrokinetics system to investigate the feasibility of remediating hydrocarbons from the subsurface. There are relatively few studies available that link laboratory-based research with field-scale trials, and until this is prioritised, innovative technologies will remain underdeveloped. There are a limited number of reported field-trials of electrokinetics, and these mostly concern the remediation of heavy metals, such as cadmium and chromium (Geokinetics International Inc: Lageman, 1993; Gent *et al.,* 2004). In addition, studies have been undertaken targeting phenol and BTEX compounds (Electro-Klean^{TM}) and the Lasagna process targeting TCE (Ho *et a*l., 1999a; 1999b). Lageman (1993) describes a number of field trials including both *in situ* and batch electrokinetic remediation projects treating up to 7 400 m^3 of soil for the remediation of heavy metals, with around 15 commercial projects (Lageman *et al.,* 2005) having been completed. The evidence gathered in the preceding chapters of this study provided a framework with which to further investigate the use of electrokinetics at field scale. Of particular interest was the use of electrokinetics to deliver nutrients and electron acceptors into the soil, coupled with control of the moisture content and pH of the soil.

Several contaminated sites were visited with RAW Consulting prior to the start of the field experiment to gain an insight into the obstacles faced at field sites. Sites visited included: a domestic oil spill at Ashington, West Sussex; a commercial oil spill at the Isle of Sheppey Community Hospital; a commercial oil spill at Andover, Hampshire; and EDF Energy sites at Guildford, Crawley and Orford. In addition, pilot electrokinetics apparatus was observed at C-Tech Innovation, Capenhurst and BNFL, Harwell.

5.1.1 EDF Energy field site

A 132 kV transformer site was supplied by EDF Energy for the *ex situ* on-site trial. Within the UK, the EDF Energy Electrical Systems section has approximately 87 000 substations, with oil insulation, transformers and a 2 000 km fluid-filled cable network. Leakage from transformers has occurred over the last 40 - 50 years. Newly installed transformers are fitted with bunding (protective barriers) and oil water separators, but older transformers remain unprotected, so there is an extensive area of land to be remediated. EDF Energy is

currently aiming to reduce its annual waste bill of £2 million by reducing its use of landfill for the disposal of contaminated land by 10%. The company plans to achieve this by investing in sustainable remediation technologies (Lawrence, 2005).

A large percentage of the transformer sites requiring remediation are located in remote areas away from public access, hence the driving force behind remediation is generally compliance with environmental legislation rather than protecting sensitive receptors, such as neighbouring properties (*e.g.* householders). Consequently, there is generally more time available for the remediation of such sites.

5.1.2 Case study site

Approximately 500 litres of transformer oil, an acid refined naphthenic product reaching BS 148 (1984) standards, were spilled from a transformer to the surrounding area at an EDF Energy site in Orford, Suffolk. The bitumen ground surfacing surrounding the transformer had disintegrated through contact with the transformer oil (Figure 5.1). The bitumen and hard core layer was removed, to a depth of ~0.15 metres below ground level (mbgl), before a portion of the hydrocarbon contaminated soil was loaded into a 4.6 m^3 (6-yard) skip and transported to Ilmer Grid 132 kV Switch station, Buckinghamshire. The relocation of the soil was necessary to ensure the field trial could be visited daily from the University of Oxford, at the same time as maintaining site conditions as close as possible to the initial spill scenario. Electroremediation at field-scale was tested in an insulated skip, making it possible to work with the contaminated soil *ex situ* from the subsurface whilst remaining on site.



Figure 5.1 Initial oil leak from a transformer, Suffolk, UK.

5.1.2.1 Site safety and utilities available at the site

A detailed health and safety risk assessment was produced in co-operation with EDF Energy and RAW Consulting Ltd. A one-day health and safety course was completed and a permit received for working on EDF Energy 132 kV transformer sites.

Electricity was made available at the site but mains water, for the electrodes, had to be transported to the site.

5.2 Chapter objectives

The objective of this chapter was to carry out electrokinetics *ex situ* at a hydrocarbon contaminated site, as a proof of concept study to determine whether electrokinetics could be used to optimise biodegradation of hydrocarbons in the subsurface (§ 1.7, Objective 5). The results and observations made in the preceding chapters were used to develop the field-scale apparatus and protocols (refer to the flow chart, Figure 6.1 [Chapter 6]). It was established, through feasibility studies, that octadecylbenzene, the principal component of transformer oil, could not be moved using electroosmosis (Chapter 2). Consequently, the

focus of the field scale investigation was to use electrokinetics to deliver nutrients and NO_3^- as an electron acceptor (Chapter 4) into the contaminated soil to facilitate biodegradation, while simultaneously controlling pH and the moisture content of the soil (Chapter 2). Furthermore, it was anticipated that an increase in soil temperature might be observed on application of the electric field, with the potential to stimulate biodegradation (Chapter 4).

5.3 Materials and methods

5.3.1 Contaminants within the soil

Five samples were taken from the contaminated transformer site; three samples to a depth of 0.5 mbgl in the direct vicinity of the transformer (from an area of about 3 m x 6 m) and two samples to a depth of 0.2 and 0.5 mbgl outside of the bunded area. The samples were analysed by a private laboratory (Severn Trent Laboratories [STL], Coventry, UK) to determine the concentrations of contaminants and constituent ions within the soil including: the relative concentrations of total petroleum hydrocarbons (TPH [>C_6 to C_8; >C_8 to C_{10}; >C_{10} to C_{12}; >C_{12} to C_{16}; >C_{16} to C_{21}; >C_{21} to C_{40}]) and PAHs using GC-FID; the concentration of anions (*e.g.* Cl^- and NO_3^-) using IC; and the concentration of metals present in the soil (*e.g.* Cu and Fe). For the full suite of the analysis undertaken refer to Appendix 8.10.

5.3.2 Characteristics of the EDF test soil at day zero of the experiment

The electroosmotic velocity was calculated using specifically designed apparatus (§2.3.4). The WHC, moisture content and pH_{H2O} were determined using the general methods described previously (refer to Sections 2.3.2, 2.3.6.1 and 2.3.6.2, respectively). In addition, as there was a four month gap between the initial spill of transformer oil in Orford and the set up of the experiment, the soil was re-analysed for the anions Cl^-, PO_4^{3-}, SO_4^{2-}, and NO_3^- using the general method described in § 2.3.6.4, at day zero of the experiment before applying an electric field.

5.3.2.1 Feasibility studies for the use of surfactants with EDF test soil

Column studies were used to investigate the feasibility of using SDS, Span 85 and/or Triton X-100 to remediate the EDF test soil. The experiments were set-up in triplicate, as described in Section 2.3.3.2, using the EDF test soil. Triton X-100, Span 85 and SDS were tested at concentrations of 0.4 and 1.0%. Two pore volumes (3.8 ml x 2) of the surfactant solutions were added to the soil columns and flow rate through the column was observed.

5.3.3 Construction of the electrodes

Six electrode frames (designed and constructed by C-Tech Innovation, Chester) were used in the field scale investigation. With assistance from engineers at the Earth Sciences Department, University of Oxford and the use of their workshops, the electrodes were refurbished in ~12 weeks and adapted for use in the field scale trial (the materials used are listed in Appendix 8.11). Daramic membranes, used in the laboratory electrokinetics chamber, were bonded to the PVC frames using Silastic, and extra support was supplied with a PVC frame screwed onto the front through the Daramic into the back PVC plate (Figure 5.2).

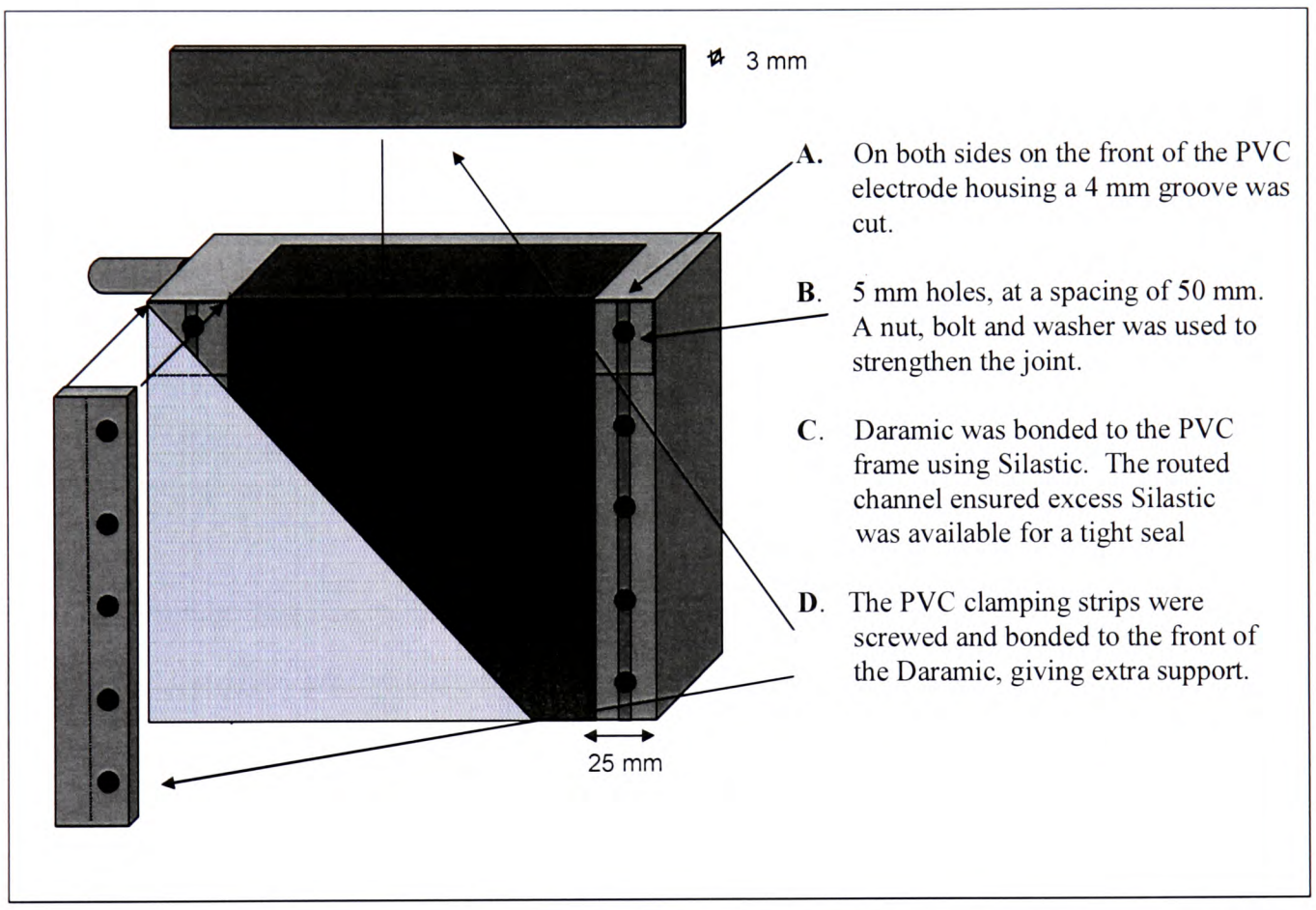


Figure 5.2 Schematic to show the construction of the electrode housing and attachment of Daramic. Both cathode and anode electrode housing were identical.

The cathode (Figure 5.3) was comprised of a stainless steel sheet (~0.45 m x 1 m) placed between three layers of polymer mesh (garden mesh; mesh size 10 mm^2), which resided in the PVC and Daramic electrode housing.

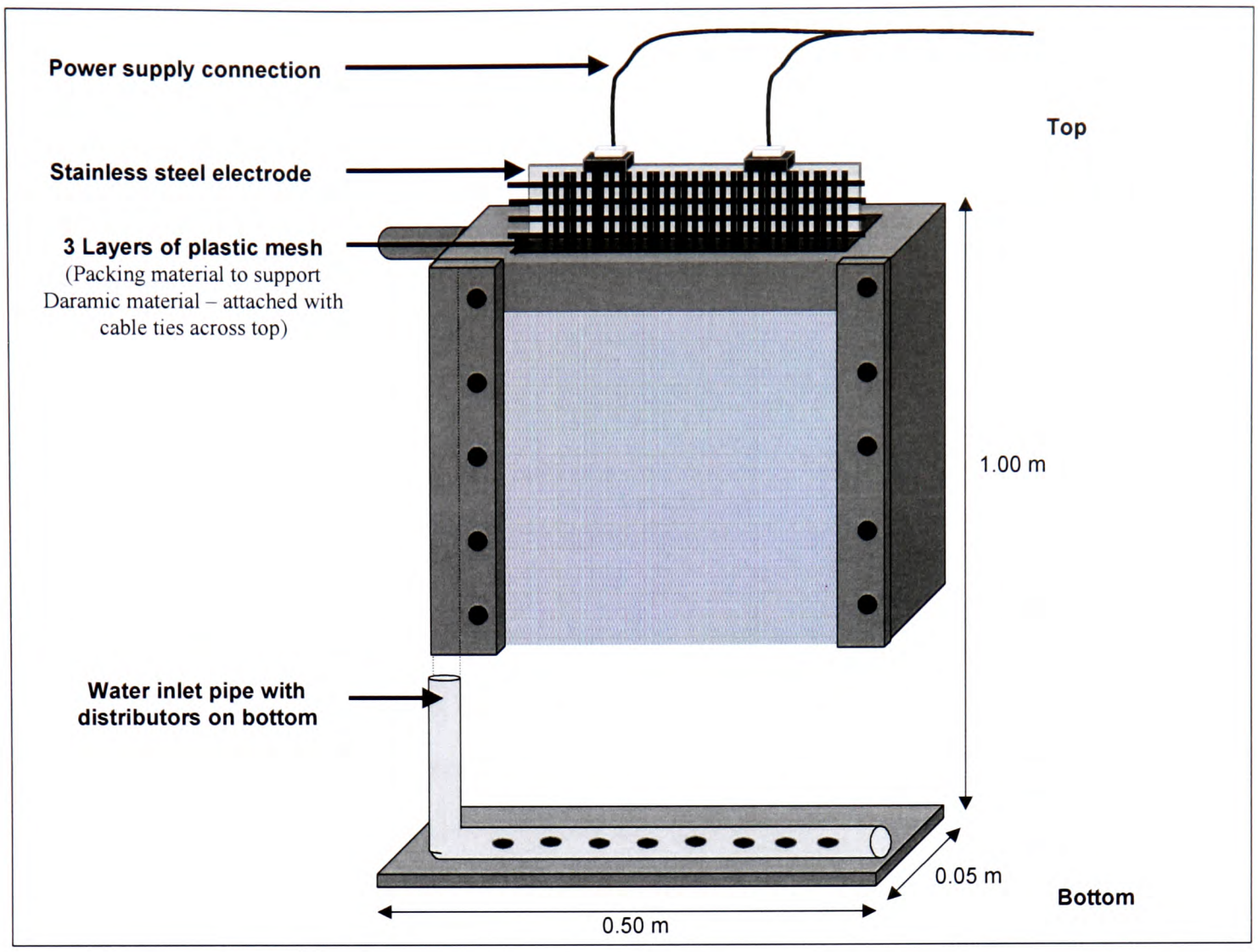


Figure 5.3 Schematic of cathode electrode chamber. At the top rear of the electrode chamber two overflow pipes were attached to withdraw water by gravity after it had flowed up through the chamber.

The anode (Figure 5.4) consisted of three tantalum oxide (Ta_2O_5) and iridium oxide (IrO_2) (resistant to corrosion and chemical attack) coated titanium strips (50 mm x 0.9 mm x 1 m) placed between three layers of polymer mesh in the electrode housing. The stainless steel sheets and the titanium strips were connected to the power supply using copper cables (Ø 2.5 mm^2) attached to the electrodes using stainless steel nuts and bolts.

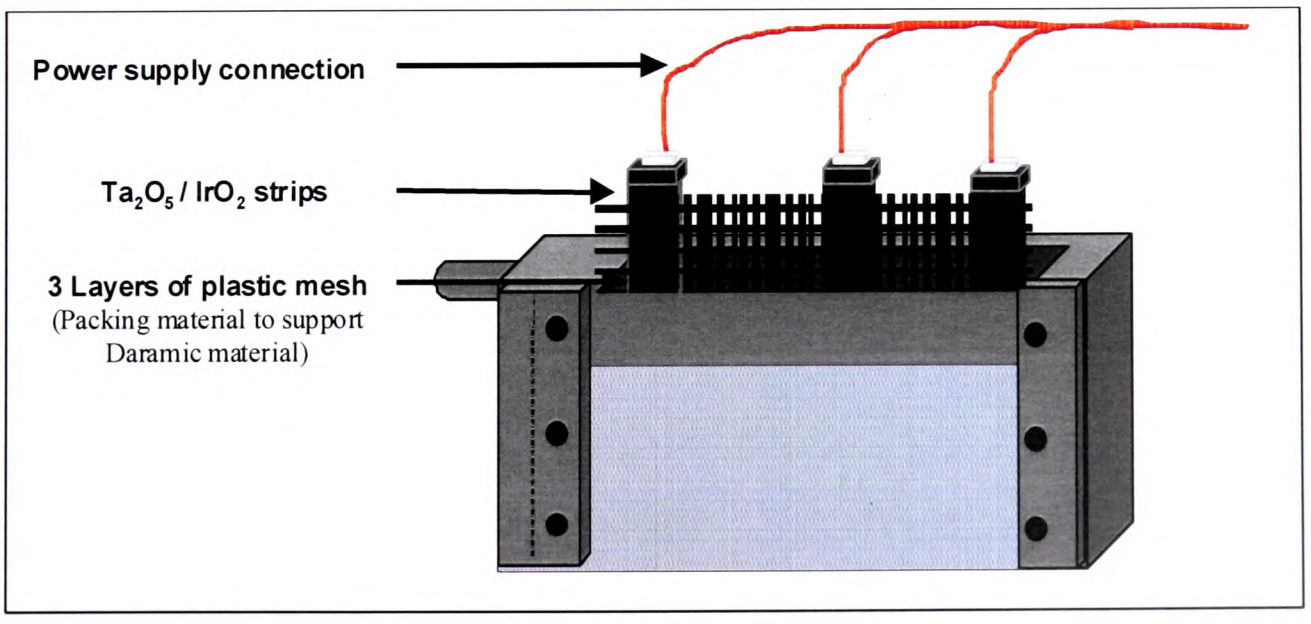


Figure 5.4 Schematic of the anode electrode chamber.

5.3.4 Set up of the field scale electrokinetic treatment cell

The field scale treatment cell was set up within a skip, which was insulated/lined with a polythene sheet (~2 mm thickness) to prevent short-circuiting. Into the bottom of the watertight skip, wooden decking sheets were placed with a permeable geotextile fabric on top (Figure 5.5; ii), onto which the soil (~2.8 m^3, 4 480 kg) was compacted (Figure 5.5; iii). Raising the soil from the bottom of the skip enabled leachate (excess liquid) to be pumped from the skip to avoid flooding. The electrodes were placed in position on top of the geotextile and wooden decking and the soil was compacted around (Figure 5.5; iv and v). Three cathodes were placed directly opposite three anodes (across the width of the skip) (Figure 5.6). The electrodes were placed into plastic troughs to provide a means of retrieving electrolyte if water was displaced in the vertical direction through hydrostatic forces exerted by the head of the water produced at the electrodes, or simply through failure of the integrity of the electrodes. The troughs were filled with shingle (Ø≥ 50 mm) allowing sufficient space for water percolation once the soil was added into the skip.

The electrodes were vented to prevent gas accumulation of the products of electrolysis (§ 1.6.4.4). The skips were covered with tarpaulin to prevent rainwater entering the skip. The power supplies, peristaltic pumps and pH controller were protected in a standard waterproof garden storage container (0.61 m x 1.25 m x 0.53 m) (Figure 5.5; vi).



(i)



(ii)



(iii)



(iv)



(v)



(vi)

Figure 5.5 Photographs depicting the set-up of the electrokinetic field-trial.

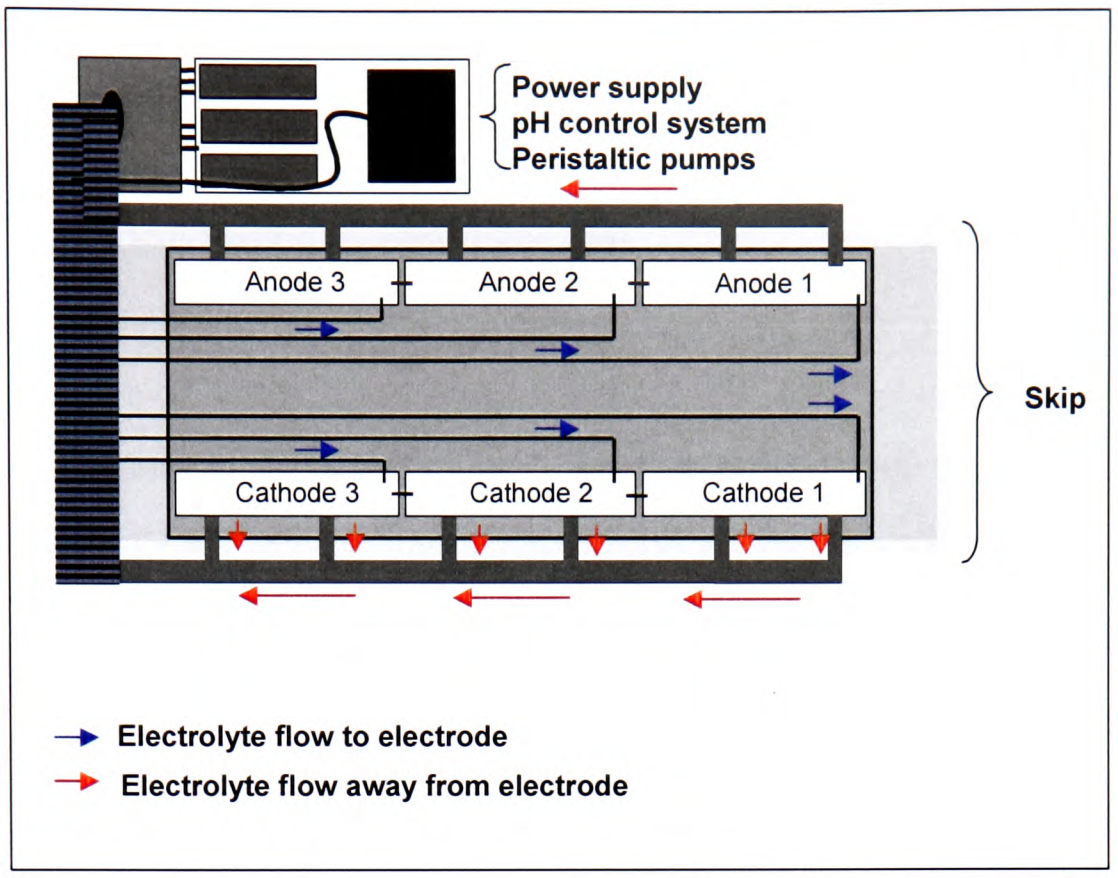


Figure 5.6 Schematic detailing layout of electrokinetics field-trial, which was set up within a skip.

5.3.4.1 Power supply

A standard transformer was used to convert the 240 V mains supply available at the substation into 110 V, which was passed through buried cables to the skip. Power was supplied to the electrodes using a Xantrex (XHR150-7) power supply capable of delivering a direct electric current of 0-5 A, at up to 100 V. A current of 10 mA was maintained for the duration of the experiment.

5.3.4.2 Regulation of the electrolyte

Each electrode chamber had a capacity of approximately 6 litres. Electrolyte was pumped via a standard peristaltic pump (Masterflex Console Drive, Cole-Palmer Instruments, London, UK) into a vertical pipe that fed electrolyte into the bottom of the electrode chamber and out through a bottom distributor pipe, which had a series of holes (refer to Figure 5.3). The electrolyte drained from each of the electrode chambers through two overflow pipes (Ø 32 mm) connected to the back of the electrode chamber, flowing passively (via gravity) to a central collection tank (an insulated cool box). Both the catholyte and anolyte solutions were recirculated together in the central collection tank and pH was regulated automatically using a Gelpas pH probe (BDH, Ltd., Poole, UK) connected to a pH monitor (3305, Jenway Ltd., Felsted, UK). A buffer solution consisting of a 10:1 mixture of 0.1 M HNO_3 and 0.1 M H_2PO_4, respectively, was used as a back-up system to maintain the electrolyte at a pH of between 6 and 7. In laboratory-scale

electrokinetic experiments it was found that an overflow system was more effective than actively pumping electrolyte directly from the chambers.

5.3.5 Set up of the field scale control cell

A second skip was used as a control cell without an electric field or nutrients added to the soil. There was insufficient soil to create an exact replica of the electrokinetic treatment cell, so the available soil (~0.22 m^3, 350 kg) was compacted into the second skip at one end to create a scaled-down version (refer to Appendix 8.12 for the schematic).

5.3.6 Spiking of the electrokinetic treatment and control cell with transformer oil

There was a prolonged period of time between the contamination incident and the start of the experiment, so additional transformer oil was added to the treatment cells. A central region of both the control and electrokinetic cells was spiked with neat transformer oil (analogous to the setup of the laboratory experiments § 2.3.5.2) to facilitate detection of the degradation and/or movement of the contaminants, from a homogeneously spiked region. A central region (1.09 m x 0.20 m x 0.44 m) (Figure 5.7) was removed from the compacted soil and spiked with additional transformer oil (~6 000 mg kg^{-1}, assuming a density 1 g ml^{-1}).

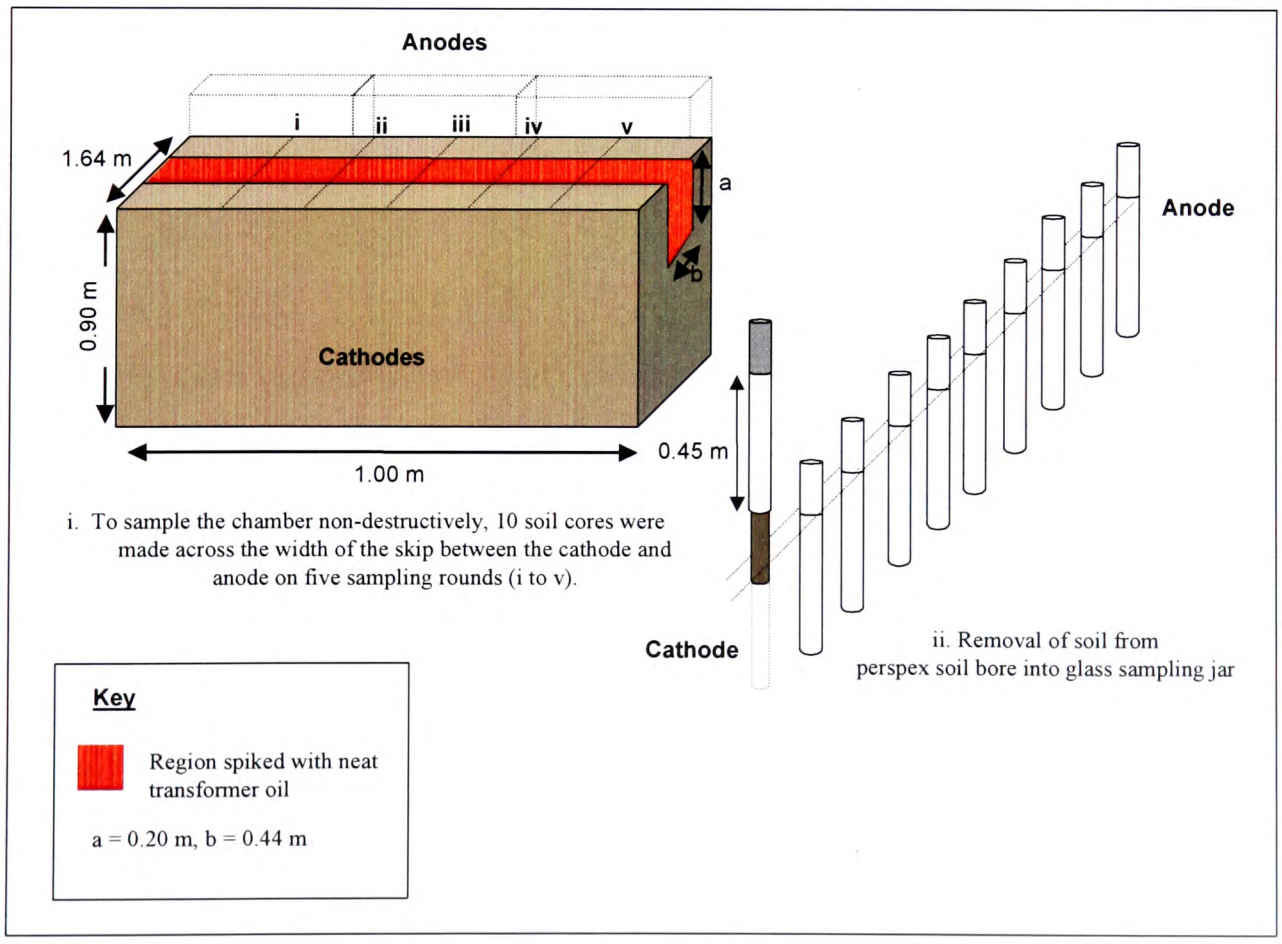


Figure 5.7 Sampling regime for the electrokinetics field trial. Ten samples were taken across the width of the skip on five separate sampling rounds. The central region (highlighted in red) was spiked with transformer oil to a concentration of ~6 000 mg kg^{-1} (assuming a density of 1 g ml^{-1}).

5.3.7 Nutrient addition

A mixture of ammonium nitrate (NH_4NO_3, ~pH 5.4) and potassium orthophosphate (KH_2PO_4, ~pH 4.5) was added to the electrolyte as a source of NO_3^- and PO_4^{3-}. Treatability studies with the transformer oil and EDF test soil were not undertaken prior to the start of the field-trial, so a C:N:P ratio of 100:10:1 was used to stimulate biodegradation (the ratio used in the standard protocol for maintaining hydrocarbon biodegradation in a biopile [Battelle, 1996]). The ratio was based on the hydrocarbon concentration spiked into the central region of the electrokinetic treatment cell, 6 000 mg kg^{-1}. For logistical reasons the TPH concentration within the soil was not determined until the end of the experiment.

5.3.8 Monitoring parameters of the electrokinetic treatment and control cell

5.3.8.1 Voltage and temperature

Ten voltage readings at a soil depth of ~50 mm, evenly spaced along the length of the electrokinetics soil chamber between each set of anodes and cathodes, were taken at intervals using a handheld Wavetek Meterman (Fisher Scientific, Loughborough, UK). The temperature was measured in close proximity (~50 mm) to each of the electrodes and in the centre of the treatment cell, between each set of anodes and cathodes, using a digital thermometer, with stainless steel sensor probe (Fisher Scientific, Loughborough, UK).

5.3.9 Sampling and analysis of the soil and electrolyte

Soil was sampled using standard PTFE tubing as a soil borer, (Ø 60 mm) to a depth of ~0.6 m. The tubes were sharpened on one edge to aid entry into the soil and a wooden mallet was used to force them into the soil with minimal disturbance. Soil samples were collected at day zero (immediately after spiking), and then after 14 days, 52 days and 133 days. From the electrokinetic treatment cell a total of 10 soil samples were collected across the width of the skip, at distances of 0.13, 0.38, 0.76, 0.88, 1.26 and 1.51 m from the anode. Five samples were collected from the control cell at distances of 0.15, 0.50. 0.82, 1.14, and 1.49 m from the edge of the skip.

Each of the soil samples was pushed from the soil borer using a PTFE rod into glass beakers where the soil was homogenised using a metal spatula before being weighed for the following analyses, defined below (§ 5.3.9.1 – 5.3.9.3). The collected soil samples were stored at –80°C until they were all analysed together at the end of the field trial.

5.3.9.1 Analysis of moisture content and pH

Moisture content and pH_{H2O} were determined using the general methods described previously (refer to Sections 2.3.6.1 and 2.3.6.2, respectively).

5.3.9.2 Analysis of the TPH concentration in the soil

To determine the TPH concentration within the soil samples, the homogenised soil samples were extracted using the general method (§ 2.3.6.3). The solvent extract was analysed for the concentration of speciated TPH by a private laboratory (SAL, Manchester) using GC-FID. (Analysis could not be carried out in-house as a suitable column for the GC-FID was not available to quantitatively assess the TPH fractions. For logistical reasons not all of the soil samples collected could be analysed for the concentration of TPH, instead a representative group of samples were selected for analysis from both the electrokinetic treatment and control cells).

5.3.9.3 Analysis of the anions in the soil and within the electrolyte

For the analysis of the anions Cl^-, PO_4^{3-}, SO_4^{2-}, and NO_3^-, soils were extracted and analysed using the general method as described in Section 2.3.6.4. To sample the electrolyte, the general method was followed but dH_2O was not added, unless a dilution was required. In addition, the soil and electrolyte samples collected at day 0 and after 133 days were also analysed for NO_2^- using the general method described in Section 4.5.1.9.

5.3.10 Statistical analyses

Paired t-tests (two sample for means) were used to compare the TPH concentration distributed across the treatment cell between days 14, 42 and 133, and across the control cell between days 42 and 133 . Where the TPH concentration from a specific location in the treatment cell was compared between sample days, unpaired t-tests (two sample, equal variance) were used. Data were considered to be significantly different (*i.e.* the null hypothesis (H_0) was rejected) if $p \leq 0.05$, where p = the probability of H_0 being true (no differences between the means of two sets of data).

5.4 Results

5.4.1 The analysis of the EDF test soil

As previously described (§ 5.3.1) soil samples were collected from the site immediately after the spill and were sent to a private laboratory for analysis. A full breakdown of the results is included in Appendix 8.10. The soil type was Newport 2, described as a 'deep well drained sandy often ferruginous soil' (Soil Survey of England and Wales, 1983). The average TPH concentration detected from the three samples collected in the vicinity of the transformer (from an area of approximately 3 m x 6 m) was 8 132.3 ± 7 934.1 mg kg^{-1} (average is ± SE; n = 3). The range of concentrations from 3 samples taken relatively close together highlights the high degree of heterogeneity in samples collected from the field. In sample 1, in which the highest TPH concentration was detected, the TPH banding was split approximately 54% $C_{16} - C_{20}$, 37% $C_{21} - C_{40}$, and 7% $C_{12} - C_{16}$, which is consistent with transformer oil that contains predominantly alkylbenzenes ($C_{18} - C_{20}$). With regard to the analysis of metals in the soil samples iron and magnesium were found at the highest concentrations, 20 000 and 1 100 mg kg^{-1}, respectively. The anions Cl^- and SO_4^{2-}, and sulphide as S were not detected in the soil and NO_3^- was detected at a concentration of approximately 1 ± 0.8 mg kg^{-1}.

5.4.2 Characteristics of EDF test soil at day zero of the experiment

The soil characteristics at day zero of the experiment are defined in Table 5.1. The moisture content and concentration of the anions SO_4^{2-}, Cl^-, and PO_4^{3-} were analogous to the results for the soils collected in Orford, immediately following the spill incident. The concentration of NO_3^- had been reduced to below laboratory detection limits. The moisture content and pH of the soil (pH 7.79 ± 0.08) were suitable for biodegradation to occur, but extractable sources of N and P were not detected.

In comparison to the WHC of the model soils used in the preceding chapters of this study the WHC of the EDF test soil was most similar to Reading soil (refer to § 2.3.2). The electroosmotic flow rate for the EDF test soil was slower than had been calculated for both Reading and Wytham soils (§ 2.4.2).

Table 5.1 Characteristics of trial soil before the application of electrokinetics.

Characteristic	Value	± SE	No. of samples
pH_{H2O}	7.79	0.08	3
Water holding capacity (WHC) (%)	20.6	0.4	3
Moisture content (%)	11.1	0.2	4
NO_3^- (mg kg^{-1})	<0.0001	n/a	9
SO_4^{2-} (mg kg^{-1})	<0.0001	n/a	9
Cl^- (mg kg^{-1})	<0.0001	n/a	9
PO_4^{3-} (mg kg^{-1})	<0.0001	n/a	9
NO_2^- (mg kg^{-1})	<0.0001	n/a	3
Electroosmotic flow rate ml in 24 h	2.6	0.09	4

5.4.3 Feasibility studies for the use of surfactants with EDF test soil

Column studies (§ 2.3.3.2) were used to investigate the potential to use surfactant solutions (0.4 and 1.0%) in the electrokinetic treatment cell containing EDF test soil. The ionic surfactant SDS and the non-ionic surfactants Triton X-100 and Span 85 did not pass readily through a soil column consisting of EDF test soil. The movement of 2 pore volumes of the surfactant solutions took > 12 h to pass through the soil column. Consequently, it was decided that surfactants would not be used in the field-scale experiment of this study. The addition of surfactant into the soil (as observed in Chapter 3) can have a detrimental effect on the soil structure and outcome of the electrokinetic experiments. It would therefore be necessary to have a number of controls so that the results could be interpreted accurately. However, the limited volume of contaminated soil and number of treatment cells prohibited further variables to be tested.

5.4.4 Voltage and temperature across the soil

A linear voltage profile across the electrokinetic treatment cell was observed throughout the duration of the experiment (Figure 5.8 and 5.9). Between day 10 and 14 the current increased from 10 mA to 810 mA. As a consequence, the voltage was reduced from 90 V to 10 V to return the current to 10 mA. No further modifications were required for the duration of the experiment.

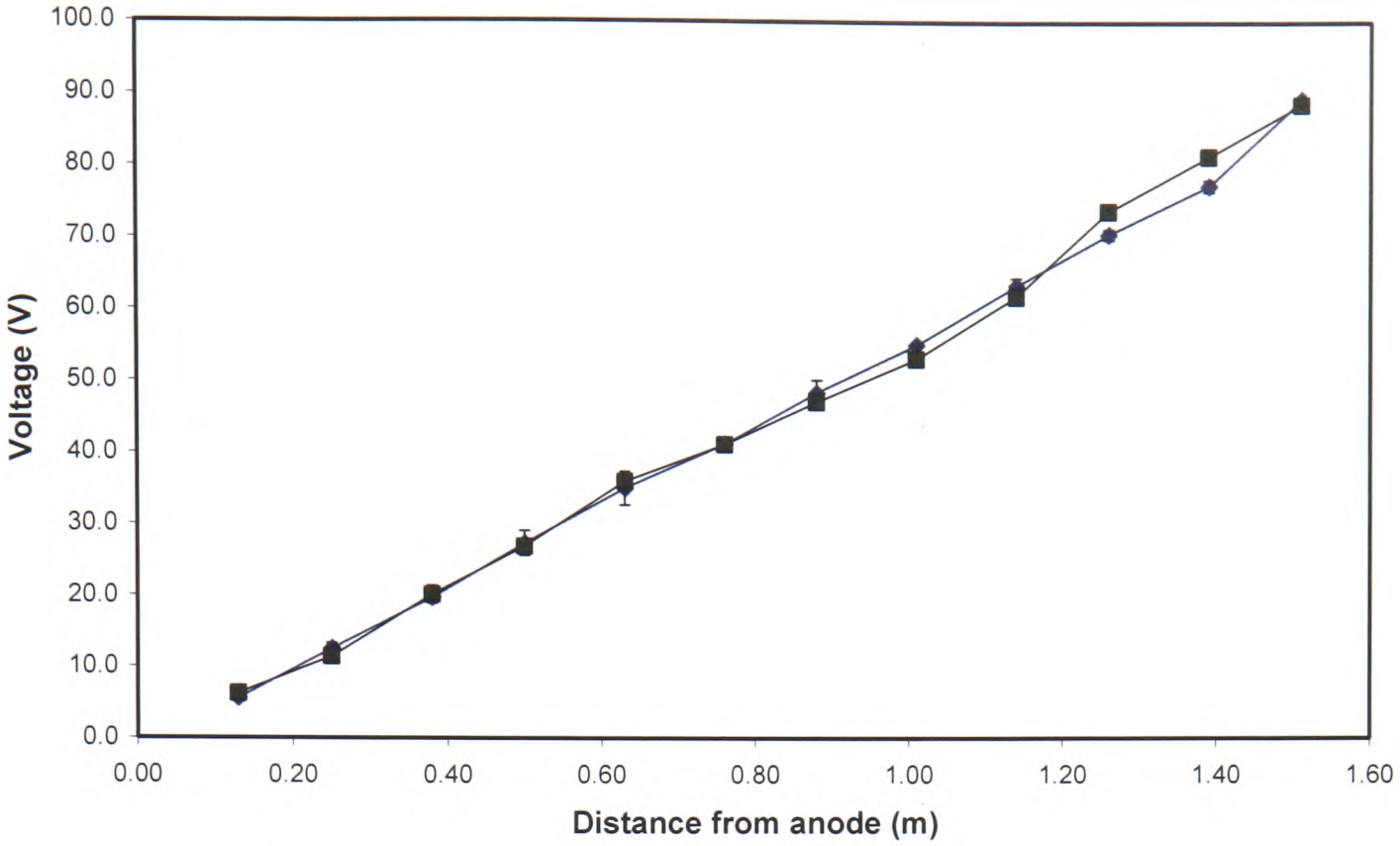


Figure 5.8 Voltage profile in electrokinetic treatment cell. Electric potential distribution across the electrokinetics treatment cell, containing EDF test soil; ◆ day 0; ■ day 14. (Data are means ± SE, n = 9).

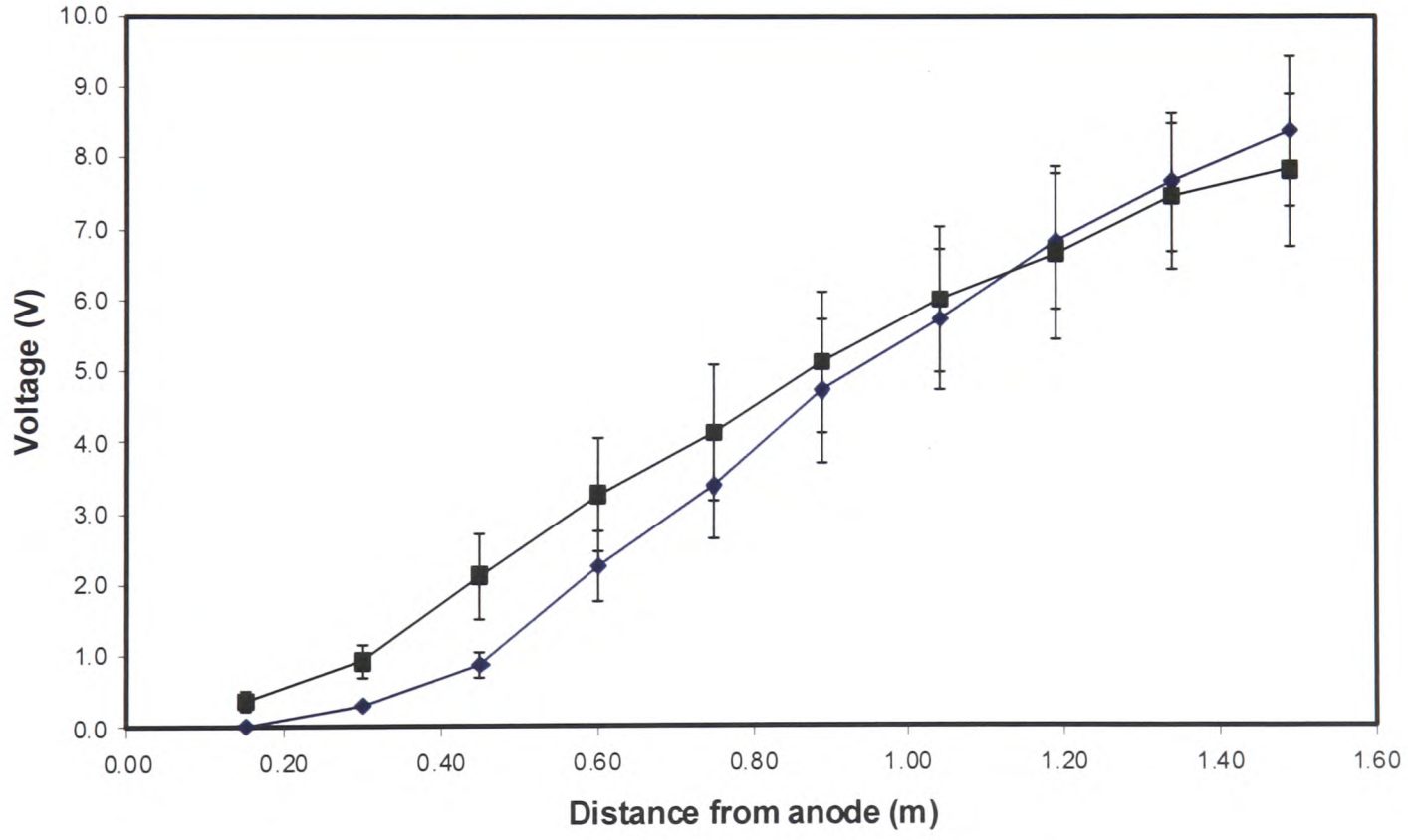


Figure 5.9 Voltage profile in electrokinetic treatment cell. Electric potential distribution across the electrokinetics treatment cell, containing EDF test soil; ◆ day 42; ■ day 133. (Data are means ± SE, n = 9).

There was no significant difference in temperature across the electrokinetic treatment cell (Figure 5.10). In comparison, the temperature of the soil within the control cell and the sub-surface soil at the field site was recorded to have a higher temperature than the soil in

the electrokinetic treatment cell (the temperature for day 14 is shown in detail as a representative example [Figure 5.11]).

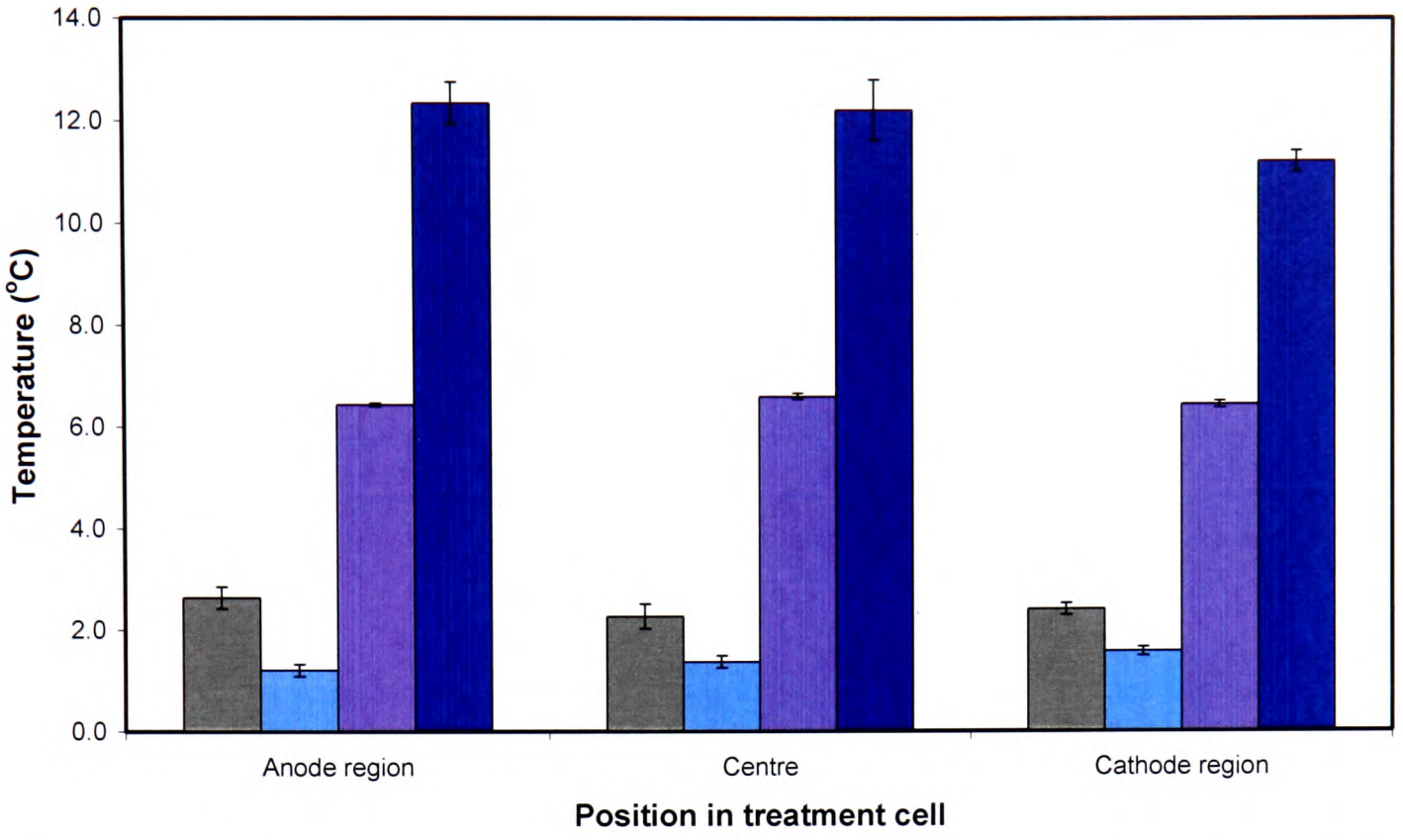


Figure 5.10 Temperature (°C) profile across the electrokinetic treatment cell (~10 mA). ■ day 0; ■ day 14; ■ day 42, ■ day 133 (data are means ± SE; n=3).

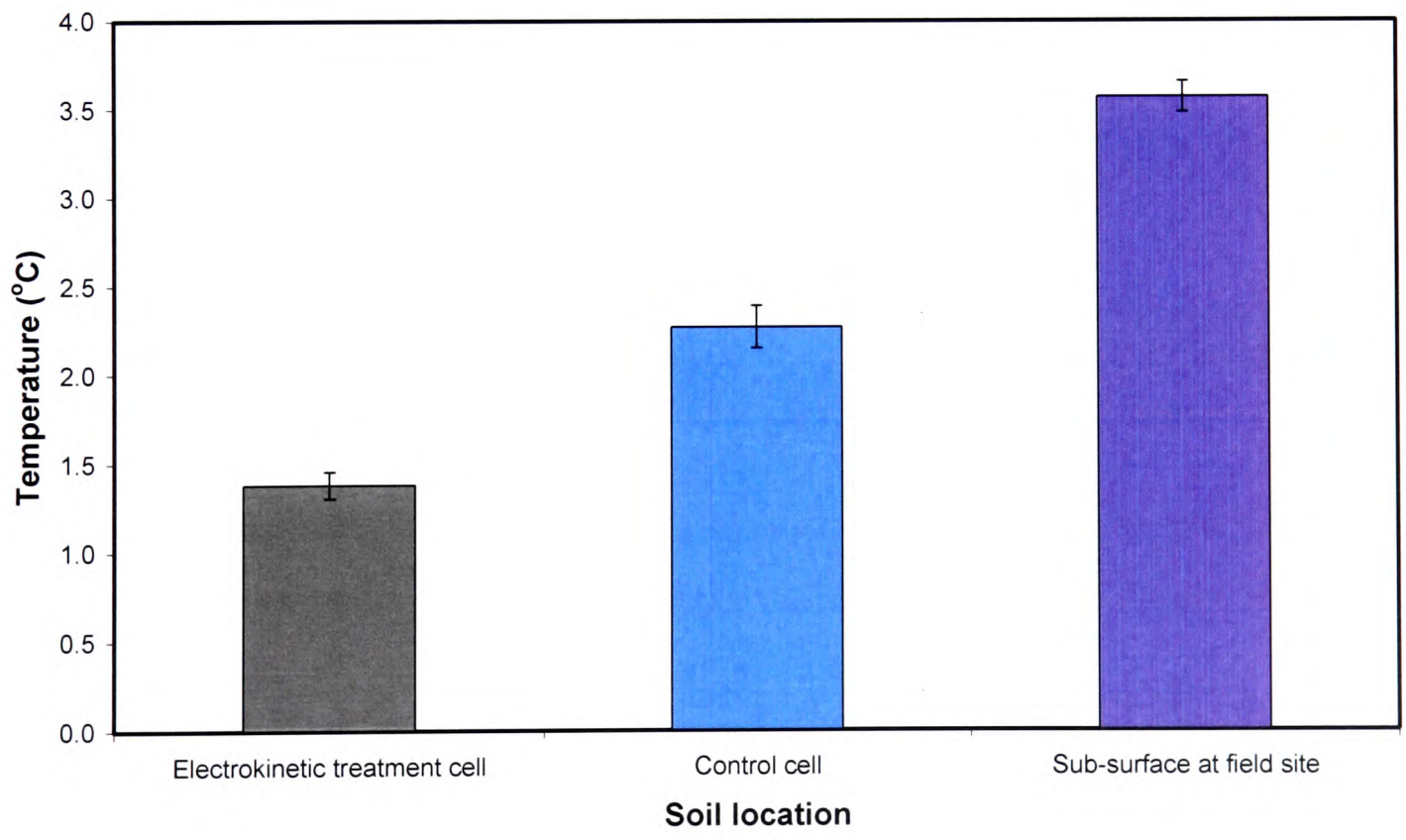


Figure 5.11 A comparison of the temperature of the soil on day 14 between the ■ electrokinetic treatment cell (~10 mA applied); ■ the control cell; sub-surface at field site ■ (data are means ± SE; n=3).

5.4.5 Moisture content and pH across the treatment and control cell

The moisture content of the soil was maintained around a concentration of approximately 11% (Figure 5.12). After 14 days of applying an electric field, the soil in the proximity of the cathode chamber was drier compared to the control cell, and with the moisture content of the soil at the beginning of the experiment (day 0). At days 42 and 133 the moisture content of the soil was observed to be relatively uniform between the anode and cathode electrodes. At the final sampling round (day 133) the soil within the electrokinetic treatment cell had a significantly higher moisture content compared with the soil within the control cell ($p = 0.02$, df = 33).

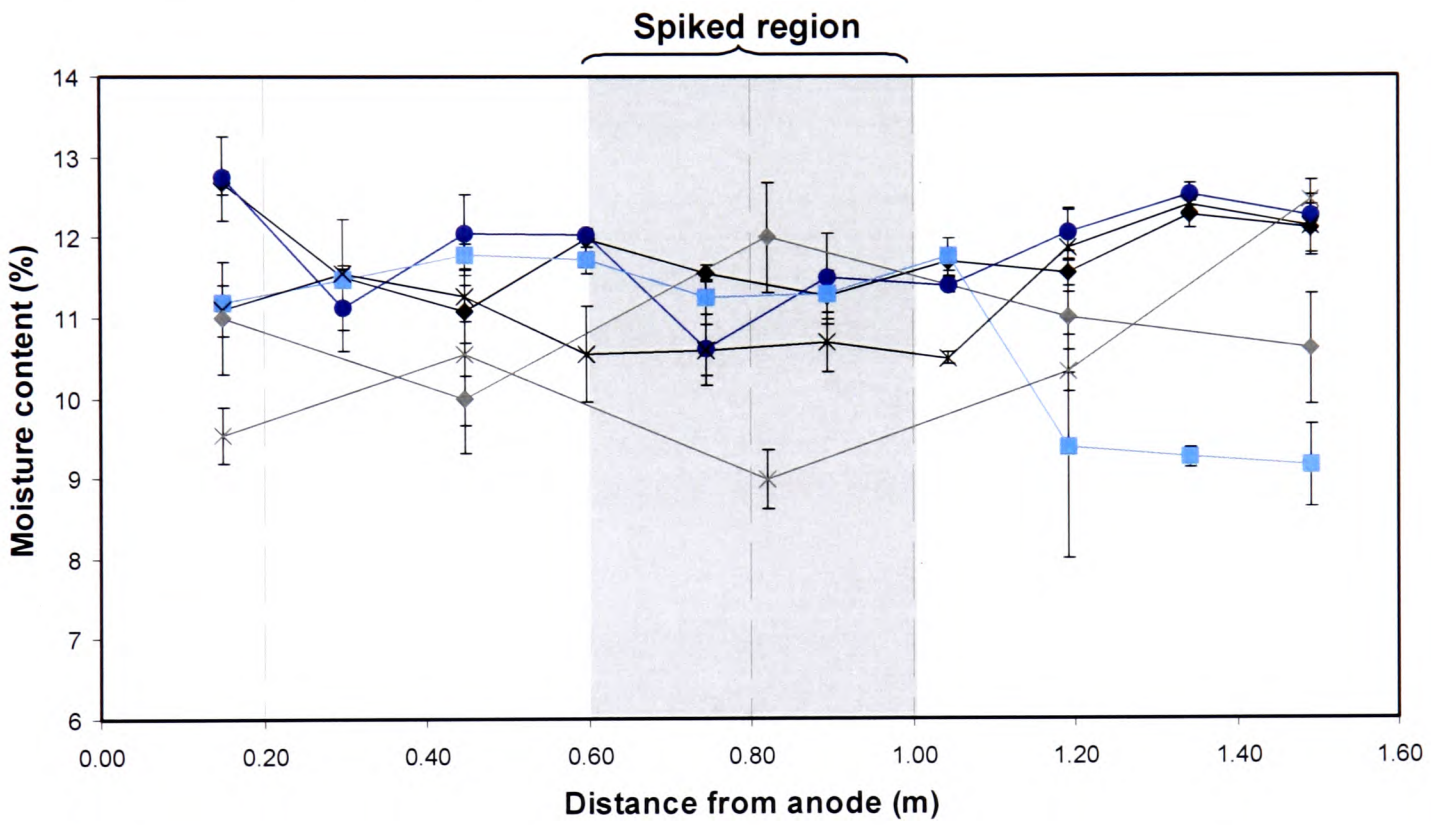


Figure 5.12 Moisture content of the soil across the electrokinetic treatment and control cells. Samples collected at ♦ EK day 0; ♦ Control day 0; ■ EK day 14; ● EK day 42; **x** EK day 133; x Control day 133 (data are means ± SE; n = 3). (EK = Electrokinetic treatment cell).

There was no marked difference in pH across the soil skip and no recordable volume of buffer solution was used to maintain the pH of the electrolyte at pH 7 (Figure 5.13).

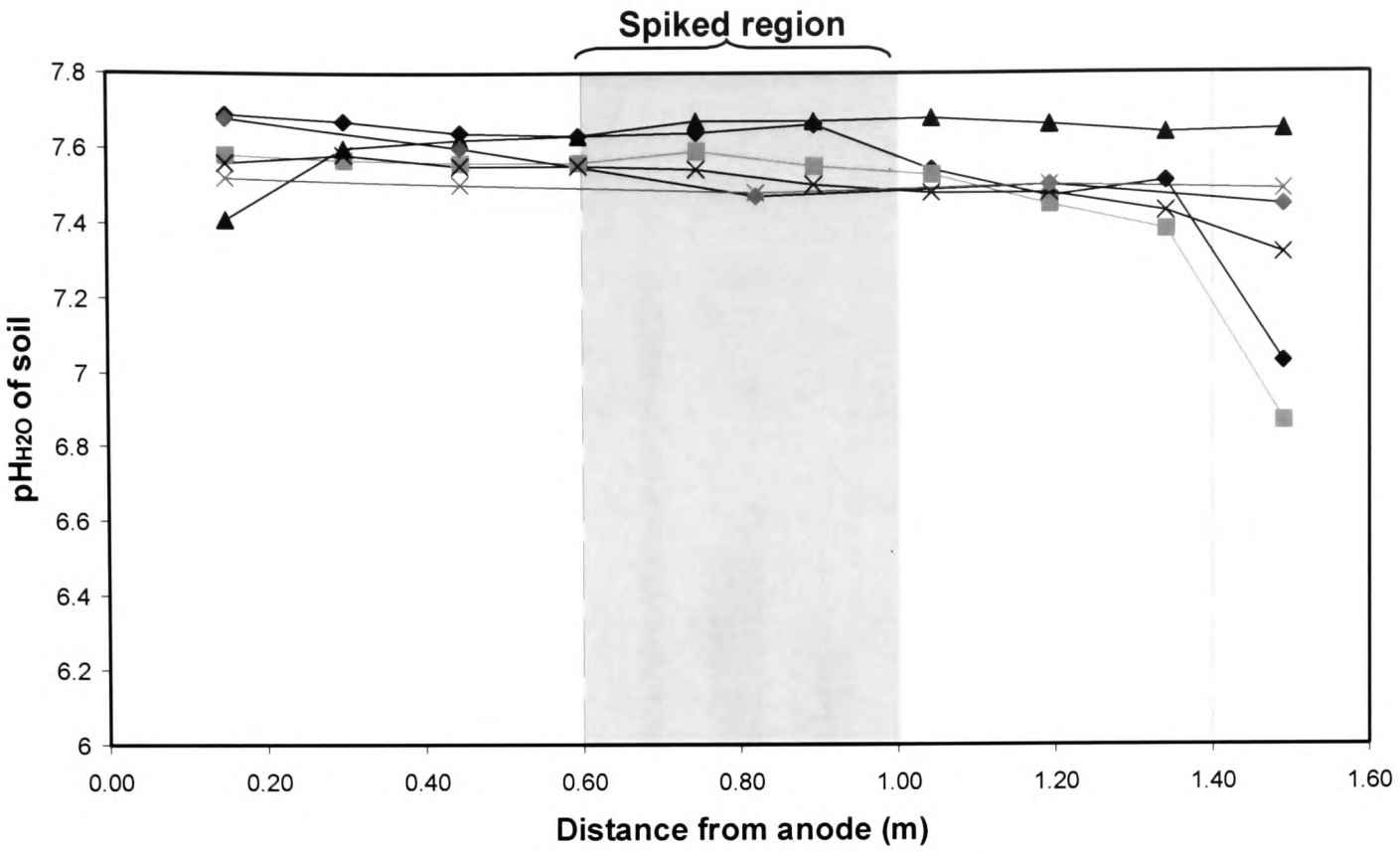


Figure 5.13 pH distribution across the electrokinetic treatment and control cells. Samples collected at intervals ♦ EK day 0; ♦ Control day 0; ■ EK day 14; ▲ EK day 42; x EK day 133; x Control day 133; (Data points are based on one reading, the error associated with the pH probe is ± 0.03). (EK = Electrokinetic treatment cell).

5.4.6 Concentration of TPH in the soil within the electrokinetic treatment cell

Soil samples from across the electrokinetic treatment cell were collected on four occasions and the TPH concentration across the $C_8 - C_{35}$ range (speciated into five carbon fractions) was determined by a private laboratory upon completion of the experiment (§ 5.3.9). Figure 5.14 details the concentration of TPH ($C_8 - C_{35}$) across the soil prior to the central region being spiked with additional transformer oil. No hydrocarbons in the range $C_8 - C_{12}$ were detected in the soil samples. The principal fractions were $C_{16} - C_{21}$ and $C_{21} - C_{35}$, consistent with transformer oil.

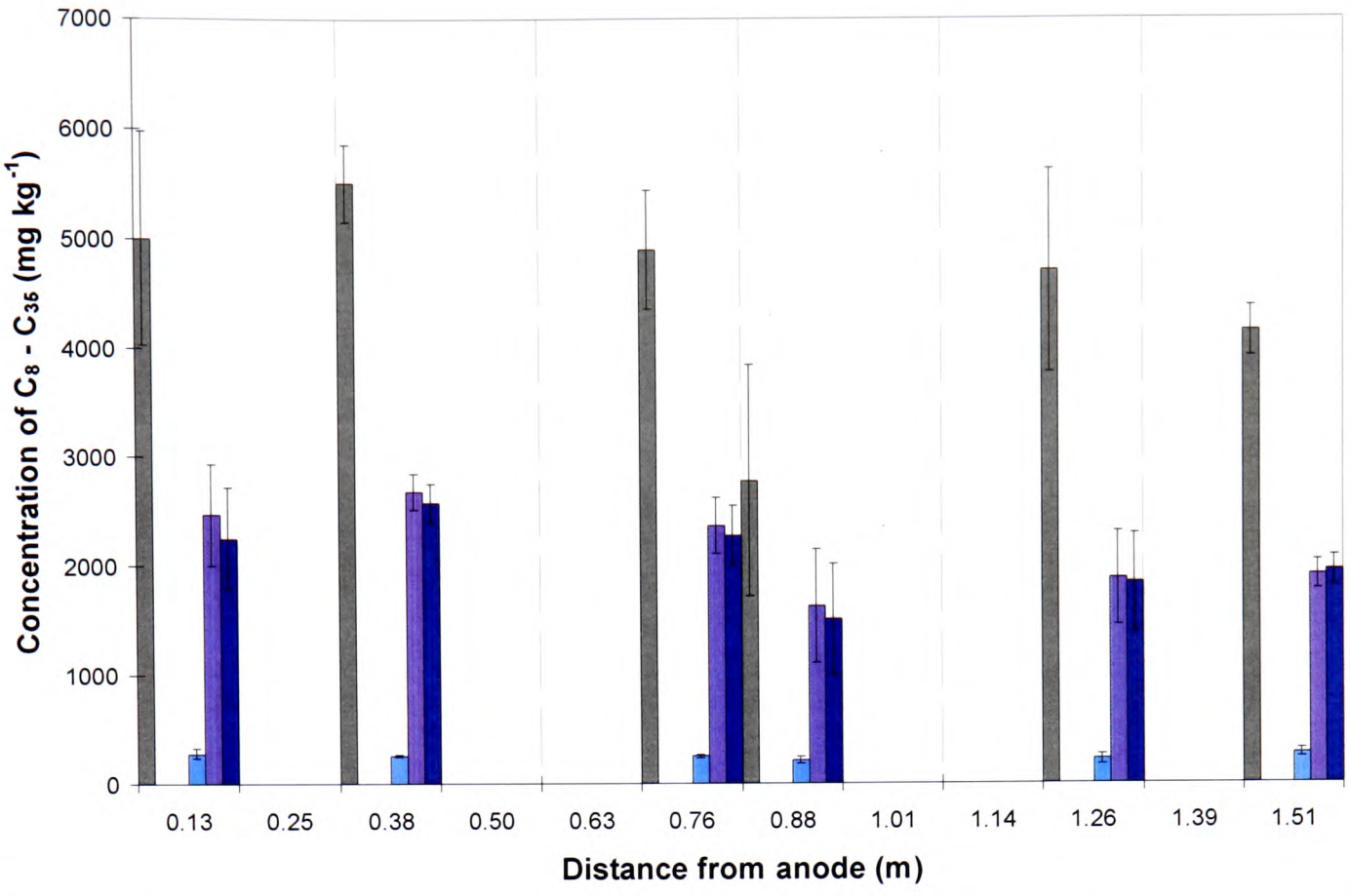


Figure 5.14 Concentration and distribution of TPH across the electrokinetic treatment cell. Soil samples collected immediately after the soil was loaded into the skip with electrodes in place before the central region was spiked. ■ Total TPH (C_8-C_{35}); ■ C_8-C_{10}; ■ C_{10}-C_{12}; ■ C_{12}-C_{16}; ■ C_{16}-C_{21}; ■ C_{21}-C_{35}. (Data are means ± SE, n = 3).

After the central region was spiked with additional transformer oil, samples were collected across the width of the skip on days 14, 42 and 133 (using the sampling method as described in Figure 5.7) . The TPH concentration across the electrokinetic treatment cell, analysed from samples collected on day 14, 42 and day 133 of the experiment, are shown in Figures 5.15 to 5.18. Figure 5.15 shows the concentration of TPH (C_8 - C_{35}), Figure 5.16 shows the TPH concentration in the range C_{12} - $C_{16,}$ Figure 5.17 the TPH concnetration in the range C_{16} – C_{21}, and Figure 5.18 the TPH concentration in the range C_{21} – C_{35}. TPH in the range C_8 – C_{12} were not detected in any of the analysed soil samples.

A comparison was made between the TPH concentration detected within soil samples collected at a distance of 0.15, 0.75, 0.89 and 1.49 m from the anode, on days 14, 42 and 133 (Table 5.2). Overall there appeared to be no significant difference between the concentration of TPH (C_8-C_{35}) across the electrokinetic treatment cell between days 14, 42, and 133 (Figure 5.15, Table 5.2). However, at a distance of 1.49 m from the anode chamber (in the proximity of the cathode chamber) a significant reduction in the concentration of TPH (C_8-C_{35}) was observed between the soil sampled on day 42 and day 133 (p = 0.02; df = 4) (Table 5.3).

Table 5.2 A comparison between the TPH concentration in the soil between all samples collected on day 14 and 42, between samples collected on day 14 and day 133, and between day 42 and day 133. The data were compared using paired t-tests (two sample for means). Data was compared between soil collected at 0.15, 0.75, 0.89 and 1.49 m from the anode.

TPH range	Day 14 and 133	Day 14 and Day 42	Day 42 and 133
$C_8 - C_{12}$	Not detected, < 1 mg kg^{-1}	Not detected, < 1 mg kg^{-1}	Not detected, < 1 mg kg^{-1}
$C_{12} - C_{16}$	Significant increase ($p = 0.01$, df = 3)	No significant difference ($p = 0.5$, df = 3)	Significant increase ($p = 0.02$, df = 3)
$C_{16} - C_{21}$	No significant difference ($p = 0.1$, df = 3)	No significant difference ($p = 0.3$, df = 3)	No significant difference ($p = 0.06$, df = 3)
$C_{21} - C_{35}$	Significant decrease ($p = 0.02$, df = 3)	No significant difference ($p = 0.2$, df = 3)	Significant decrease ($p = 0.01$, df = 3)
$C_8 - C_{35}$	No significant difference ($p = 0.06$, df = 3)	No significant difference ($p = 0.2$, df = 3)	No significant difference ($p = 0.5$, df = 3)

■ shaded cells indicate that there was a significant difference between the data sets, $p \leq 0.05$.

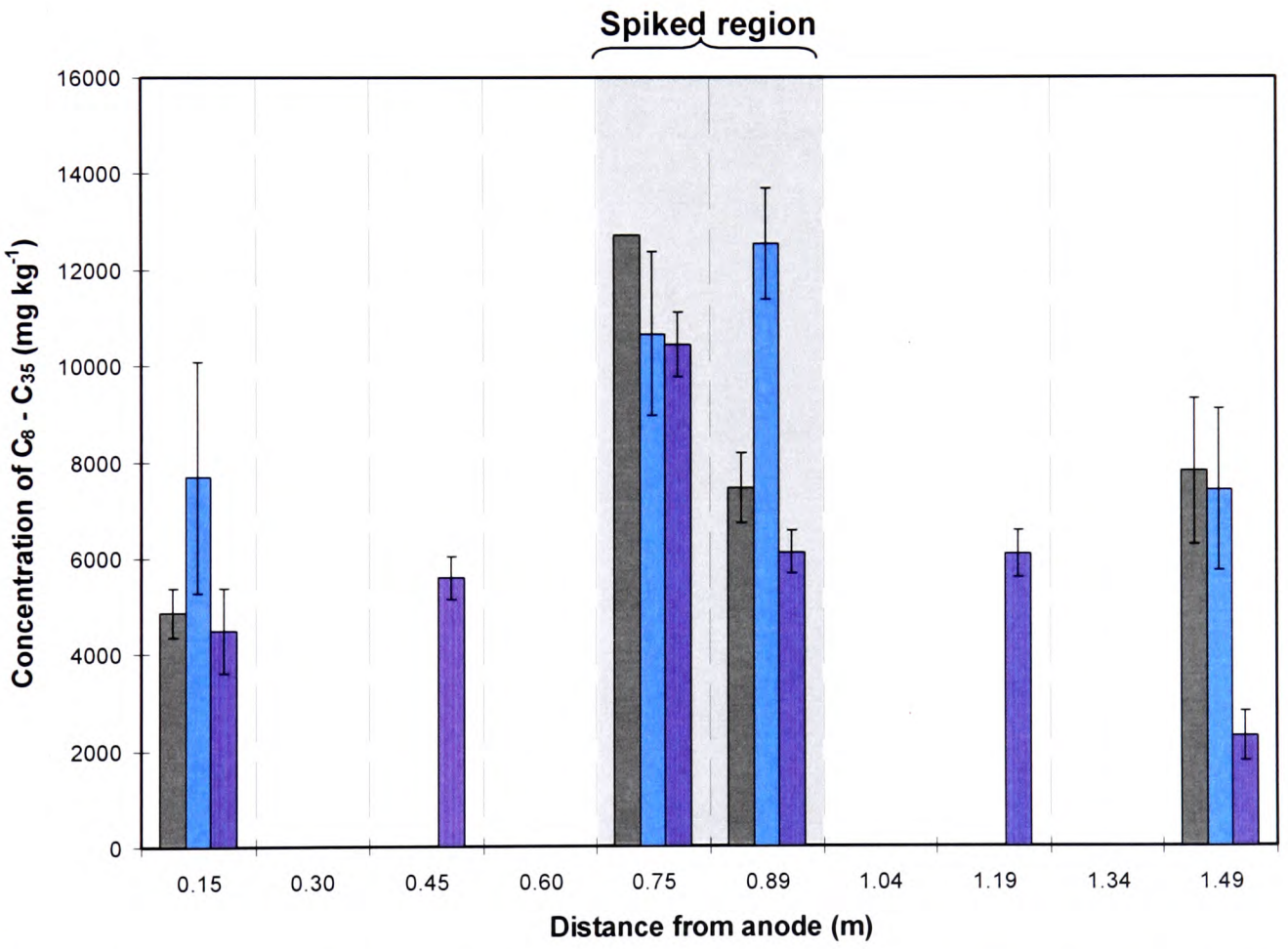


Figure 5.15 Concentration and distribution of TPH (C_8-C_{35}) across the electrokinetic treatment cell. Samples collected at intervals ■ day 14; ■ day 42; ■ day 133 (data are means ± SE, n = 3). Error bars are not included for day 14 at 0.75 m, as the soil sample was not successfully analysed in triplicate.

Table 5.3 A comparison between the TPH concentration in the soil in close proximity to the cathode chamber (~1.49 m from the anode) between samples collected on day 14 and 42, between samples collected on day 14 and day 133, and between day 42 and day 133. The data were compared using unpaired t-tests (two-sample, equal variance).

TPH range	Day 14 and Day 133	Day 14 and Day 42	Day 42 and Day 133
$C_8 - C_{12}$	Not detected, < 1 mg kg^{-1}	Not detected, < 1 mg kg^{-1}	Not detected, < 1 mg kg^{-1}
$C_{12} - C_{16}$	No significant difference ($p = 0.06$, df = 4)	No significant difference ($p = 0.4$, df = 4)	No significant difference ($p = 0.06$, df = 4)
$C_{16} - C_{21}$	Significant decrease ($p = 0.01$, df = 4)	No significant difference ($p = 0.4$, df = 4)	Significant decrease ($p = 0.02$, df = 4)
$C_{21} - C_{35}$	Significant decrease ($p = 0.01$, df = 4)	No significant difference ($p = 0.5$, df = 4)	Significant decrease ($p = 0.01$, df = 4)
$C_8 - C_{35}$	Significant decrease ($p = 0.01$, df = 4)	No significant difference ($p = 0.4$, df = 4)	Significant decrease ($p = 0.02$, df = 4)

■ shaded cells indicate that there was a significant difference between the data sets, $p \leq 0.05$

With reference to the specific TPH fractions, a significant increase in the concentration of $C_{12} - C_{16}$ across the soil was observed between days 42 and 133 of applying an electric field (~10 mA) (Figure 5.16).

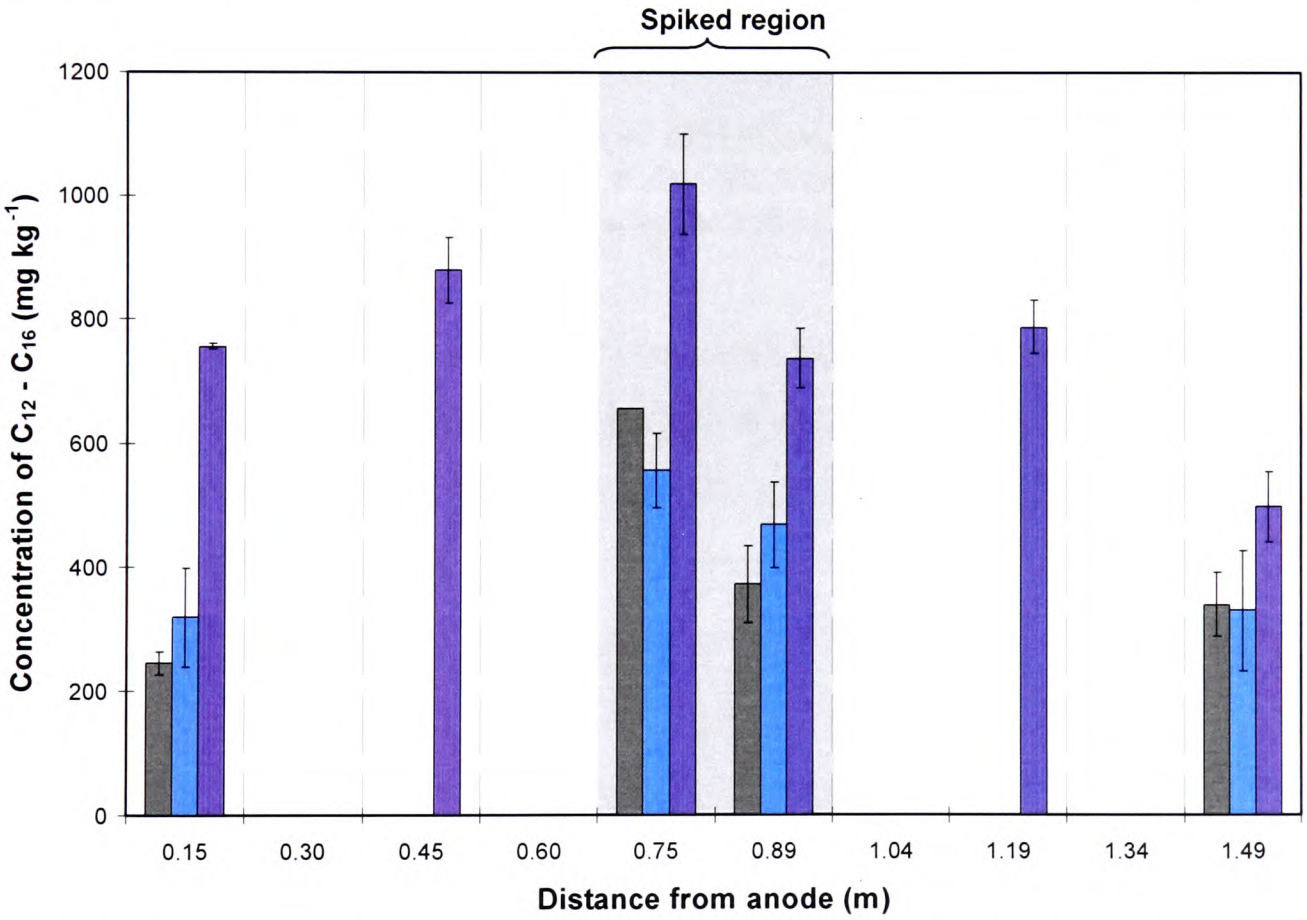


Figure 5.16 Concentration and distribution of TPH (C_{12}-C_{16}) across the electrokinetic treatment cell. Samples collected at intervals ■ day 14; ■ day 42; ■ day 133 (data are means ± SE, n = 3). Error bars are not included for day 14 at 0.75 m, as the soil sample was not successfully analysed in triplicate.

There was no significant difference between the concentration of the TPH fraction $C_{16} - C_{21}$ analysed in the soil across the electrokinetic treatment cell at day 14 compared with the concentration detected at the end of the experiment (day 133) (Figure 5.17). In close

proximity to the cathode chamber a signficant decrease in concentration of TPH ($C_{16} - C_{21}$) was observed between days 42 and 133 of the experiment ($p = 0.02$; df = 4) (Table 5.3).

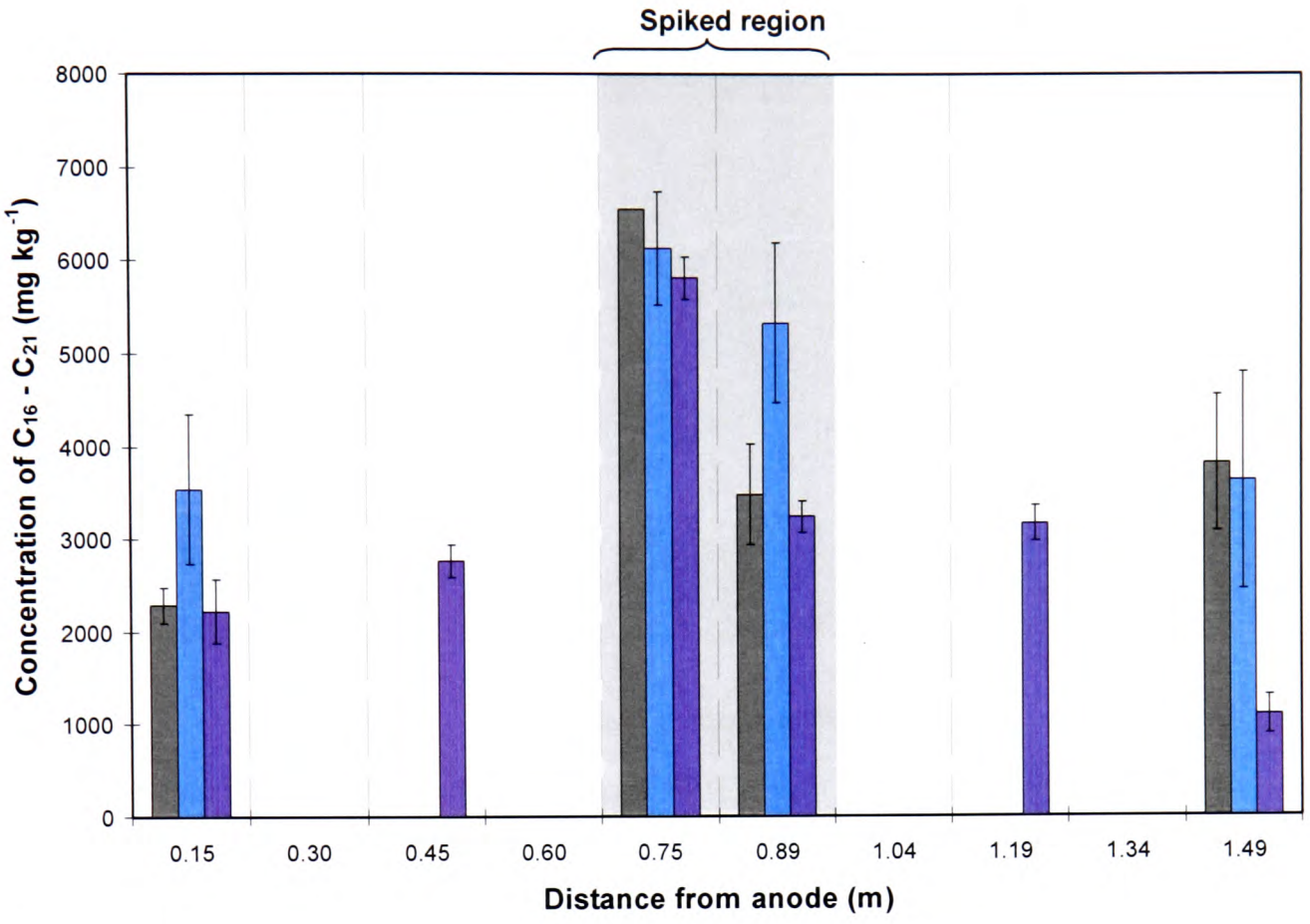


Figure 5.17 Concentration and distribution of TPH (C_{16}-C_{21}) across the electrokinetic treatment cell. Samples collected at intervals ■ day 14; ■ day 42; ■ day 133 (data are means ± SE, n = 3). Error bars are not included for day 14 at 0.75 m, as the soil sample was not successfully analysed in triplicate.

A reduction in the concentration of the TPH fraction $C_{21} - C_{35}$ was observed across the soil within the electrokinetic treatment cell between day 42 and day 133 ($p = 0.01$; df = 3) (Figure 5.18). The reduction in concentration was particularly noticeable in the proximity of the cathode chamber (1.49 m from the anode reservoir).

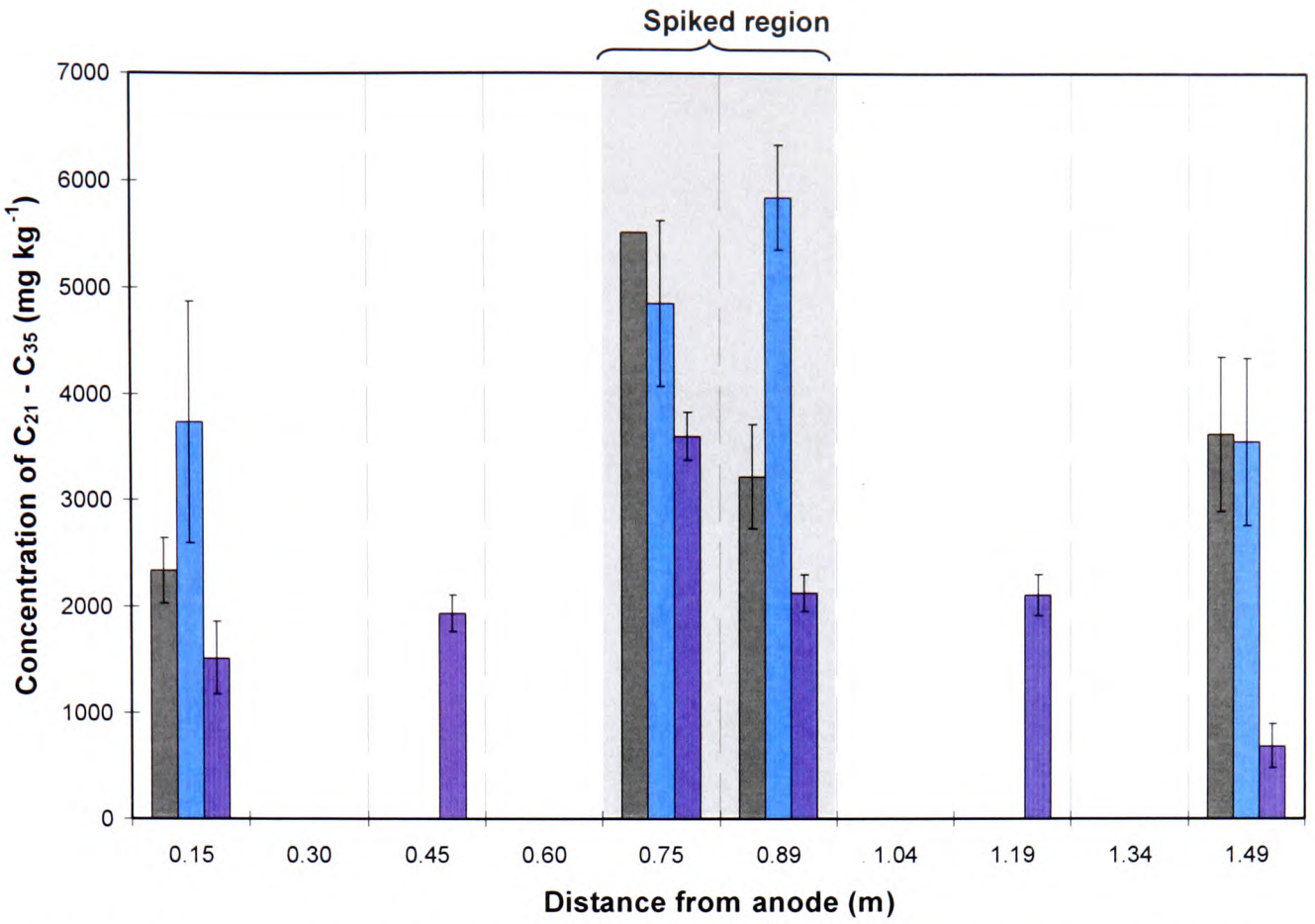


Figure 5.18 Concentration and distribution of TPH ($C_{21} - C_{35}$) across the electrokinetic treatment cell. Samples collected at intervals ■ day 14; ■ day 42; ■ day 133 (data are means ± SE, n = 3). Error bars are not included for day 14 at 0.75 m, as the soil sample was not successfully analysed in triplicate.

5.4.7 Concentration of TPH in the soil within the control cell

The concentration of TPH ($C_8 - C_{35}$) detected in the three sampling locations of the control cell (0.15, 0.82 and 1.49 m from across the width of the skip) was observed to be reduced by 11.7 ± 1.0% between day 42 and 133 of the experiment. Within the electrokinetic treatment cell, the concentration reduced by 28.9 ± 3.5% between days 42 and 133.

Analogous to the data collected from the electrokinetic treatment cell, there was a significant increase in the concentration of TPH ($C_{12} - C_{16}$) (Figure 5.20) and a decrease in the concentration of TPH ($C_{21} - C_{35}$) (Figure 5.22) observed in the control cell, between days 42 and 133 of the experiment ($p = 0.03$, df = 2 [Table 5.4]) (these observations are based on three samples collected in triplicate across the control cell [§ 5.3.9.2]). No significant difference was observed between the overall concentration of TPH in the range $C_8 - C_{35}$ and $C_{16} - C_{21}$ (Figures 5.19 and Figure 5.21, respectively). In contrast to the significant reduction in the concentration of TPH (C_8 to C_{35}) within the electrokinetic treatment cell, 1.49 m from the anode, of the three locations sampled within the control cell (0.15, 0.82 and 1.49 m across the width of the skip) there was no significant difference

between the concentrations of TPH (p = 0.2, 0.3 and 0.06, df = 4, respectively) between days 42 and 133.

Table 5.4 A comparison between the TPH concentration in the soil within the control cell, between samples collected on days 42 and 133. The data was compared using paired t-tests (two sample for means). Data was compared between soil collected at 0.15, 0.82 and 1.49 m across the width of the skip.

TPH range	Day 42 and 133
$C_8 - C_{12}$	Not detected, < 1 mg kg^{-1}
$C_{12} - C_{16}$	Significant increase (p = 0.03, df = 2)
$C_{16} - C_{21}$	No significant decrease (p = 0.5, df = 2)
$C_{21} - C_{35}$	Significant decrease (p = 0.03, df = 2)
$C_8 - C_{35}$	No significant decrease (p = 0.1, df = 2)

■ shaded cells indicate that there was a significant difference between the data sets, $p \leq 0.05$

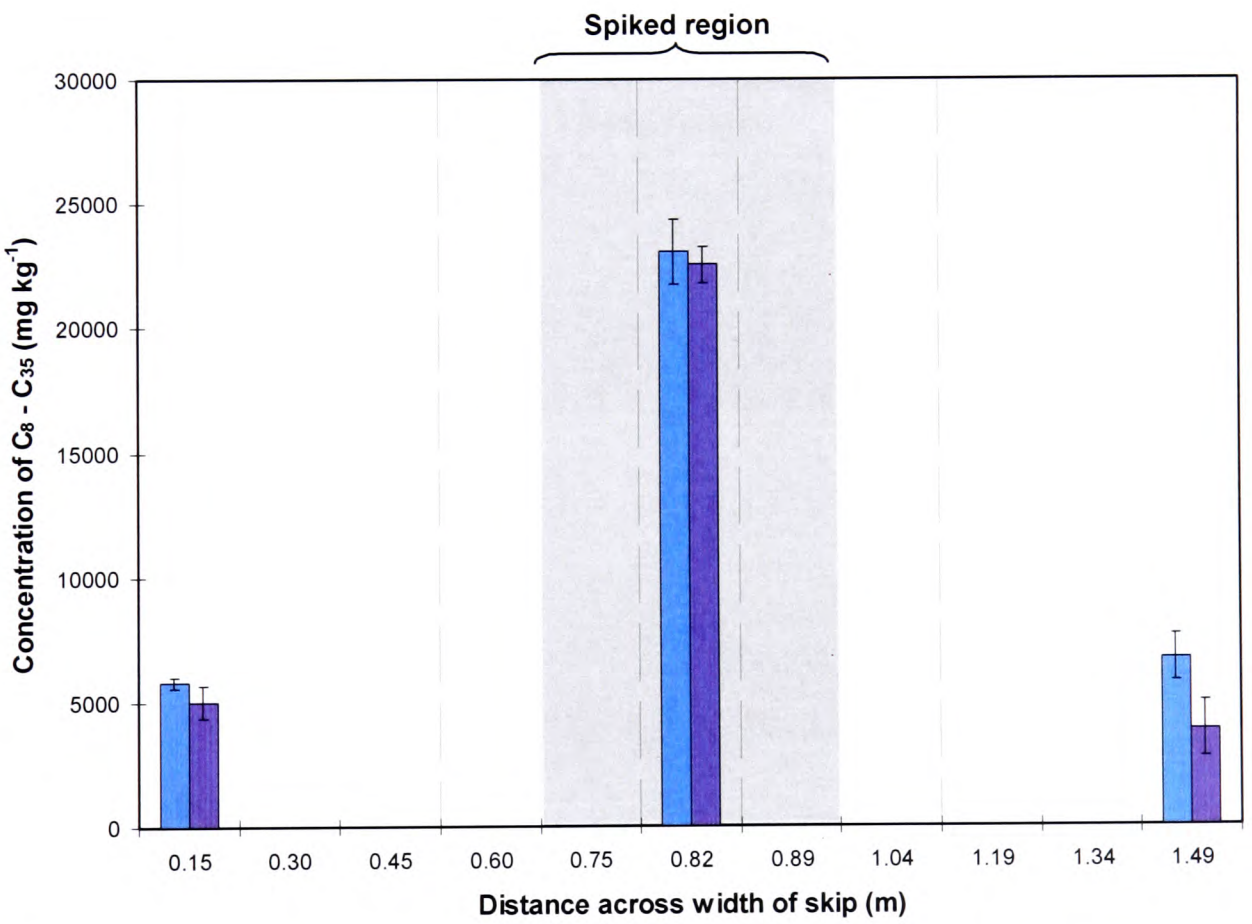


Figure 5.19 Concentration and distribution of (C_8-C_{35}) across the control cell. Samples collected at ■ day 42 and ■ Day 133 (data are means ± SE, n = 3).

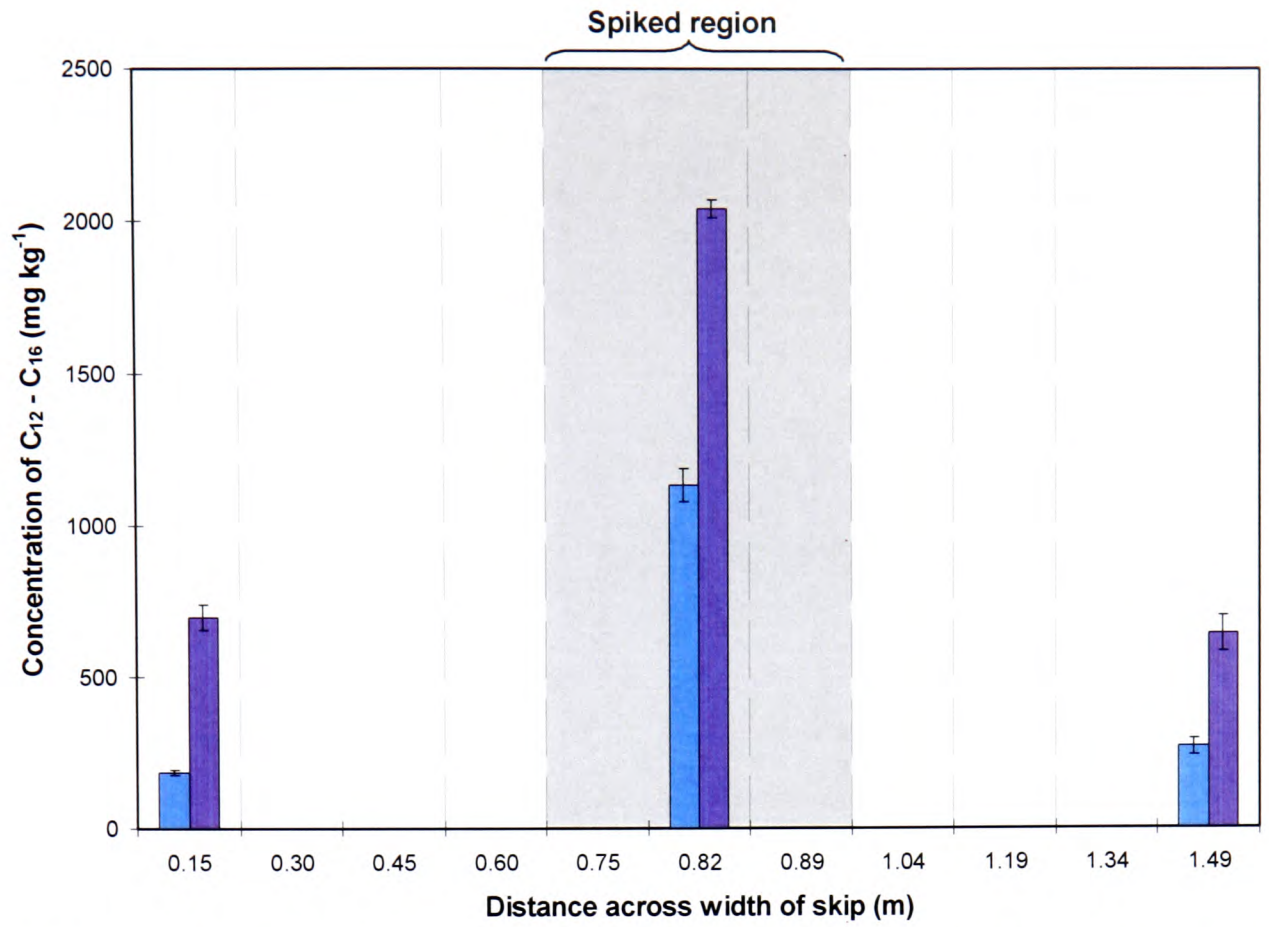


Figure 5.20 Concentration and distribution of (C_{12}-C_{16}) across the control cell. Samples collected at ■ day 42 and ■ Day 133 (data are means ± SE, n = 3).

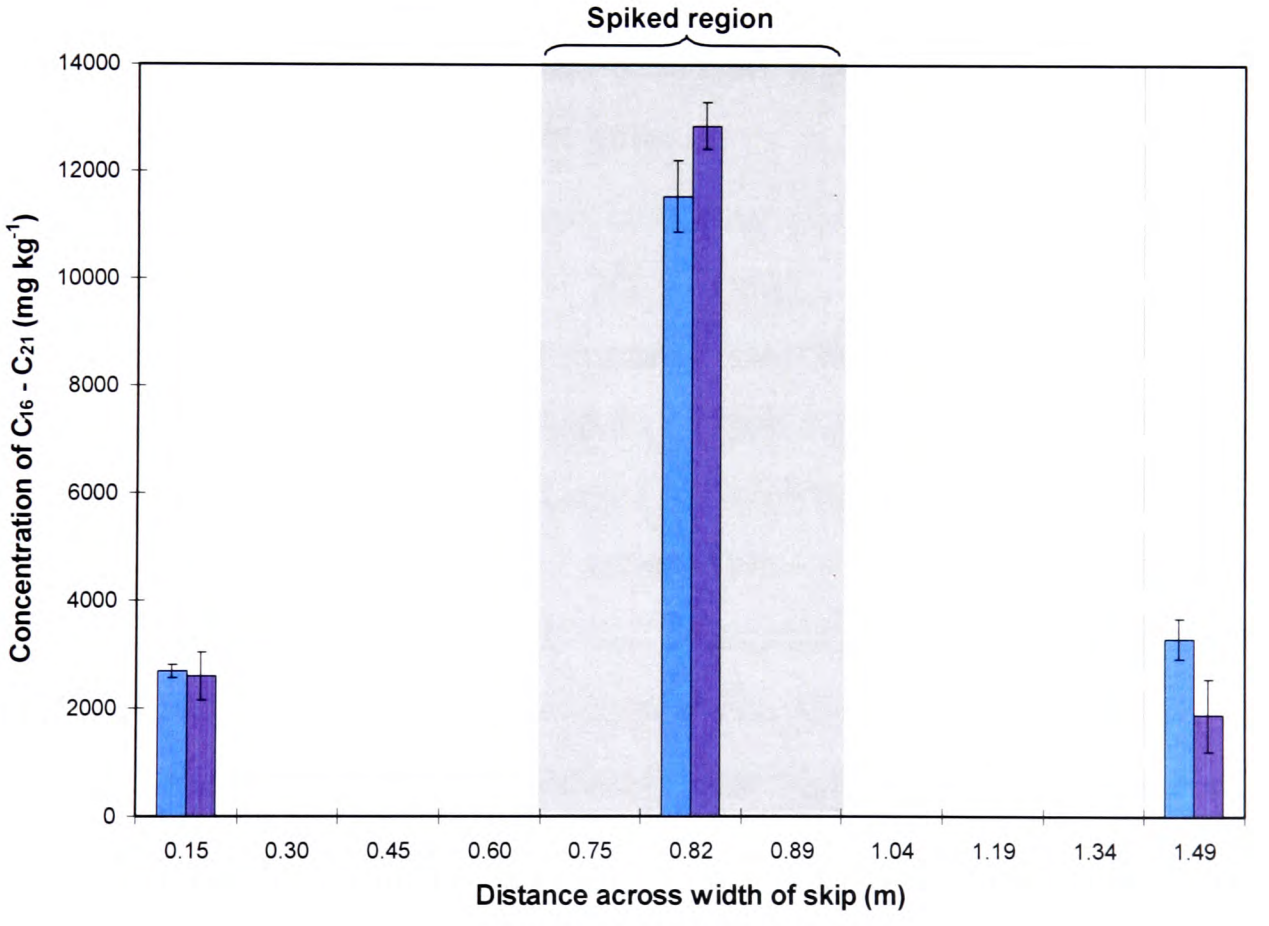


Figure 5.21 Concentration and distribution of (C_{16}-C_{21}) across the control cell. Samples collected at ■ day 42 and ■ Day 133 (data are means ± SE, n = 3).

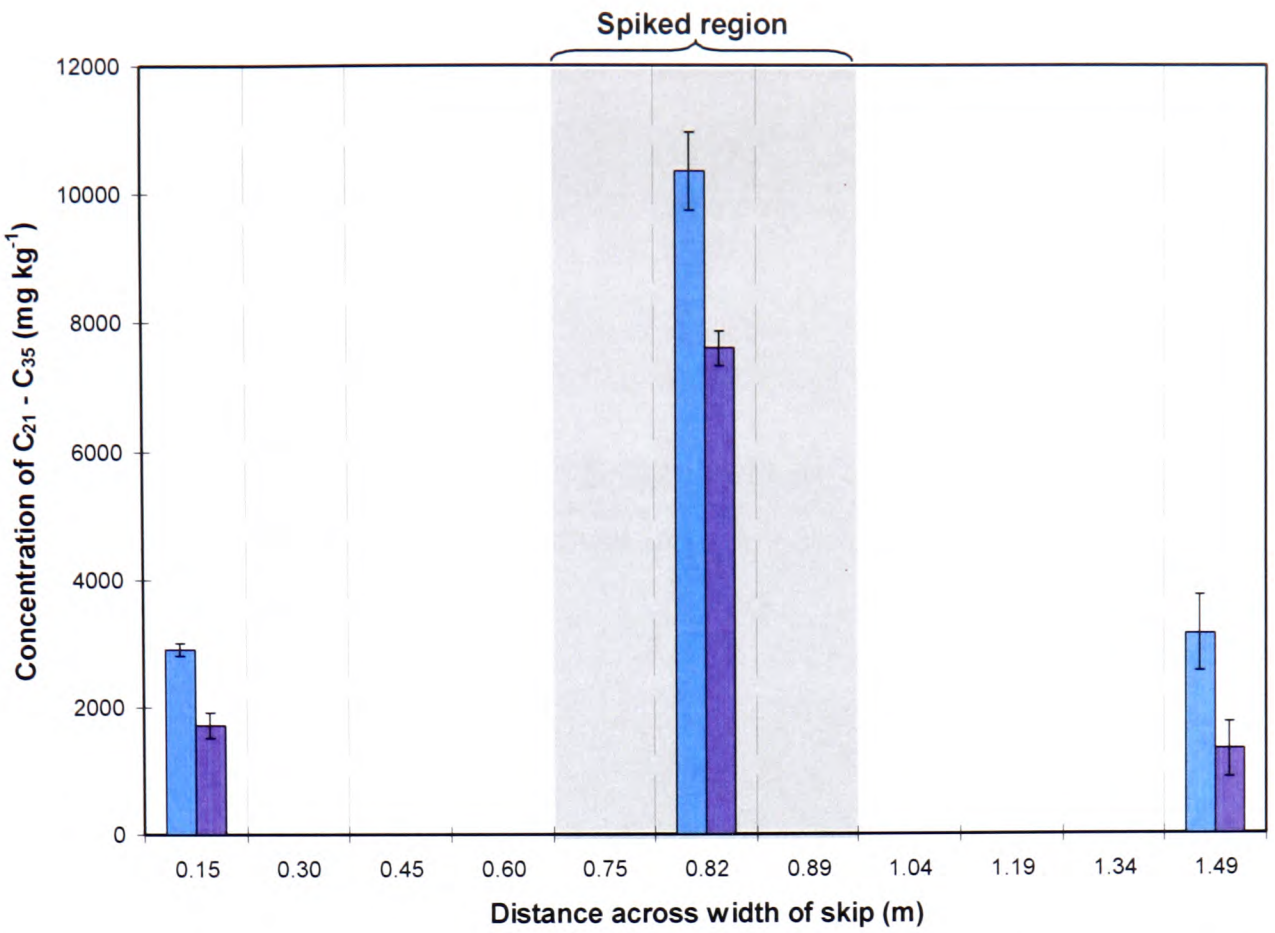


Figure 5.22 Concentration and distribution of (C_{21}-C_{35}) across the control cell. Samples collected at ■ day 42 and ■ Day 133 (data are means ± SE, n = 3).

5.4.8 Concentration and distribution of target anions in the soil of the electrokinetic treatment and control cells

Soil within the electrokinetic and control cells was analysed for the concentration and distribution of the anions NO_3^-, NO_2^-, Cl^-, PO_4^{3-}, and SO_4^{2-} (§ 5.3.9.3). The concentration of NO_3^-, Cl^-, and PO_4^{3-} was determined in mains water and in the electrolyte solution on day 133 of the experiment (refer to Table 5.5). Figure 5.23 gives evidence that NO_3^- had been delivered into the soil under the influence of the electric field. Within the control cell NO_3^- was present across the soil at a low concentration, ~4 mg kg^{-1}. In comparison, within the electrokinetic treatment cell elevated NO_3^- concentrations were detected at 0.15, 1.34 and 1.49 m from the anode reservoir at concentrations of 12.3 ± 1.3 mg kg^{-1}, 43.1 ± 4.2 mg kg^{-1}, and 156.8 ± 21.5 mg kg^{-1}, respectively. The highest concentration of NO_3^- was detected in close proximity to the cathode reservoir, providing some evidence that migration of NO_3^- had occurred via electromigration into the soil.

Table 5.5 Concentration of target anions in mains water and the electrolyte reservoir at the final sampling point (day 133).

	Cl^- mg l^{-1} (mean ± SE)	**NO_3^- mg l^{-1} (mean ± SE)**	**PO_4^{3-} mg l^{-1} (mean ± SE)**
Mains water	117.3 ± 0.3	11.3 ± 0.3	0.8 ± 0.6
Electrolyte Reservoir	90.0 ± 1.2	2617.3 ± 58.4	517.1 ± 58.4

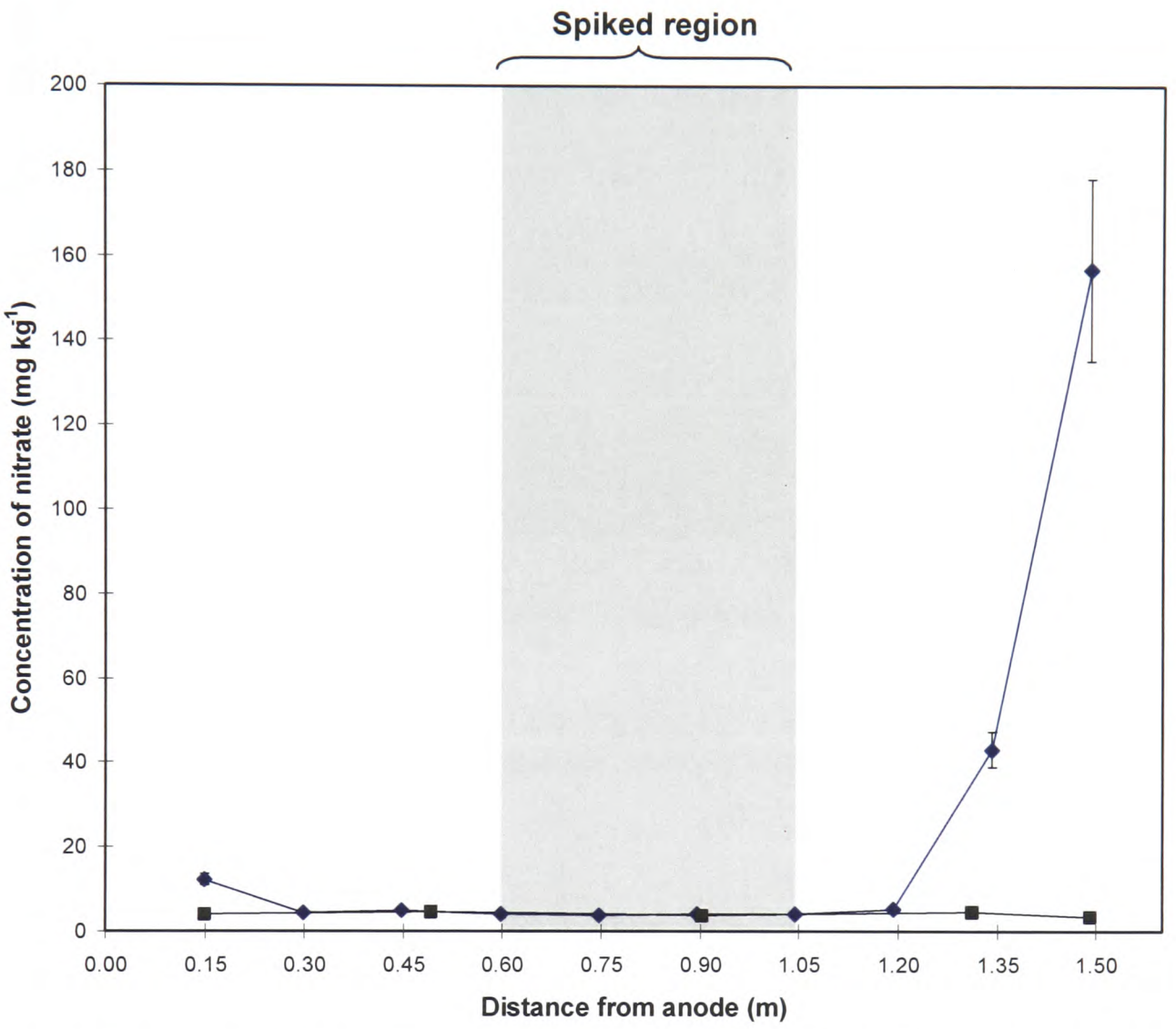


Figure 5.23 Horizontal distribution of **nitrate** (mg kg^{-1}) in field treatment cells after 133 days, ◆ electrokinetic treatment cell; ■ control cell (data are means ± SE, n = 3).

From the three samples collected NO_2^- was not observed in the soil at the beginning of the experiment (Table 5.1). After 133 days of applying an electric field (~10 mA), NO_2^- was not detected in the soil of either the electrokinetic treatment or control cell, or within the electrolyte.

Chloride was not detected in the 9 soil samples collected at day zero of the experiment (Table 5.1). After 133 days of applying an electric field (~10 mA) a peak of Cl^- was detected at 1.04 m from the anode chamber, at a concentration of 18.4 ± 7.4 mg kg^{-1} (Figure 5.24). Within the control cell Cl^- appeared to be uniformly distributed throughout the soil at a concentration of ~3.8 mg kg^{-1}.

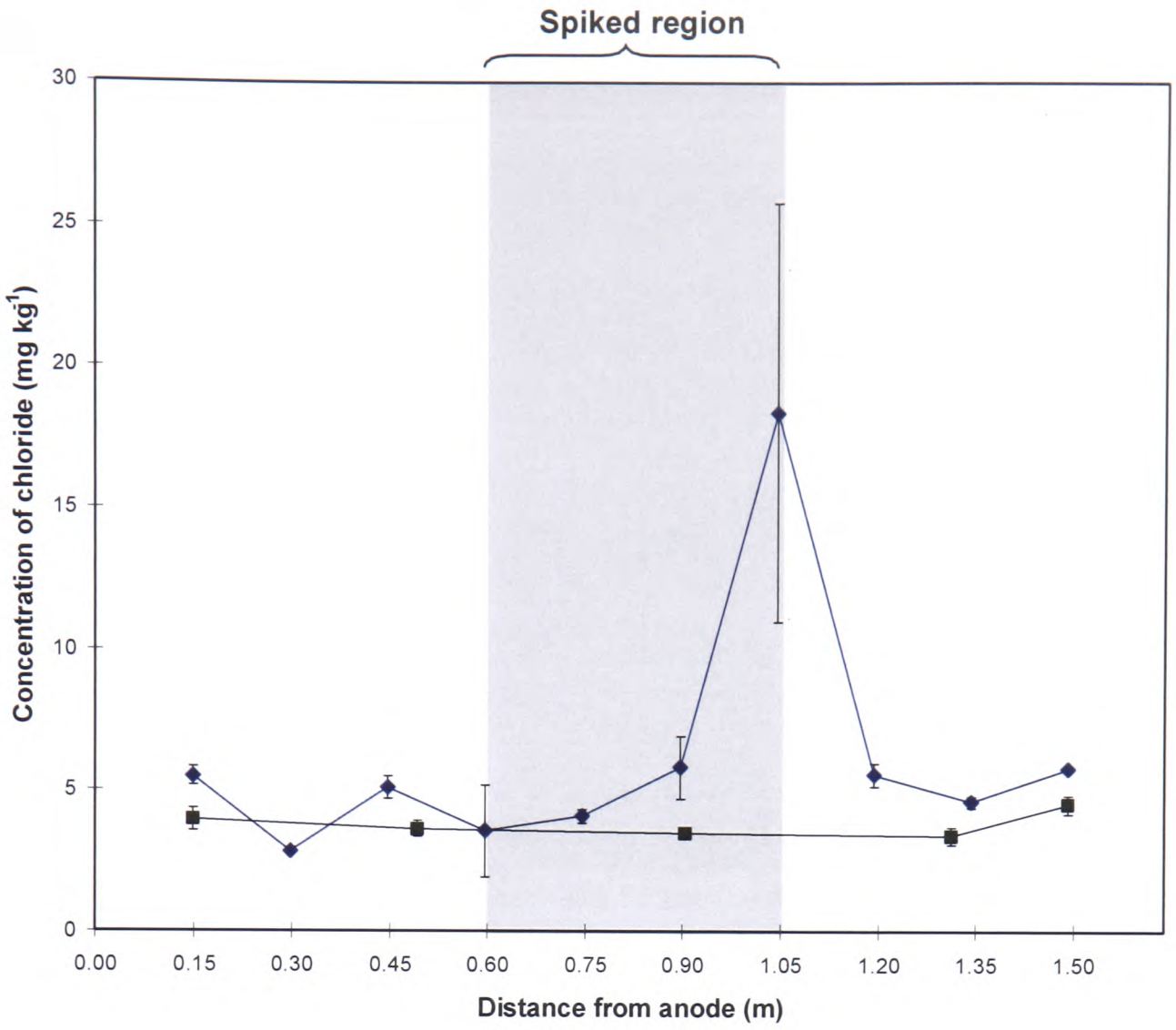


Figure 5.24 Horizontal distribution of **chloride** (mg kg^{-1}) in the field treatment cells after 133 days, ◆ electrokinetic treatment cell; ■ control cell (data are means ± SE, n = 3).

The distribution and concentration of SO_4^{2-} and PO_4^{3-} in the soil of the electrokinetic treatment and control cell appeared to be highly varaiable and there were no significant differences between the two soils (Figure 5.25 and 5.26).

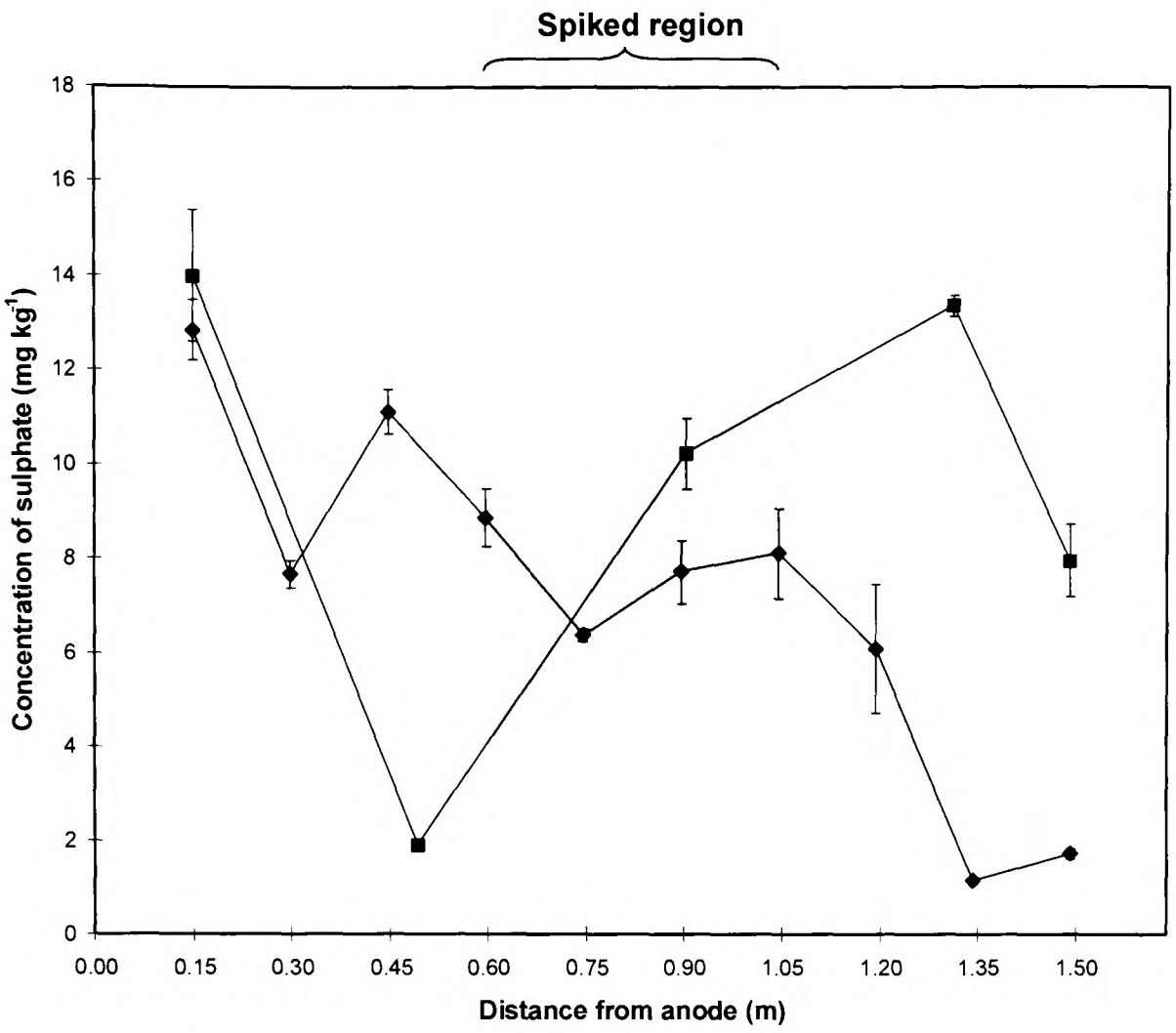


Figure 5.25 Horizontal distribution of **sulphate** (mg kg^{-1}) in the field treatment cells after 133 days, ◆ electrokinetic treatment cell; ■ control cell (data are means ± SE, n = 3).

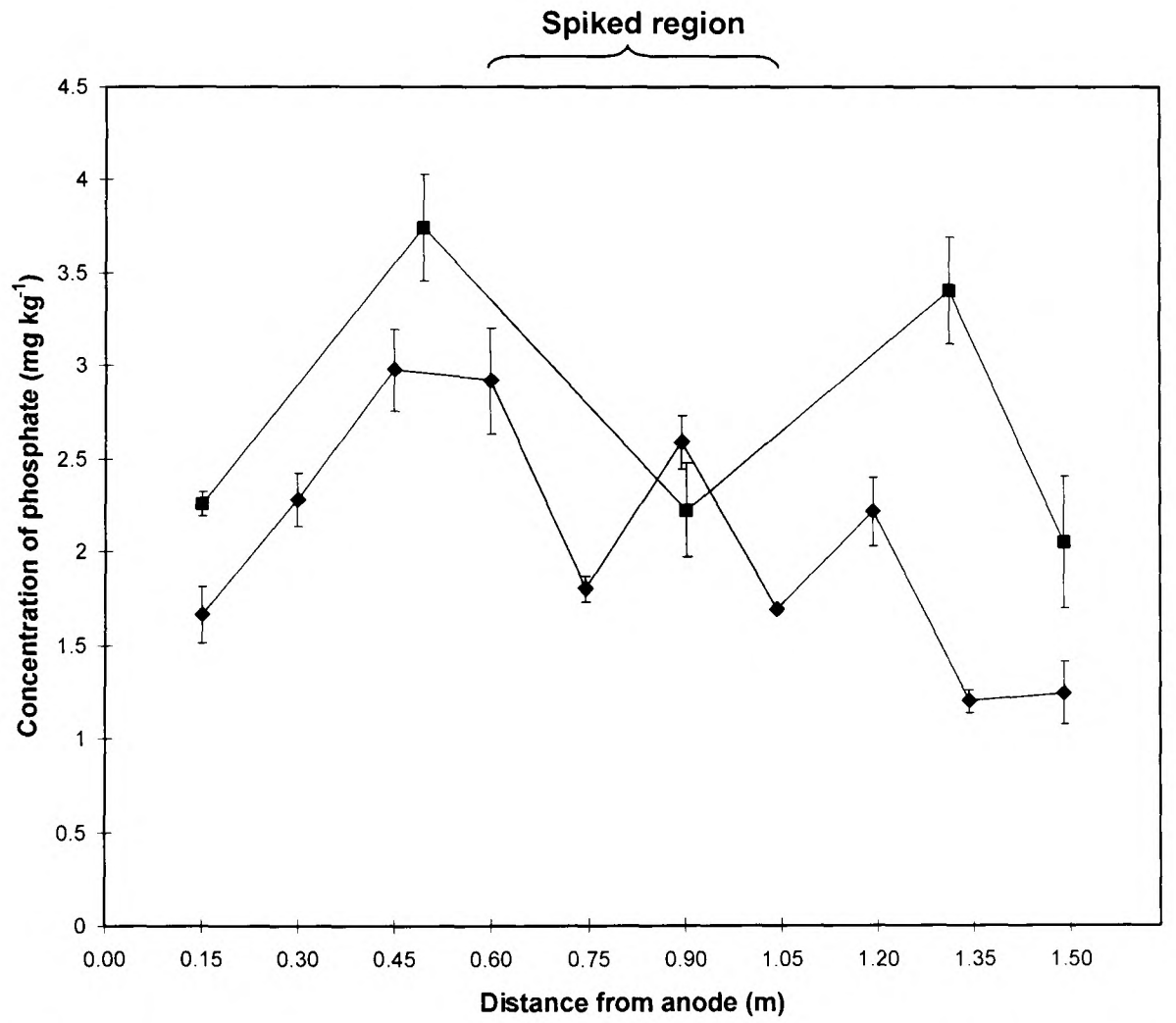


Figure 5.26 Horizontal distribution of **phosphate** (mg kg^{-1}) in the field treatment cells after 133 days, ◆ electrokinetic treatment cell; ■ control cell (data are means ± SE, n = 3).

5.5 Discussion

5.5.1 Set up of the electrokinetic treatment and control cells

The aim the investigations carried out within this chapter was to evaluate the use of electrokinetics at field-scale to remediate hydrocarbons from the sub-surface. The electrokinetic treatment and control cells were set up, operated and validated within a restricted time-frame and budget, as would be the case for any commercial application of electrokinetics.

5.5.2 Voltage, temperature, moisture content and pH distribution within treatment cells

Between day 10 and 14 of the experiment the current increased from 10 mA to 810 mA, at a constant voltage of 90 V. The elevation of the current is likely to be a consequence of a reduction in resistance, which may be related to an increase in the ionic concentration of the pore fluid upon addition of NO_3^- and PO_4^{3-} salts into the electrolyte. After the voltage was reduced to 10 V the voltage profile remained relatively linear and the current remained at approximatly 10 mA.

Based on evidence from the laboratory trials it was predicted that an increase in soil temperature would be observed upon application of the electric field. This was not observed: although there was a slight variation in temperature across the electrokinetic treatment cell (between anode and cathode), compared to the soil within the control cell and the neighbouring sub-surface (at the field site) there was no significant increase in temperature. The lower temperature observed in the electrokinetic treatment cell is considered to be linked to the relatively large surface area of soil in contact with the metal skip (which would conduct a cold temperature) as compared to the control cell, and soil in the sub-surface. Furthermore, with relatively low resistance within the soil, the influence of ohmic heating would be negligible (§ 2.5.4.1)

After 133 days of applying an electric field the moisture content within the electrokinetic treatment cell was relatively uniform between the anode and cathode, but significantly higher overall, compared with the control cell. Furthermore the pH was maintained at approximately 7. The results indicate that electrokinetics can be used to maintain soil moisture and pH, but there may be insufficient differences between the control and the

electrokinetic treatment cell to warrant the expenditure of resources to apply electrokinetics.

5.5.3 Reduction in the concentration of transformer oil from the soil

In both the electrokinetic and control cell the concentration of TPH ($C_8 - C_{35}$) was reduced. Overall, a higher percentage of TPH removal was observed in the electrokinetic treatment cell (~30%) compared to the control cell (~12%). A direct comparison between the concentration of TPH found in the electrokinetic treatment cell and the control cell were not made because of the apparent differences in the starting concentration of TPH ($C_8 - C_{35}$) and the limited number of samples that were collected from the control cell. The TPH ($C_8 - C_{35}$) concentration in the central region of the control cell, detected in the soil 42 days after the start of the experiment, was 22 514.3 ± 719.6 mg kg^{-1}, whereas the concentration in the electrokinetic treatment cell was 11 593.8 ± 1 014.1 mg kg^{-1}.The difference in the concentration of transformer oil in the spiked region between the electrokinetic treatment and control cell is likely to be attributable to heterogeneities with the distribution of contaminants in the soil. Analysis of the soil samples could not be undertaken until after the experiment had gone to completion and therefore an assumption had to be made that the TPH concentration throughout the soil was near uniform after manually mixing (with a shovel) on set-up of the cells. The volume of soil within the control cell (8% of the electrokinetic treatment cell – Appendix 8.12) was small and therefore more susceptible to 'hot spots or pockets' of contaminants skewing the data.

A significant increase in the concentration of TPH in the range $C_{12} - C_{16}$ was observed in both the electrokinetic treatment and control cell. The simultaneous increase in the concentration of shorter chain hydrocarbons, with a significant decrease in the concentration of longer chain hydrocarbons ($C_{21} - C_{35}$) could be evidence of degradation (§ 1.4.2.2 and 1.4.2.3). The increase in concentration of $C_{12} - C_{16}$ observed in the soil appeared to be consistent with the concentration of TPH. For instance, the highest concentration of $C_{12} - C_{16}$ was present in the central spiked region. However, extensive analysis for potential TPH metabolites was not undertaken.

Within the electrokinetic treatment cell it was observed that the most significant reduction in TPH (C_8 - C_{35}) was at the sampling point in close proximity to the cathode (~1.49 m from the anode) (refer to Table 5.3). It is postulated that the increased degradation/disappearance of TPH at this position may be linked to the presence of NO_3^- and PO_4^{3-} at this location (§ 5.5.4), which may have supported microbial metabolism of the

target hydrocarbons (§ 1.4). As discussed in the following section, there was evidence that NO_3^- had been delivered into the soil via electromigration, and at 133 days PO_4^{3-} was also detected in the soil.

5.5.4 The distribution and concentration of the target anions within the soil

Nitrate migration into the soil was observed under the influence of the electric field (10 mA). After 133 days elevated concentrations of NO_3^- were present ~0.30 m from the cathode chamber and in close proximity to the anode chamber (a distance of 0.30 m). Movement of anions into the soil may have occurred as a result of the hydrostatic forces exerted by the head of water within the electrode chamber, driving the ionic electrolyte into the soil. However, the forces would be similar in each of the chambers and therefore the concentration of anions distributed into the soil would be the same. This was not observed; instead there was greater than a 10-fold higher concentration of NO_3^- within the cathode region of the soil compared to the anode region, indicating that movement of NO_3^- was as a consequence of electromigration.

The concentration of Cl^- in the electrokinetic treatment cell was elevated (18.4 ± 7.4 mg kg^{-1}) at a distance of 1.04 m from the anode chamber. Chloride was not detected from samples collected immediately after the spill incident or at the start of the experiment (day 0). It is postulated that the elevated concentration of Cl^- may be attributed either to the presence of Cl^- in the mains water (117.3 ± 0.3 mg l^{-1}) that was delivered into the soil via electromigration or is present as a result of degradation of a chlorinated contaminant. As discussed (§ 1.2.1) PCBs (not included in the analysis suite) were historically used as a constituent of transformer oil and it is therefore feasible that they would be present in the contaminated soil and subsequently degrade. A definitive conclusion could not be drawn because of the number of complex variables. Further study is warranted, with the use of deionised water as the electrolyte and analysis for chlorinated hydrocarbons performed for the soil samples. It was not possible to calculate a mass balance to compare the concentration of anions added to those present in the electrolyte at the end of the experiment as on a number of occasions snow and ice resulted in the feed-pipes becoming dislodged resulting in loss of electrolyte solution.

There was no significant difference between the concentration and distribution of PO_4^{3-} and SO_4^{2-} detected in the electrokinetic treatment cell and the control cell on day zero. Both PO_4^{3-} and SO_4^{2-} were not detected in the soil at day zero of the experiment. The presence of PO_4^{3-} and SO_4^{2-} in the soil of the control cell after 133 days is likely to be as a

result of rainwater entering the control skip during the experiment, as discussed in Section 5.5.5.

5.5.5 Operational difficulties and limitations

The endpoint of the field-trial was determined by both operational and financial constraints. There had to be a cut-off between the number of samples to be taken to maximise the effectiveness of the study, whilst recognising that discrete sampling may not give a sufficient resolution. The heterogeneity of soil samples introduces a degree of uncertainty when analysing the results. For instance, at 0.89 m from the anode in the electrokinetic treatment cell the TPH concentration was higher on day 42 compared with day 14 of the experiment. Furthermore, the error bars, for the TPH analysis of soil samples collected at 0.15 m from the anode on day 42 are large (± 31%). A larger sample size would improve confidence levels with the data, but there is always a limitation in the number of samples that can feasibly collected and analysed.

At periods during the experiment the temperature fell below freezing and subsequently problems arose with the peristaltic tubing, which was designed predominantly for laboratory use. The use of an insulated cool box as the central reservoir provided some protection against the electrolyte becoming frozen but further amendments, such as lagging of pipes for future 'Winter-time' trials, would be required.

Throughout the experiment a layer of algae (slime) was observed to have developed on the surface of the electrodes (Appendix 8.13). Although the presence of a slime layer did not appear to affect the integrity of the Daramic membrane it is likely that with longer duration experiments this could contribute to deterioration of the performance of the electrodes. The electrodes were full of nutrient rich electrolyte providing the right conditions for growth. As stated in other studies that have investigated the use of biostimulation to enhance biodegradation (*e.g.* Bewley and Webb, 2001), nutrient addition can result in biofouling around the injection wells as well as blocking the pores of the soil resulting in reduced permeability of the soil, and thus inhibiting further penetration of nutrients into the contaminated soil. To remedy this, nutrient addition can be added in batches rather than continually overwhelming the soil system, which can result in eutrophication.

As discussed (§ 5.3.5), the size of the control cell was restricted by the quantity of contaminated soil available and therefore the control was not an exact replica of the electrokinetic treatment cell. This limits the value of the control because of the

introduction of more variables, such as a higher chance of sampling a contamination 'hotspot or pocket' and a difference in the surface area exposed to the air. Additionally, contractors working at the transformer site had access to the skip, and despite clear signage prohibiting interference, the tarpaulin was pulled back and items were placed into the skip during the course of the experiment. For a maximum period of 6-days, the soil within the control cell was left exposed to the environment.

5.6 Summary of findings

1. A field-scale electrokinetic treatment cell was successfully set up at a 132 kV transformer site to investigate the feasibility of remediating hydrocarbons from soils (§ 1.7, Objective 5). The field-scale experiment was run for 133 days with a current of 10 mA applied across the treatment cell.

2. Feasibility studies, used to examine the potential to use surfactants in the test soil highlighted that the surfactants impeded the hydraulic conductivity of the soil (§ 5.3.2.1). Consequently, as only one control cell could be run the use of surfactants to facilitate remediation was not investigated during the field trial.

3. The moisture content and pH of the soil at day zero were conducive for biodegradation to occur within the treatment cell, but the principal macronutrients, N and P, were not detected in the soil obtained from the contaminated site. Soil moisture content and pH were maintained for the duration of the experiment (133 days) in both the control and electrokinetic treatment cells.

4. There was no evidence in the field that electrokinetics at low voltage could be used to raise the soil temperature. The low resistance of the soil and exposure of the soil to the cold metal work of the skip appeared to limit the effect of ohmic heating on the soil temperature in the field.

5. The migration of NO_3^- into the soil under the influence of the electric field (~10 mA) was observed. At day 133 elevated concentrations of NO_3^- were detected at distances of 0.15, 1.34 and 1.49 from the anode chamber.

6. In close proximity to the cathode chamber (1.49 m from the anode chamber) a significant reduction in the concentration of TPH (C_8 – C_{35}) was observed between the soil sampled on day 42 and day 133. This decrease in TPH concentrations coincided with a significant increase in NO_3^- concentration at 1.49 m from the anode. In the control cell no significant difference in the disappearance of TPH was observed, providing evidence of electrokinetic enhanced biodegradation.

6 Conclusions

6.1 Introduction

The clean-up of hydrocarbon contamination from the subsurface poses a significant engineering challenge where the contamination lies beneath buildings, or other infrastructure such as electricity transformers. The high costs and the level of disruption caused by conventional remediation methods, such as 'dig and dump', provide a driving force for the development of alternative remediation technologies and strategies. Bioremediation, when used within a risk-based framework, can offer cost-effective and sustainable remediation strategies. However, at present there is a large gap between bioremediation research undertaken in the laboratory and introducing the technologies into the field (Randall, 1999). Predictions of the success of bioremediation, and timeframes for clean-up determined in the laboratory, do not translate well to field-scale (Roote, 1997). There has been an increase in the commercial use of bioremediation products *in situ*. However, many of these products are used without sufficient understanding by the operator of the mechanisms of remediation, and they are therefore applied inappropriately without adequate monitoring. Consequently, there is a risk that the credibility of bioremediation as an *in situ* remediation technology will be jeopardised before techniques are fully validated (Carter *et al.*, 2006).

This study evaluated the use of electrokinetics coupled with biodegradation to remediate hydrocarbons from sub-surface soils. To summarise, the key investigations carried out within this study included: use of electroosmosis as an *in situ* flushing technique; use of surfactants to aid electroosmosis in remediating hydrocarbons from the sub-surface; and the use of electrokinetics to enhance biodegradation, by using electrokinetics to deliver nutrients/ electron acceptors and to remove toxic metabolites. Figure 6.1 illustrates the decision tree developed in this study for the use of electrokinetics to bioremediate hydrocarbon contamination, based on the objectives defined in Chapter one (§ 7.1). The study progressed in two principal stages, commencing with laboratory studies and progressing to an *ex situ* on-site field trial.

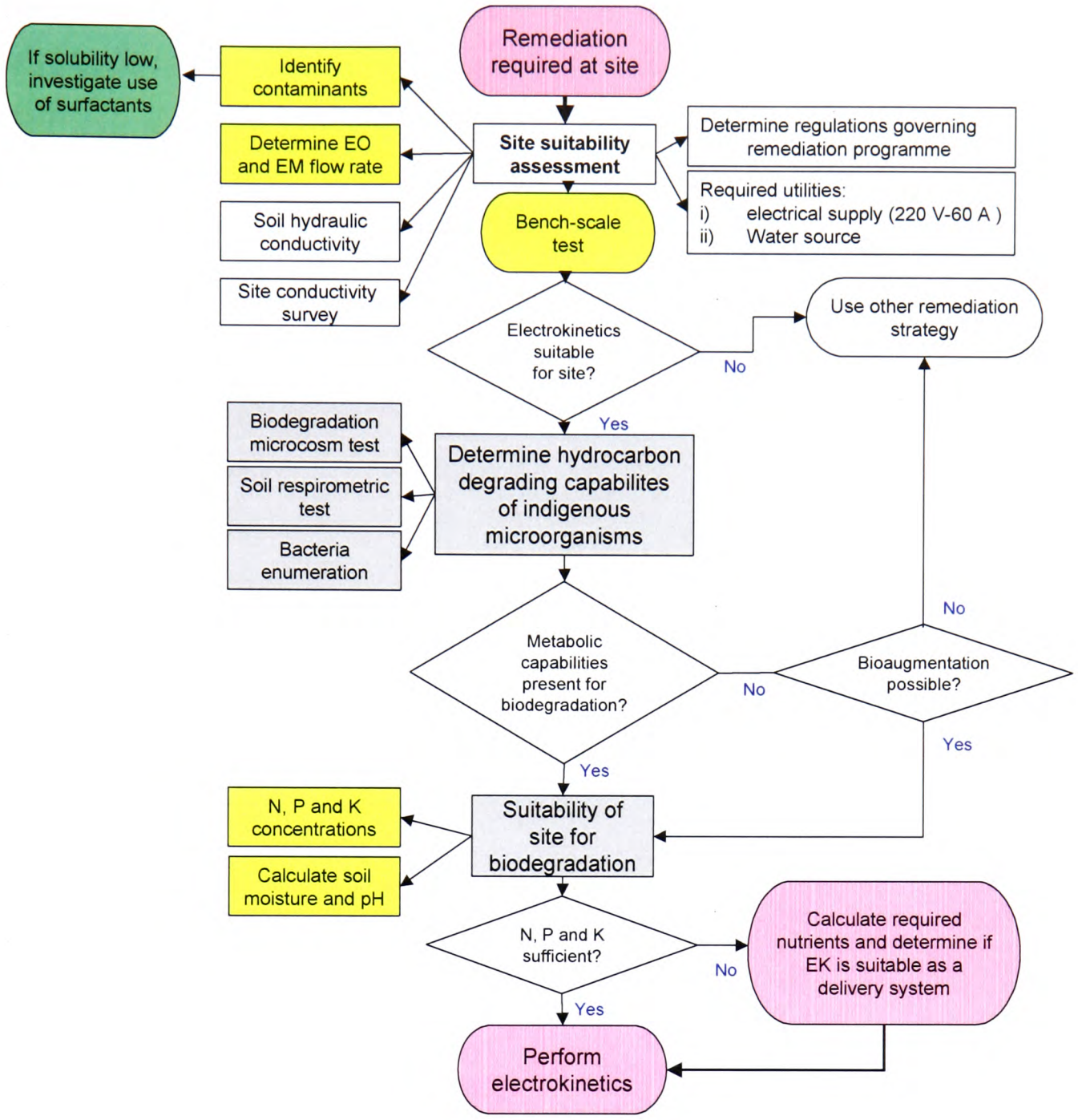


Figure 6.1 Selection flow chart to determine site suitability to electrokinetic remediation. Chapter two; Chapter three Chapter four; Chapter five.

6.2 The use of electrokinetics as an *in situ* flushing technique

Electrokinetics was evaluated for its potential to be used as an *in situ* flushing technique. In normal circumstances, under the influence of an electric field, electroosmosis results in the movement of water from the anode towards the cathode. It was postulated that the movement of water may be sufficient to mobilise hydrocarbons from the soil. Hydrocarbons are hydrophobic, so feasibility studies were undertaken to investigate their likely partitioning behaviour between soil and water, and the potential to mobilise contaminants from a soil column under the pressure of an hydraulic gradient (Chapter 2). It was determined from the feasibility studies that the target contaminants (dodecane, xylene [*p*-, *m*- and *o*-] and tetradecane) partitioned from the soil to the water phase, but this was considered to be a result of the concentration of contaminant used exceeding the

retention capacity of the soil. Where mobilisation of the excess contaminant occurred through the soil, upon entering the adjacent clean soil (at the peripheries of the spiked region) immediate sorption appeared to prevent further flushing through the soil. There was evidence that electroosmosis could be used to facilitate the flushing of the more soluble and more toxic contaminants, including xylenes, 3-chlorobiphenyl and toluene (§ 2.5.3.1), through the soil. However, the use of electroosmosis to flush the straight chain hydrocarbons (including octadecylbenzene) through the soil did not prove to be successful.

6.2.1 The use of surfactants with electrokinetics

In Chapter 3, surfactants were investigated to determine whether they could promote the movement of the target contaminants under the influence of electroosmosis by increasing the concentration of contaminants in the aqueous phase. The delivery of the anionic surfactant SDS into, and through, the soil was achieved by both electroosmosis to the cathode and electromigration towards the anode. Mobilisation of the non-ionic surfactants (Triton X-100 and Span 85) occurred under the influence of electroosmosis towards the cathode. The enhanced delivery of surfactants into the sub-surface has potential application in remediating contaminants from soil when the sub-surface can not be directly accessed. In soils with low hydraulic conductivity, electrokinetic delivery could offer an effective alternative to ineffective delivery techniques, such as direct application to the surface of the soil.

The inclusion of surfactants with the electrokinetics system did not significantly enhance mobilisation of the target contaminants through the soil. However, both anionic and non-ionic surfactants promoted the dissolution of the target contaminants from the spiked region into the adjacent soil (§ 3.5.2.3), which is likely to further increase bioavailability of the contaminants. The enhanced degradation/disappearance of the target contaminants compared with experiments without surfactants provides evidence of enhanced biodegradation.

As highlighted in the laboratory-based experiments, it is necessary to undertake feasibility studies to assess the risk of surfactants causing detrimental effects on the condition of the sub-surface, inducing changes such as swelling and cracking of clay-rich soils (§ 3.4.3.1, Figure 3.11). Furthermore, it is essential that if surfactants are used to promote the movement of contaminants into the aqueous phase, appropriate measures are in place to ensure contaminants do not migrate from the treatment site.

6.3 Using electrokinetics to promote the degradation of hydrocarbons

Although mobilisation of the straight chain hydrocarbons was not achieved using electroosmosis as a flushing technique, there was evidence that the application of the electric field promoted the removal of the target contaminants from the soil. In 14-day experiments, all of the principal target contaminants were depleted from the soil to a greater extent under the influence of the electric field (~10 mA) compared to the control (§ 2.5.3.2). It is hypothesised that the increased disappearance is attributed to enhanced biodegradation by increased bioavailability and a rise in the soil temperature, through ohmic heating. Electrokinetics may increase bioavailability by promoting the removal of hydrophobic contaminants from small soil pores, where they are discrete from the degraders (Harbottle, 2003; Lear, 2004). A relatively small distance of movement from within intra-aggregate pore spaces (nm to μm pore size Ø) can increase the interfacial contact between the degraders (average Ø 0.2 – 2.0 μm) and the contaminants, and thus increase the efficiency of biodegradation (Harms and Wick, 2006). With regard to the increase in soil temperature, upon application of the electric field, microcosm experiments have demonstrated that a small increase in temperature can significantly enhance the degradation of the target contaminants (§ 4.6.2.3).

The feasibility of delivering anionic nutrients into the soil to enhance biodegradation was investigated (Chapter 4). Under the influence of electromigration, NO_3^- and PO_4^{3-} were delivered into and through the soil. As well as being used as a macronutrient there was some limited evidence suggesting that NO_3^- was utilised as an alternative electron acceptor to oxygen in the degradation of hydrocarbons from the spiked region. Further investigation is required to evaluate the injection of the inorganic nutrients with their utilisation in biodegradation. This is of importance to ensure compliance with environmental legislation, especially with regard to nitrate concentrations. In addition, further investigation is required to assess the balance between adding NO_3^- to enhance biodegradation and the potential to inhibit biodegradation via increased toxicity and the effects of eutrophication. The use of electrokinetics to deliver charged chemical oxidants into soil with a low hydraulic conductivity, has also been shown to be feasible (§ 4.6.6).

Electromigration was successfully used in this study for the mobilisation of potentially toxic metabolites. During the study it was observed that 3-chlorobenzoic acid could be mobilised through the soil. As discussed in Chapter 4 (§ 4.7.4), the mobilisation of metabolites that inhibit further biodegradation of the target contaminant could potentially be of significant use for sustaining *in situ* biodegradation.

6.4 Electrokinetics field-trial

The laboratory-scale experiments were invaluable for identifying and overcoming engineering challenges before the field-scale apparatus was designed and set up. For example, the method of delivering the electrolyte solution to the central reservoir by gravity ensured that flooding did not occur, and recirculation enabled nutrients to be recycled, and removed the requirement to add a buffer solution. As discussed in detail (§ 5.5), the field-scale experiment gave evidence that NO_3^- could be delivered into the soil under the influence of an electric field. The distance of anion movement through the soil in 133 days appeared to be relatively short. However, it was observed that in the soil where NO_3^- had been successfully delivered, and PO_4^{3-} was present, the removal of transformer oil was enhanced. Overall, the field trial achieved its targets on a relatively small budget and within a short time period. For further investigations, the creation of a working group would be beneficial. For instance, for the Lasagna™ trials, a consortium of five companies/research organisations was in place (Ho *et al.*, 1995), which increases the potential scope of the investigations.

6.5 Limitations to the laboratory and field experiments

6.5.1 Following the movement of contaminants in the electrokinetic cells

One of the principal limitations with the electrokinetics experiments at laboratory and field-scale was the inability to detect small movements of hydrocarbons through the soil. It is likely that biodegradation may have been faster than the partition rate of the contaminants through the soil under the influence of the electric field. Therefore contaminants may have been removed from the soil before their movement was detected by subsequent sampling and analysis. This may have been overcome by the use of abiotic layers (sand) either side of the spiked region so that minimal movement could be detected over and above biodegradation of the target contaminants (the principle of surrounding a spiked area with abiotic regions has been previously described in the literature [Ho *et al.*, 1995]).

6.5.2 The study of biodegradation

Throughout the study an emphasis was placed on analysis of the functional capabilities of the soil as a whole ('pseudo-unit') rather than following individual components, such as changes to microbial communities or the effects of the electric field on the physiology of the bacteria. Length heterogeneity – PCR (LH-PCR) was used in a short pilot study to assess the community structure within two samples of Reading soil: the first sample was

collected from the spiked region of a 14-day electrokinetics experiment soil chamber, and the second from the spiked region of a control soil chamber after 14 days. The method and results are detailed in Appendix 8.14, and demonstrate the potential for microbial community changes to be observed under the influence of the electric field. With regard to changes to the physiology of the bacteria under the influence of the electric field, there other studies have been undertaken (as reviewed by Weaver and Chizmadzhev, 1996) that have provided evidence that the electric field can create reversible pores in the lipid bilayer of bacterial membranes, which may increase the efficiency of nutrient uptake.

There was no differentiation made between the biodegradative actions of either fungi or bacteria. Within the soil chamber experiments (Chapters 2 and 3), the presence of fungi was evident in the spiked region of the soil, with and without the presence of the electric field, providing evidence of their involvement at the 'spiked' region (Appendix 8.15). It is likely that the fungi are capable of using the target contaminants as a carbon source, but their presence does not provide conclusive confirmation. For example, bacterial proliferation or modifications in the soil structure in the 'spiked' region may also promote fungal growth. The hydrocarbon degrading capabilities of some fungi (*e.g.* white rot fungi) have been demonstrated in both laboratory and field-studies (*e.g.* Shah *et al.*, 1992).

The laboratory experiments carried out in this study involved the use of spiked soils. It is recognised that contaminants that have been present in the soil for long periods of time, in excess of one month, are likely to have become sequestered within the soil aggregates, dramatically decreasing their readiness to enter the aqueous phase. Predicting the effect of electromigration and soil flushing on aged contaminants involves a large number of variables (as discussed by Jennings and Mansharamani, 1999). The main benefit of spiking soils for each experiment is reproducibility, due to a known and consistent starting concentration. Within aged soils the contamination is more likely to be heterogeneously distributed and the starting concentration variable. However, in some instances, for example following recent oil spill incidents, the contamination may be analogous to spiked soils, with remedial works being initiated within weeks of the spill incident occurring.

Several experiments were performed with radio-labelled toluene in an attempt to determine the fate of the contaminant in the electrokinetics soil chamber compared with a control. Although a small fraction of radio-labelled CO_2 was detected in CO_2 traps (a 1 M NaOH solution) indicating that biodegradation had occurred, the mass balance of carbon in the soil system gave inconclusive results. Investigating the link between the degree and rate of

biodegradation of hydrocarbons versus volatilisation, in the presence of the electric field warrants further investigation.

6.5.3 The influence of the hydraulic gradient

The electrokinetics chambers used, at both laboratory and field-scale, were closed systems without an hydraulic gradient. In the field-scale installation, the only sources of hydraulic gradient were electroosmosis and the hydraulic pressure exerted from the height of fluid in the electrode chambers. Consequently, the effect of an hydraulic gradient on the introduction of enhancing agents into the sub-surface and/or the mobilisation of the hydrocarbons has not been examined. The selectively permeable Daramic membrane, used in the electrode chambers, prevented the passage of water through it in the absence of an electric current. Therefore, even the addition of a larger head of water in the electrode chambers was insufficient to reproduce the effects of an hydraulic gradient in the soil. A complete change to the design of the electrokinetics equipment would be necessary to assess the influence of the hydraulic gradient on the electromigration of enhancing agents.

6.5.4 Limitations to the number of variables investigated

There are numerous variables that exist in soil systems, and many of these are difficult to control in laboratory and (especially) field- scale experiments. Inevitably, there is a degree of uncertainty as to the observed outcome, but this can be overcome by increasing the number of repeat experiments performed. In the case of the field-trial, repeat experiments could not be undertaken due to time and cost restrictions. Furthermore, there were many intrinsic complex factors that may have influenced the outcome of the electrokinetics experiments that could not be monitored experimentally in this study. For instance, Rayner *et al.* (1997) report that wet soil can act as an electrochemical solid bed reactor, with soil acting as a microconductor surface with direct oxidation and reduction reactions occurring at its surfaces. In addition to processes induced by the electric field, other chemical reactions and movement mechanisms may occur concurrently in the soil, including dissolution-precipitation reactions, sorption-desorption, redox reactions and movement through passive hydraulic advection and dispersion (Reed *et al.*, 1996; Rayner *et al.*, 1997; Yu and Neretnieks, 1997a).

In the field-scale experiment, there were many variables that could not be investigated within the scope of the experiment because of the restricted budget (as discussed § 5.5.5). For instance, the effect of the electric field on the distribution of metals in the soil, and their subsequent impact on the degradation of target contaminants was not examined and

could be the subject of further investigation. For example, there were large concentrations of Fe observed in several of the initial soil samples taken from the site (Appendix 8.10). Fe is a catalytically active transition metal and therefore could have an influence on the redox processes of hydrocarbon degradation (Rayner *et al.*, 1997).

6.6 Areas for further research

6.6.1 Overcoming the obstacles to applying electrokinetics *in situ*

There are few studies that consider the effect of sub-surface remedial technologies on the integrity of structures and underground services present at contaminated sites. This is not only relevant to electrokinetics but to other technologies, such as chemical oxidation (§ 4.3), the addition of surfactants (Chapter 3), and to any technology that has the potential to change the structure of the sub-surface. Before remediation can be undertaken beneath and in the vicinity of sensitive structures, a comprehensive understanding on the impact of the remedial technology on the sub-surface should be attained. As discussed (§ 1.6.7.1), the presence of conducting materials within the sub-surface is likely to have a detrimental effect on the application of the electric field, with the current preferentially flowing along the conducting material rather than through the soil. In addition, insulating materials, such as wooden beams, may result in restricted current flow.

In situ biodegradation is a site-specific process and therefore treatability tests should be carried out prior to initiating bioremediation at a contaminated site to ensure the technology is appropriate for use. However, it is not always practical, for example in the case of bioaugmentation, to tailor bacteria for every individual site because of financial and time constraints (Thompson *et al.*, 2005). There remains a requirement for a field-tool that makes it possible to evaluate the *in situ* metabolic capabilities of microorganisms indigenous to a contaminated site to determine if biodegradation is a viable option for remediation of that site. A pilot study was undertaken to assess the use of MT2 Plates (Biolog, Oxoid, Hampshire, UK) to be used as a rapid assay for assessing the hydrocarbon-degrading capabilities of microorganisms indigenous to a contaminated site (refer to Appendix 8.16 for the method). However, the limited aqueous solubility of the target contaminants limited the success of the technique and the hydrocarbons reacted chemically with the plastic 96-well plate.

6.6.1.1 The installation of electrodes in the sub-surface

Planar electrodes were used in the electrokinetic treatment cell. It is far easier to ensure that current is distributed uniformly with the cathodes and anodes parallel to each other, compared with tubular electrodes. However, it has been discussed that tubular electrodes may be best suited for installation *in situ* (G Sunderland, C-Tech Innovation, Pers. Comm. 2004). Tubular electrodes could be inserted into the ground utilising already inserted boreholes or alternatively, with the use of an auger. With tubular electrodes, the geometrical relationship of the anode with respect to the cathodes needs to be considered to ensure that there are no 'dead' areas, where there is no flow of current. Murdoch and Chen (1997) investigated the use of hydraulic fractures to insert conductive carbon chips at depths of between 1 and 3.5 m, and hydraulic fractures were used to install the electrodes of the Lasagna process (refer to § 1.6.6). Furthermore, an hydraulic-driven mole, as used for the installation of water mains, could be adapted to create conduits through the soil without having to excavate the sub-surface. This technique may be used for the installation of horizontal electrodes.

6.6.2 The use of electrokinetics to remediate other contaminants

This study has focused on the remediation of kerosene and transformer oil from soil, but there is great potential for electrokinetics to be used for the clean-up of many other organic contaminants. For example, it may be feasible for electrokinetics to be used to remediate nitroaromatic compounds. Nitroaromatics are common environmental contaminants because of their use in munitions, insecticides, herbicides, pharmaceuticals, and as industrial feedstock chemicals for dyes and plastics (Achnich *et al.*, 1999). For example 2,4,6-trinitrotoluene (TNT) has a reported aqueous solubility of 86 mg l^{-1} at 13.9°C and 115 mg l^{-1} at 23°C (Phelan and Barnett, 2001), which may be sufficient for electroosmosis to be used as an *in situ* flushing technique. Within this study the electroosmotic mobilisation of toluene, xylene and 3-CB through the soil was achieved and their solubilities are approximately 530, 164, and 1.3 mg l^{-1}, respectively (Table 2.1).

In addition, electrokinetics may be used to enhance and sustain biodegradation of TNT by avoiding the build-up of toxic metabolites. It has been reported (*e.g.* Chew & Zhang, 1998) that the accumulation of NO_3^- and NO_2^- can be toxic to the microbial degraders and therefore the metabolites eventually prevent further degradation of the parent compound. Metabolite repression has also been noted to negatively affect the degradation of benzo[*a*]pyrene and dibenz[*a,h*]anthracene (Juhasz *et al.*, 2002) and is therefore an area

that would merit further study to identify whether electrokinetics could be used to sustain biodegradation.

6.6.2.1 The use of electrokinetics to treat waste materials

The disposal of building materials such as plasterboard is becoming significantly expensive following the ban of its disposal in landfills with biodegradable waste in July 2005 (Defra, 2006). Plasterboard consists of approximately 21% SO_2^{2-} and in the reducing conditions of a landfill may form the toxic gas hydrogen sulphide (H_2S). Electrokinetics could be used to remove the SO_2^{2-} from chipped plasterboard so that the waste material can be disposed of in landfill. Furthermore, electroremediation could feasibly be investigated for its use to remediate SO_2^{2-} from landfills that have historically accepted large volumes of plasterboard waste. Leachate from landfills is a particular environmental problem and electrokinetics may be used to strip ionic contaminants (both inorganic and organic) from the wastewater to reduce its impact on the environment.

6.6.3 The use of electrokinetics to enhance other remediation technologies

From both the literature review and investigations carried out in this study it has become clear that many of the innovative remediation technologies are being developed in isolation. Frequently, it can be seen that one technology may complement another but, as the operator and the scientist are so often separate entities, further progress is not made. Through this Industrial Case Studentship the impetus of the research has been to investigate electrokinetics in a way that gives insight to the underlying principles and how these can be exploited to remediate hydrocarbon contaminated land in the field. Electrokinetics should not simply be regarded as a technology to be used in isolation but should be used as a tool, and as such used alongside other remediation technologies where appropriate.

It was recognised within this study that electrokinetics as a remediation strategy may offer a cost effective, efficient solution when used as a hybrid process, combining both biological and engineering based technologies. For example, the incorporation of electrokinetics with techniques such as landfarming and biopiling could significantly improve the efficiency of contaminant remediation. Biopiles and landfarms (§ 1.3.2) involve the *ex situ* treatment of contaminated soil by enhancing biodegradation by controlling the moisture content, pH, aeration, temperature and nutrient concentrations. Conventionally, nutrients are either sprayed on or are added in granule form at the

beginning of the treatment process. Penetration of the nutrients into the biopile relies on diffusion into the soil, which is subsequently dependent on the permeability of the soil. Electrokinetics could feasibly be used for simultaneously delivering nutrients and electron acceptors, heating the soil, and controlling the pH and moisture content (as identified in Chapters 2 and 4). One of the limitations of using landfarms in the UK is the air temperature and at present the use of landfarms is restricted to the summer months (Prandi and Di Louise, 2004). Unfortunately, it is during the cold months within the UK (October to March) that the highest number of domestic heating oil spills occur.

6.7 Electrokinetic bioremediation as a viable remediation strategy

The evidence gathered in this study indicates that electrokinetics can facilitate biodegradation by reducing/eliminating the spatial separation between microorganisms, the target contaminants, and nutrients. In addition, it has been demonstrated that electrokinetics may be used for delivering enhancing agents to promote degradation, and also to remove toxic metabolites to sustain biodegradation in the sub-surface. However, before electrokinetics can be used effectively *in situ*, further feasibility studies need to be undertaken to fully establish the link between the delivery of enhancing agents and facilitating biodegradation in the sub-surface. Engineering obstacles must also be overcome, such as the presence of conducting materials in the sub-surface.

As with many innovative remedial technologies, little progress has been made in the use of electrokinetics to remediate soils *in situ* since its first successful application in the field in 1987 by Lageman in the Netherlands. At present, there are a limited number of incentives for the use of electrokinetics over conventional remediation techniques. For instance, the practice of excavating contaminated land and sending the waste to landfill ('dig and dump') can be operated within a well-defined time scale, and there is an immediate transfer of liability as the contamination is removed from the site. Conversely, it is difficult to predict the time required for the completion of an *in situ* bioremediation programme even with the use of optimised conceptual and numerical models. Furthermore, there are inherent uncertainties involved with *in situ* electrokinetic bioremediation including the presence of unidentified conduits and services (which could act as preferential pathways for the electrical current, or provide pathways for contamination to migrate off-site), and potential limitations of the indigenous microorganisms to degrade the contaminants to an acceptable end-point. Alternative pathways and contamination hot spots can be easily identified using a 'dig and dump'

approach through excavation of the impacted site, but for less disruptive bioremediation techniques these may go unnoticed.

Electrokinetics may be chosen over conventional remediation techniques where remediation of the contamination is logistically difficult, and consequently expensive. For example, there are no landfills in Ireland or on the Isle of Wight that accept hazardous soils, and it is therefore necessary to transport soils by ferry and road to the nearest landfill. It is considered that in this scenario enhanced biodegradation may offer a suitable alternative solution. Within Ireland treatment centres have been established for processing contaminated soils, and there is already a significant market for *in situ* bioremediation, using commercially available products.

Prior to the implementation of any remediation strategy at a contaminated site it is essential that an assessment is made to evaluate the likelihood of reaching an acceptable end-point. A full site investigation with regard to hydrogeology (hydraulic conductivity, permeability, pH, conductivity, depth to the water table, rate and direction of groundwater flow, the size of the aquifer and sensitive receptors) is required, followed by detailed analysis of the contaminants and site history, free product recovery, and a determination of the nutrient requirements (Thomas and Ward, 1989; Will, 1995). It may not be cost effective, or an attractive option, for landowners and/or insurers to invest time and money to undertake feasibility studies to determine whether electrokinetic bioremediation is applicable at a given site. However, through collaboration with EDF Energy it was evident that a large number of their sites are fundamentally similar. Across the company the layout of sites, the type of contaminants, and underground services is relatively generic. It may therefore be feasible in the future to invest time and money to develop an electrokinetic system for a common set of parameters, which can then be used effectively to remediate contaminants from numerous sites with similar characteristics.

It is hoped that this study, with its emphasis on both laboratory and field-scale experiments, will serve as an impetus for further development of electrokinetics as a useful tool for enhancing the *in situ* bioremediation of a range of contaminants.

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Appendix 8.1 LQM CIEH generic assessment criteria for petroleum hydrocarbons for the residential with plant uptake land-use.

Standard Land Use	LQM CIEH Generic Assessment Criteria (mg kg^{-1} dry weight soil)		
	1% SOM	2.5% SOM	5% SOM
Aliphatic			
EC 5-6	2.11	3.72	6.38
EC >6-8	5.37	11.9	22.7
EC >8-10	1.46	3.55	7.00
EC >10-12	8.53	20.8	40.1
EC >12-16	40.7	93.4	163
EC >16-35	16400	16400	16300
EC >35-44	16400	16400	16300
Aromatic			
EC 5-7 (benzene)	57.5	1.33	2.57
EC >7-8 (toluene)	62.4	1.46	2.85
EC >8-10	1.09	2.67	5.30
EC >10-12	1.94	4.76	9.44
EC >12-16	2.19	5.39	10.7
EC >16-21	115	132	133
EC >21-35	157	161	157
EC >35-44	157	161	157
Aliphatic and Aromatic			
EC >44-70	174	179	174

Reference: Nathanail *et al.*, 2006 – Generic assessment criteria for human health risk assessment, Land Quality Press.

SOM = Soil organic matter

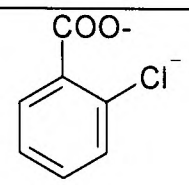
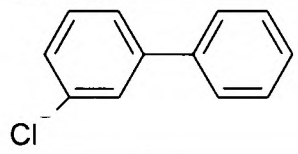
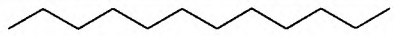
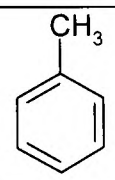
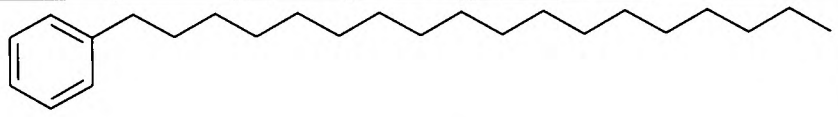
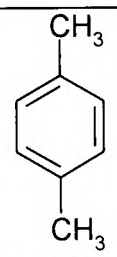
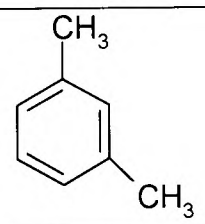
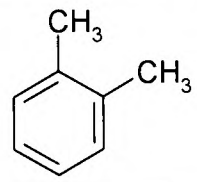
Appendix 8.2 Constituents of kerosene.

Compound class	Carbon Number	Compound
Alkyl-monoaromatics	10	1,2,3,4-Teramethylbenzene
Branched alkanes	10	Isodecane
	11	Isoundecane
	12	Isododecane
	13	Isotridecane
	14	Isotetradecane
Diaromatics (Except naphthalenes)	13	Fluorene
Monoaromatics	9	Indene
	10	Tetralin
	11	1-Methyltetralin
	11	2-Methyltetralin
n-Alkanes	7	n-Heptane
	8	n-Octane
	9	n-Nonane
	10	n-Decane
	11	n-Undecane
	12	n-Dodecane
	13	n-Tridecane
	14	n-Tetradecane
	15	n-Pentadecane
	16	n-Hexadecane
	17	n-Heptadecane
	18	n-Octadecane
	19	n-Nonadecane
	20	n-Eicosane
	21	n-Heneicosane
Naphthalenes	10	Naphthalene
	11	1-Methylnaphthalene
	11	2- Methylnaphthalene
	12	1,4-Dimethylnaphthalene
Polycyclic aromatic hydrocarbon (PAH)	12	Acenaphthene
	12	Acenaphthylene
	12	Anthracene
	14	Phenanthrene
	15	2-methylanthracene
	16	9,10-Dimethylanthracene
	16	Fluoranthene
	16	Pyrene
	17	2,3-Benzofluorene
	17	Benzo(a)fluorine
	20	7,12-Dimethylbenz(a)anthracene

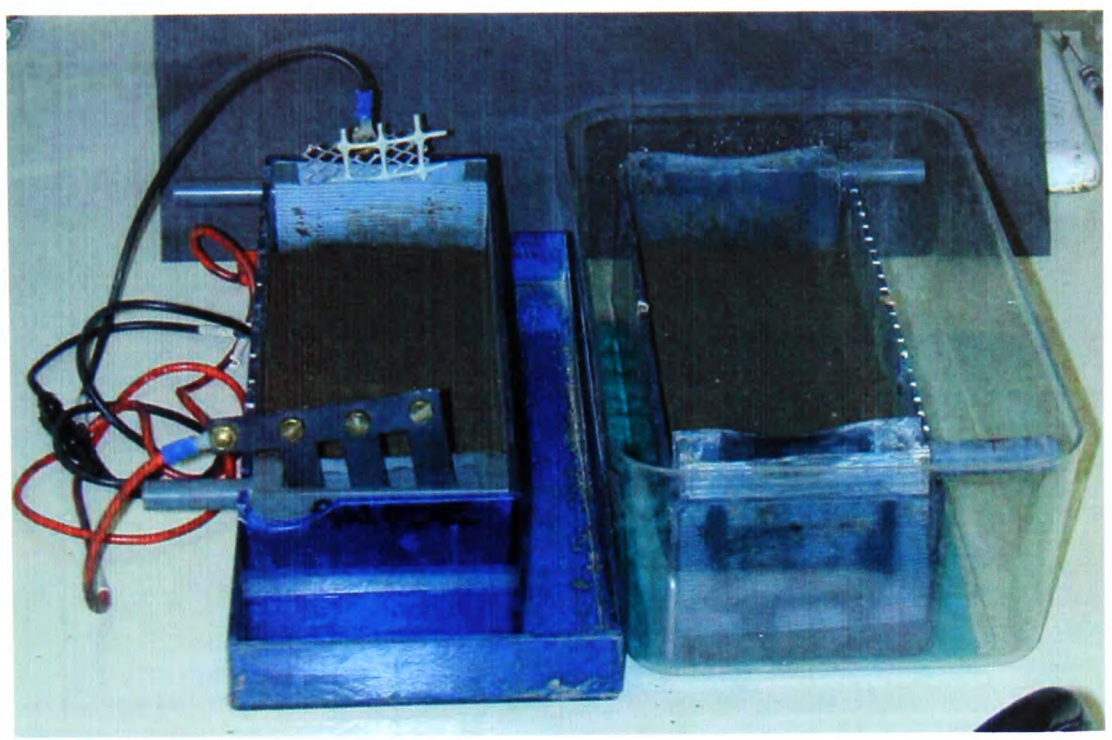
Reference: Potter & Simmons, (1998) – Total Petroleum Hydrocarbon Criteria Working Group Series.

In addition it is reported that benzene, butadiene, cyclohexane, cyclohexene, cyclopropane, ethyl benzene, methylcyclohexene, toluene, and xylene isomers are constituents of kerosene (*e.g.* Irwin, 1997).

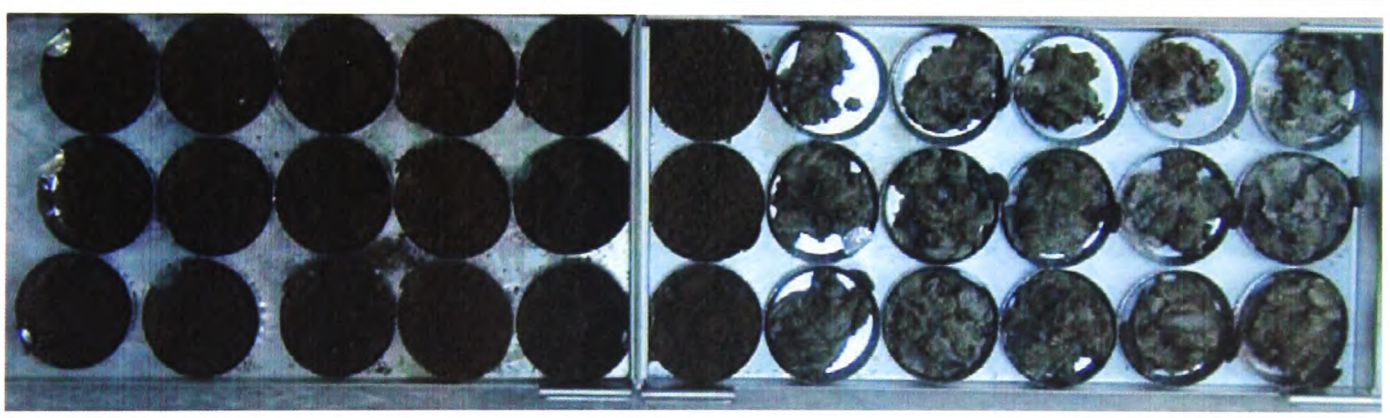
Appendix 8.3 Structures of target contaminants.

Compound	Structure
3-Chlorobenzoate	
3-Chlorobiphenyl	
Dodecane	
Tetradecane	
Toluene	
Octadecylbenzene	
p-Xylene	
m-Xylene	
o-Xylene	

Appendix 8.4 Photograph of soil chambers. Electrokinetics chamber (left hand-side) and control chamber.



Appendix 8.5 Photograph of Wytham soil samples taken across the soil chamber after two weeks of applying an electric field (60 V, ~10 mA). A white precipitate can be observed in soil samples collected at a 13.3 cm distance from the anode.



Appendix 8.6 Distribution of iron and potassium in the electrokinetic soil chamber.

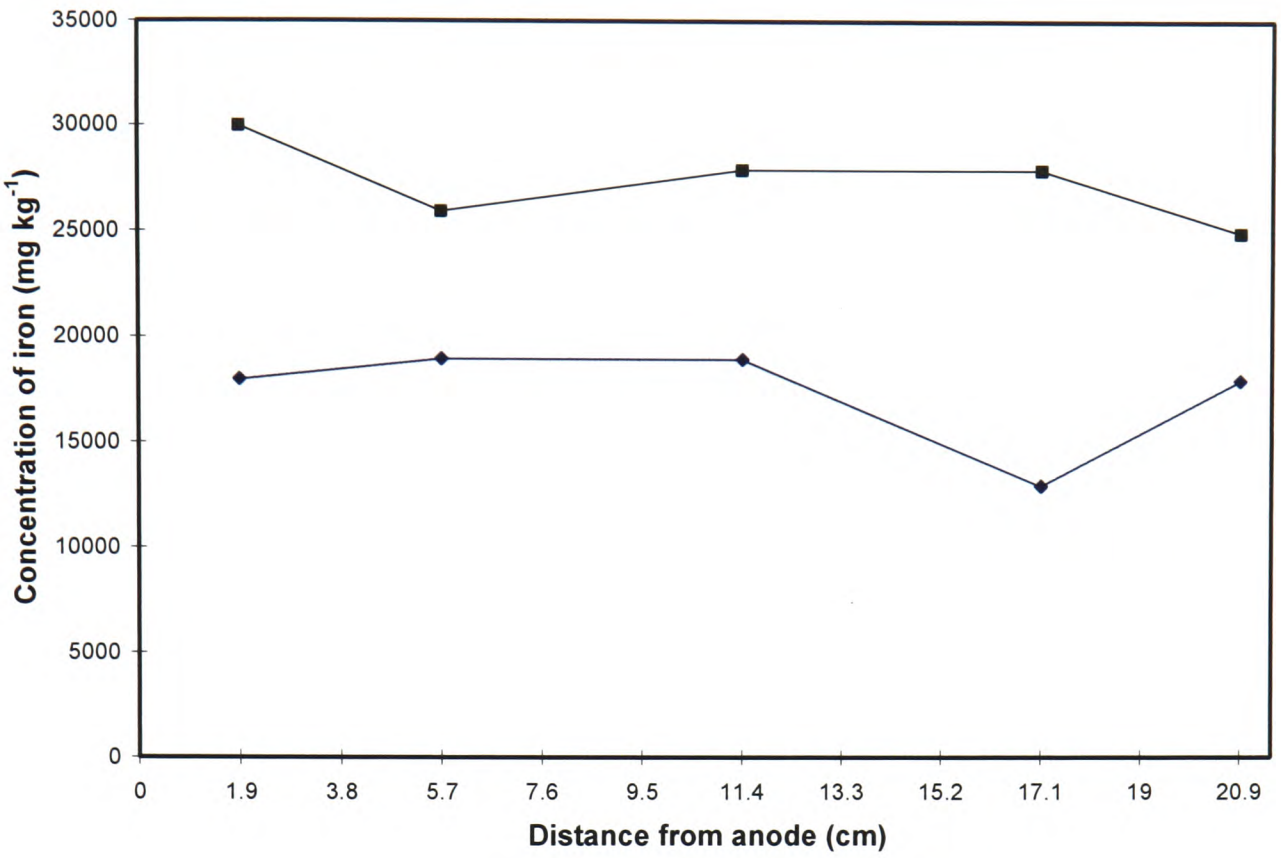


Figure i Distribution of iron (mg kg^{-1}) in the soil chamber after 2 weeks of applying an electric field (60 V, ~10 mA). ♦ Soil chamber packed with Reading soil; ■ soil chamber packed with Wytham soil. (Data set is based on one experiment).

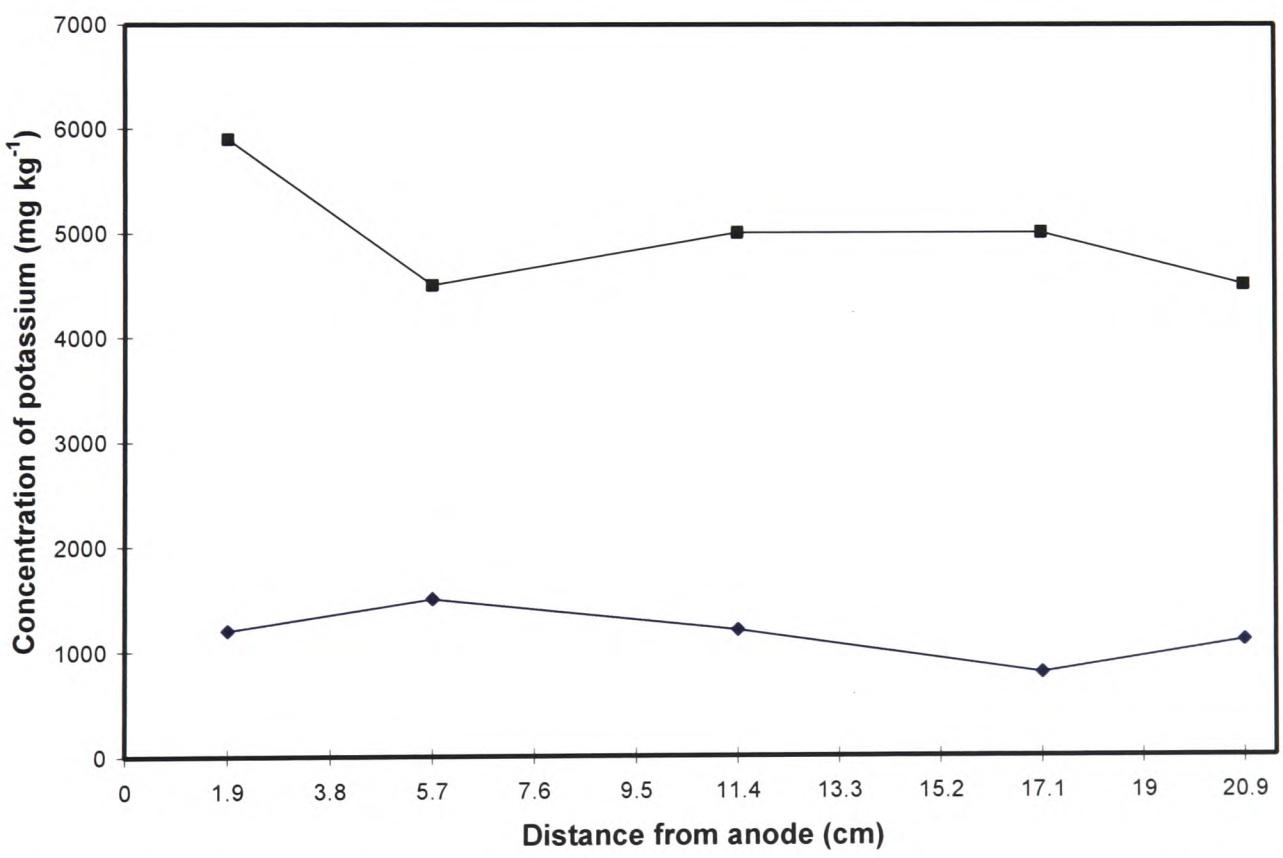


Figure ii Distribution of potassium (mg kg^{-1}) in the soil chamber after 2 weeks of applying an electric field (60 V, ~10 mA). ♦ Soil chamber packed with Reading soil; ■ soil chamber packed with Wytham soil. (Data set is based on one experiment).

Appendix 8.7 Effect of SDS on the structure of the soil during 14 days of electrokinetics (60 V).

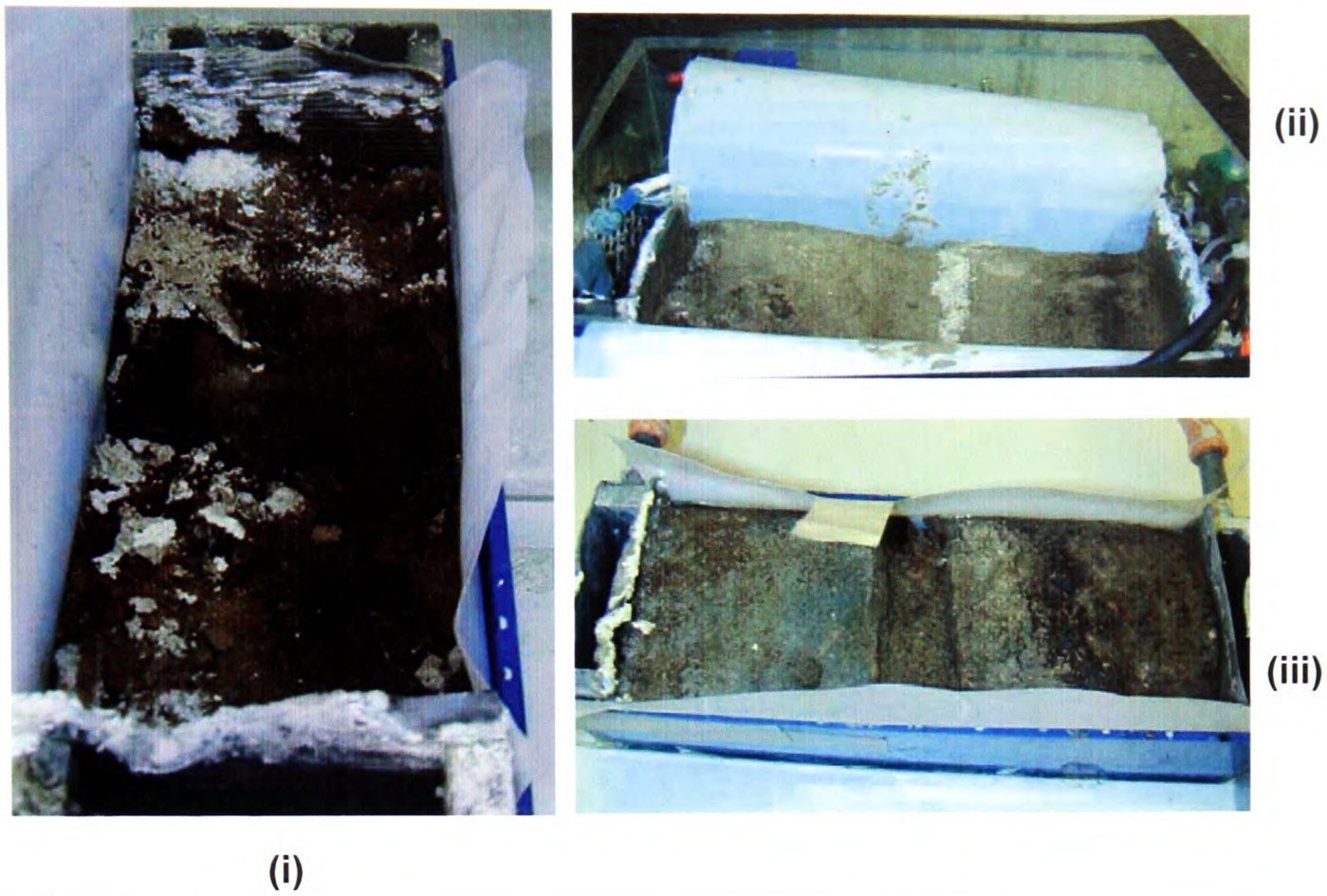


Figure i Disruption of soil structure on addition of 0.4% SDS to the electrokinetics soil chamber during 2 weeks of applying an electric field (60 V, ~10 mA). (i) Wytham soil chamber (anode at far side of photograph); (ii) soil chamber packed with Reading soil with line of precipitation on anode side of the spiked region and crusting of the soil surface in the vicinity of the electrodes; (iii) soil chamber packed with Wytham soil showing evidence of cracking and lines of precipitation.

Appendix 8.8 The use of NO_3^- as a nutrient and/or electron acceptor.

Refer to § 4.6.3. for details of the experimental set-up. In the microcosms with amendments of NH_4^+ no concentration of either NO_3^- or NO_2^- was detected in the soil samples.

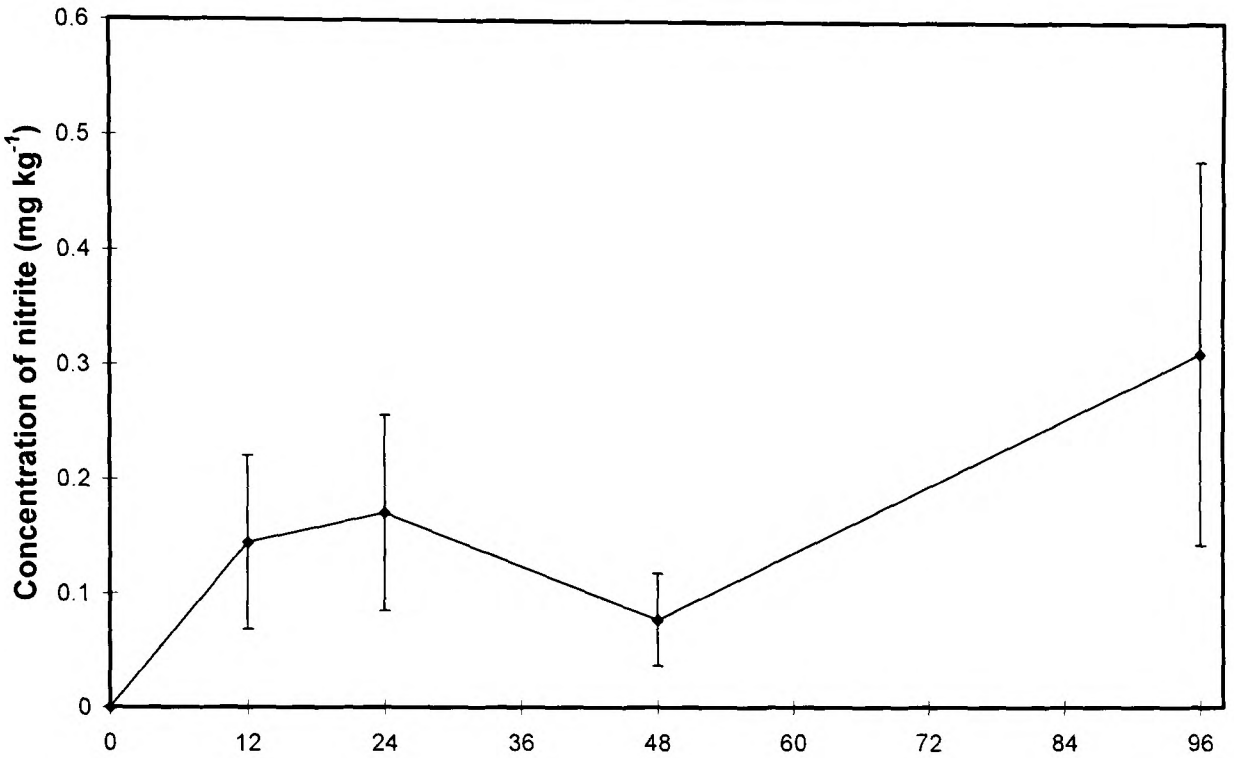


Figure i Concentration of NO_2^- after the addition of 5 mM NO_3^- to a microcosm containing the target contaminant mix (DXT) at a concentration of ~11 000, 9 100, 4 000 mg kg^{-1}, respectively.

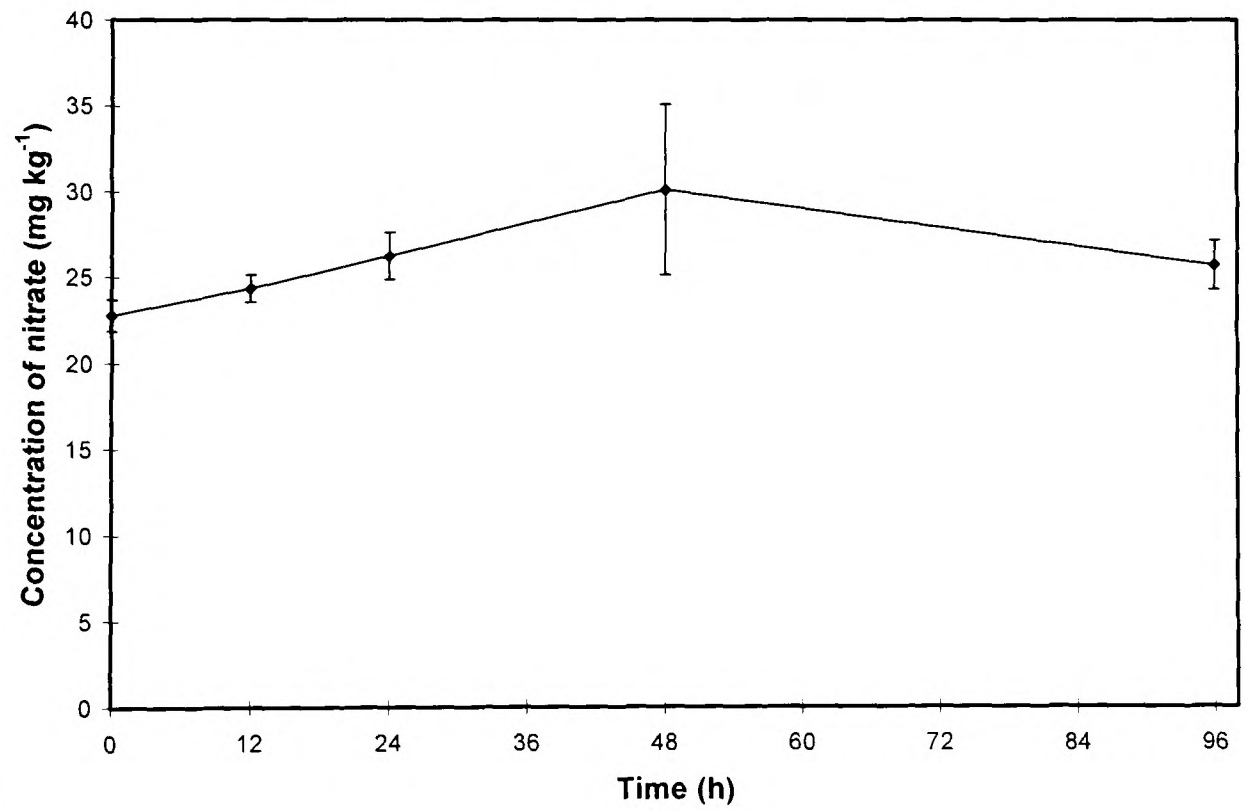


Figure ii Concentration of NO_3^- after the addition of 5 mM NO_3^- to a microcosm containing the target contaminant mix (DXT) at a concentration of ~11 000, 9 100, 4 000 mg kg^{-1}, respectively.

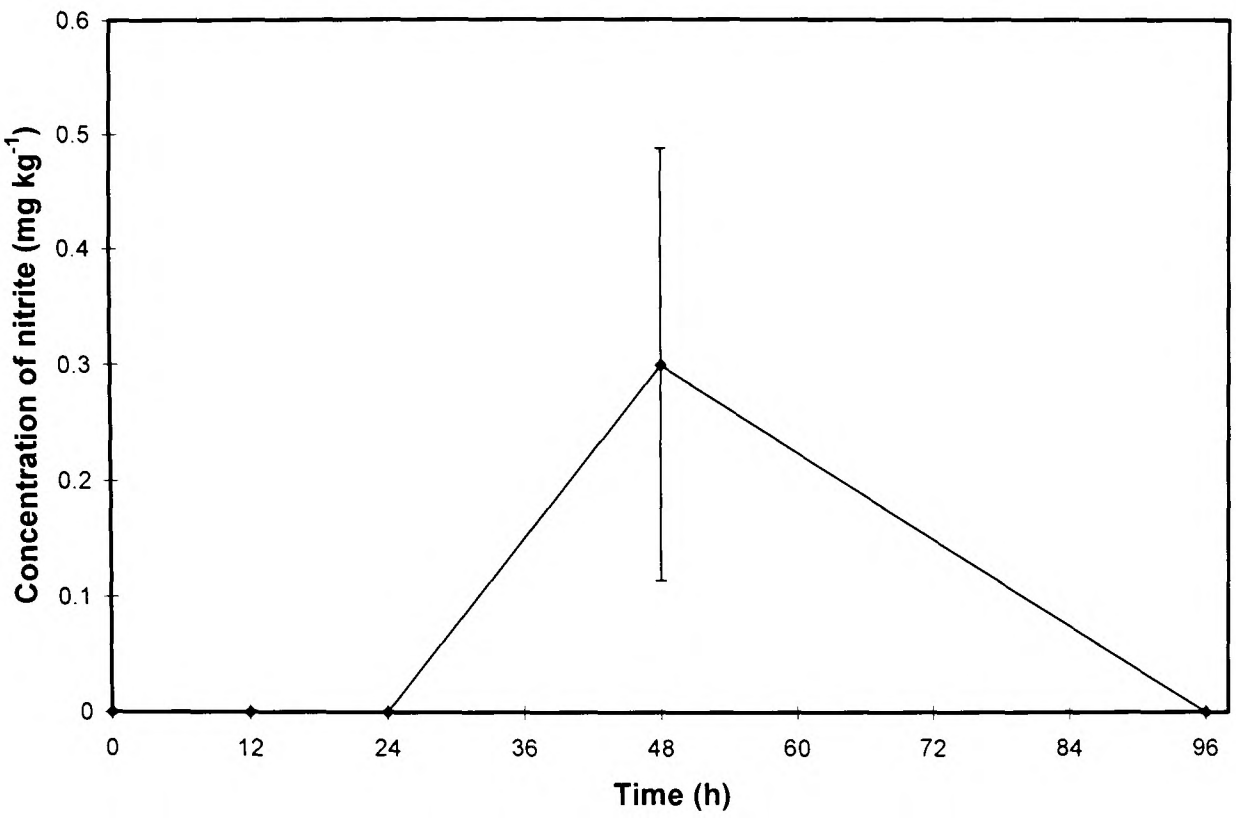


Figure iii Concentration of NO_2^- after the addition of 5 mM NO_3^- and 5 mM NH_4^+ to a microcosm containing the target contaminant mix (DXT) at a concentration of ~11 000, 9 100, 4 000 mg kg^{-1}, respectively.

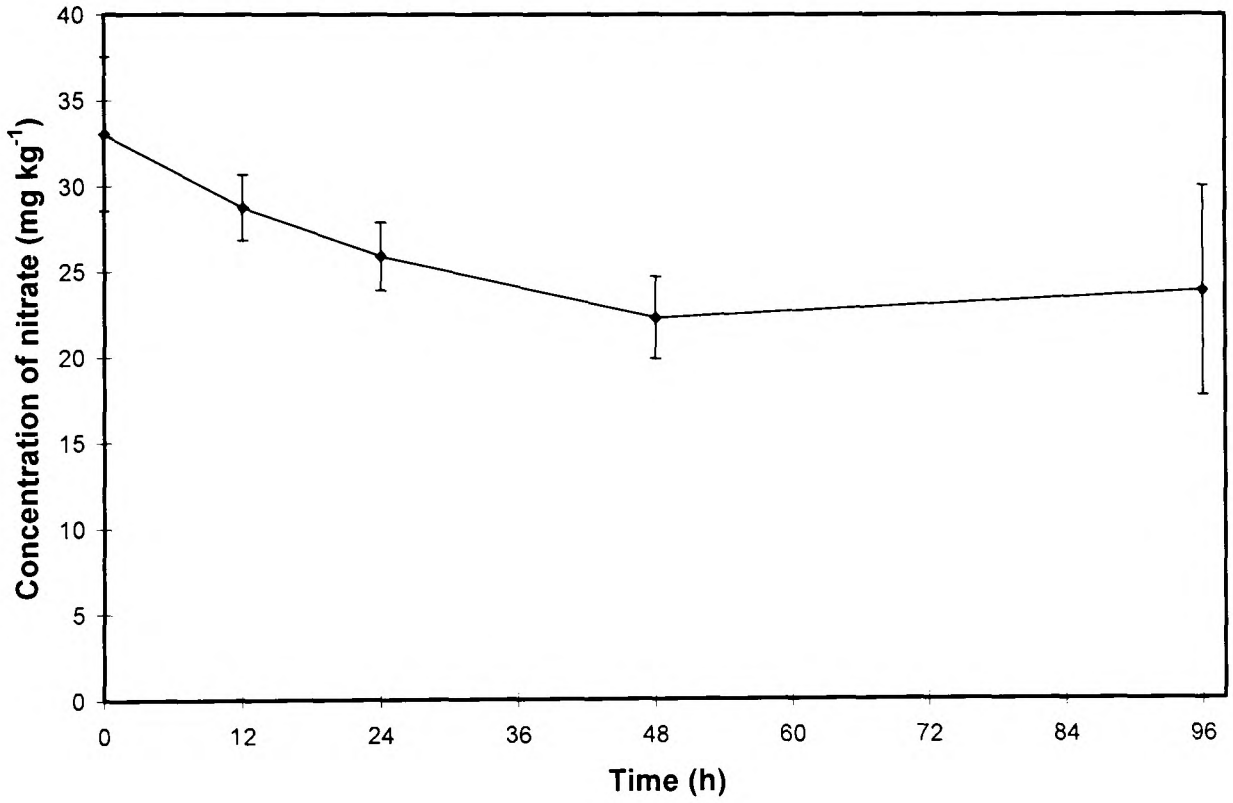


Figure iv Concentration of NO_3^- after the addition of 5 mM NO_3^- and 5 mM NH_4^+ to a microcosm containing the target contaminant mix (DXT) at a concentration of ~11 000, 9 100, 4 000 mg kg^{-1}, respectively.

Appendix 8.9 Required soil pH for effective chemical oxidation

For the use of Fenton's reagent in the soil as an oxidising agent a pH of between 3 and 5 is required. Conventionally to acidify the soil prior to ISCO acid solutions (*e.g.* H_2SO_4) are flushed through the soil. A brief investigation was performed to determine the efficacy of electrokinetics to acidify a soil prior to the use of Fenton's Reagent. 60 V was applied to the soil chamber for 62 h and the pH was analysed (§2.2.2.5) at time points 0, 24, 36 and 62 h. The anode chamber was unbuffered, but to sustain the integrity of the Daramic membrane the cathode reservoir was buffered to pH 6.5 using 0.1 M H_2SO_4. It was identified that the buffering capacity of the soil restricted the acidification of the soil. After applying 60 V (~10 mA) for 62 h acidification was only achieved as little as 60 mm from the anode chamber.

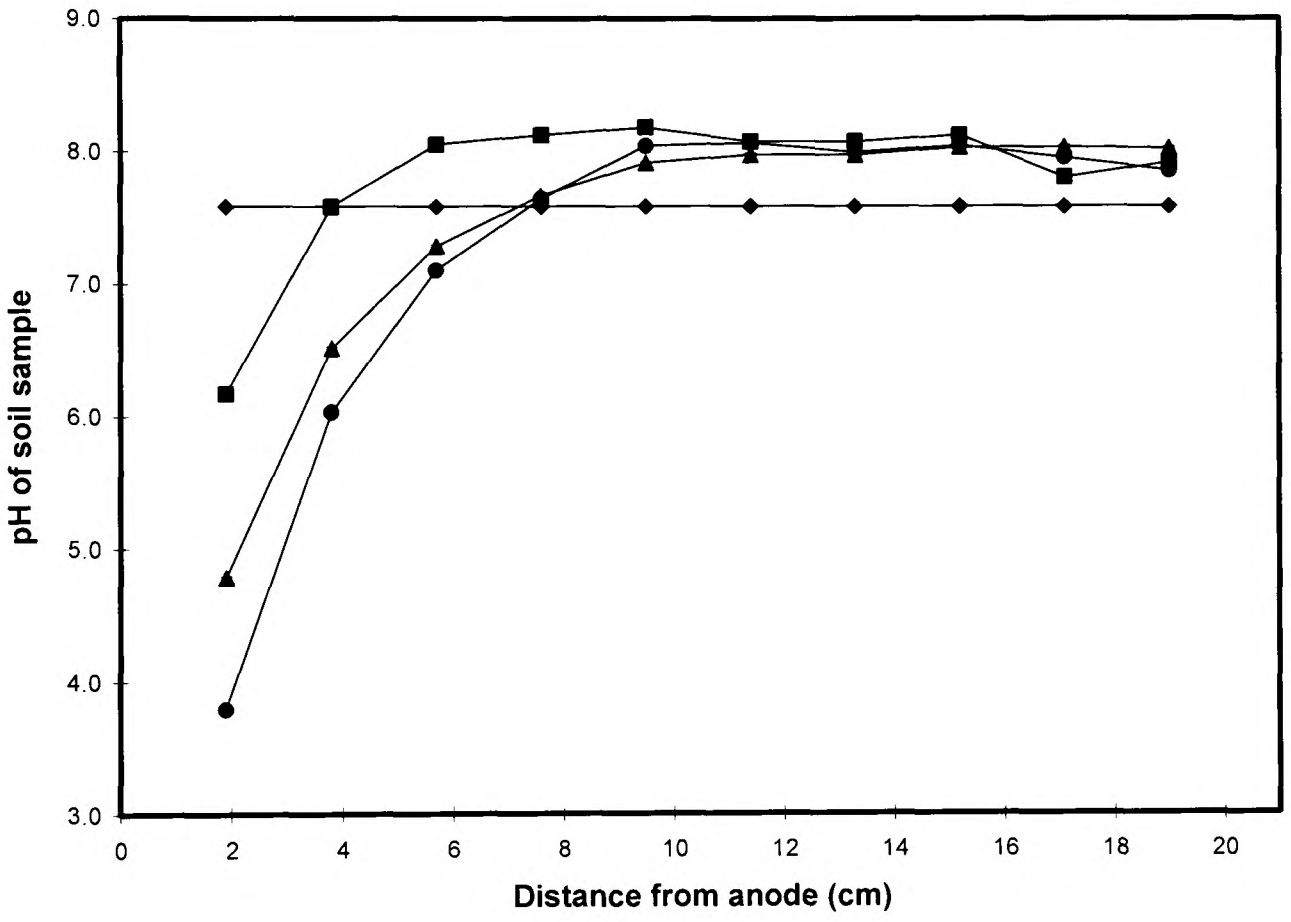


Figure i Spatial and temporal pH changes in the soil chamber with 60 V applied (10 mA), ◆ 0 h; ■24 h; ▲ 36 h; ● 62 h. (NB: y-axis crosses at pH 3).

Appendix 8.10 Concentration of soil constituents. Soil samples taken from EDF Transformer Site, Orford, Suffolk and analysed by Severn Trent Laboratories, Reading, UK. (N/S = sample not scheduled for analysis).

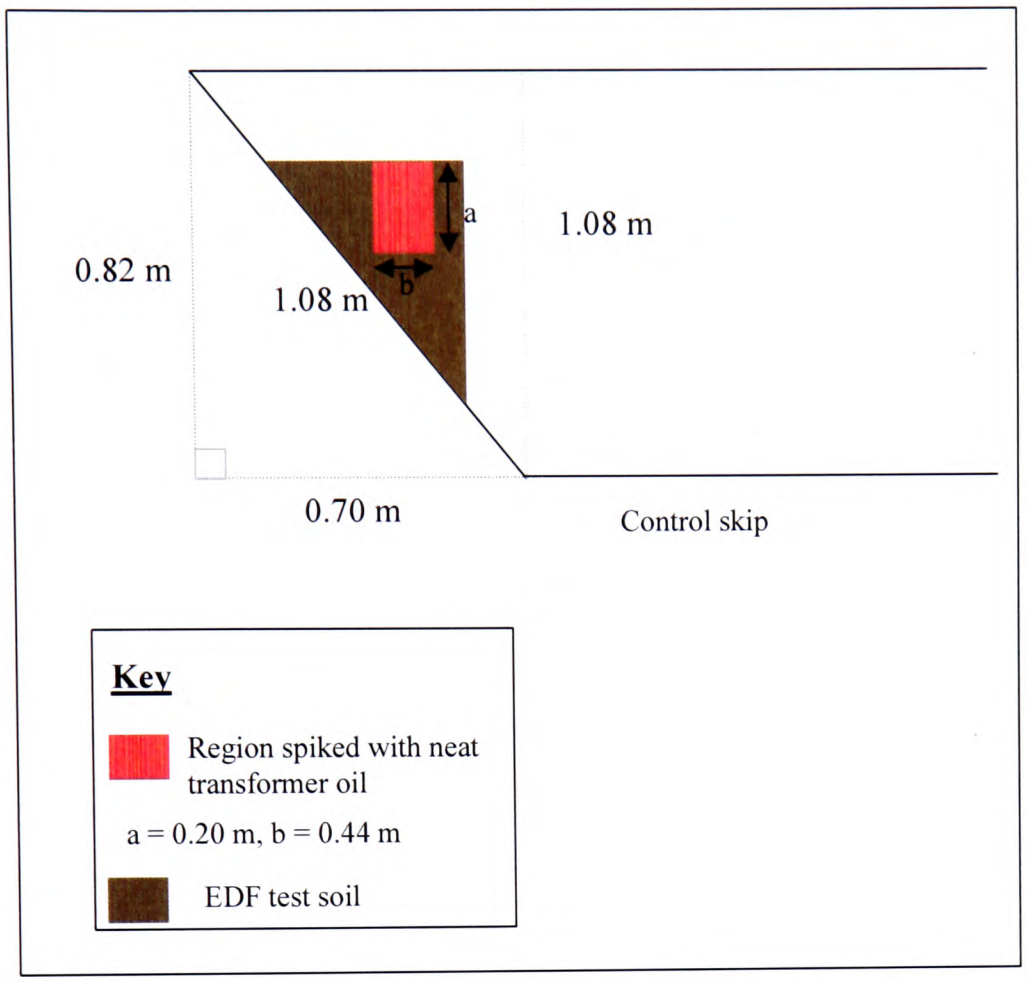
	Units	1 0.50m	2 0.50m	3 0.50m	4 0.50m	5 0.20m
Arsenic (Total)	mg/kg	11	8.8	9.7	11	12
Boron (Soluble)	mg/kg	0.81	1.3	0.92	1.1	2
Cadmium (Total)	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
Chromium (Total)	mg/kg	21	17	21	21	19
Copper (Total)	mg/kg	12	26	8.3	7.9	21
Iron (Total)	mg/kg	20000	N/S	N/S	N/S	21000
Lead (Total)	mg/kg	30	91	23	20	49
Magnesium (Total)	mg/kg	1100	N/S	N/S	N/S	1100
Manganese (Total)	mg/kg	670	N/S	N/S	N/S	530
Mercury (Total)	mg/kg	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20
Nickel (Total)	mg/kg	8.1	7	6.7	7.3	7.7
Potassium (Total)*	mg/kg	980	N/S	N/S	N/S	1200
Selenium (Total)	mg/kg	< 0.30	< 0.30	< 0.30	< 0.30	< 0.30
Sodium (Total)*	mg/kg	130	N/S	N/S	N/S	130
Zinc (Total)	mg/kg	41	54	41	37	120
Ammonia as N	mg/kg	< 25	N/S	N/S	N/S	< 25
Chloride (2:1 Water Extract)*	mg/kg	< 100	N/S	N/S	N/S	< 100
Cyanide (Total)	mg/kg	3.9	< 0.50	< 0.50	< 0.50	< 0.50
Organic Matter	%	4.6	1.2	1.1	0.9	2.3
Phenols (Monohydric)*	mg/kg	0.31	< 0.25	< 0.25	< 0.25	0.36
Sulphate as SO4 (2:1 Water Extract	g/l	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06
Sulphide as S	mg/kg	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
Fraction Of Organic Carbon		0.029	0.013	0.0059	0.013	0.021
TOC by Ignition in Oxygen	%	2.9	1.3	0.59	1.3	2.1
pH	pH units	8.3	8.3	8.3	8.1	7.8
Nitrate as NO3 (2:1 Water Extract)*	g/l	0.0026	N/S	N/S	N/S	0.0035
TPH						
>C6 to C8	mg/kg	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
>C8 to C10	mg/kg	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
>C10 to C12	mg/kg	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
>C12 to C16	mg/kg	1700	5.3	< 1.0	< 1.0	15
>C16 to C21	mg/kg	13000	100	7.8	< 5.0	580
>C21 TO C40	mg/kg	8900	210	69	< 10	930
Total TPH	mg/kg	24000	320	77	< 25	1500
PAH Suite						
naphthalene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
acenaphthylene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
acenaphthene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
fluorene	mg/kg	1.1	< 0.50	< 0.50	< 0.50	< 0.50
phenanthrene	mg/kg	0.78	< 0.50	< 0.50	< 0.50	< 0.50
anthracene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
fluoranthene	mg/kg	0.62	< 0.50	< 0.50	< 0.50	< 0.50
pyrene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
benzo(a)anthracene	mg/kg	0.99	< 0.50	< 0.50	< 0.50	< 0.50
chrysene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
benzo(b)fluoranthene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
benzo(k)fluoranthene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
benzo(a)pyrene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
dibenzo(ah)anthracene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
benzo(ghi)perylene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
indeno(123cd)pyrene	mg/kg	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50
PAH (Total)	mg/kg	6	< 2	< 2	< 2	< 2
Moisture*	%	11	9	13	11	9.6
Stones %*	%	6.6	18	2.1	2.1	6.5

Appendix 8.11 Materials for field-scale experiment

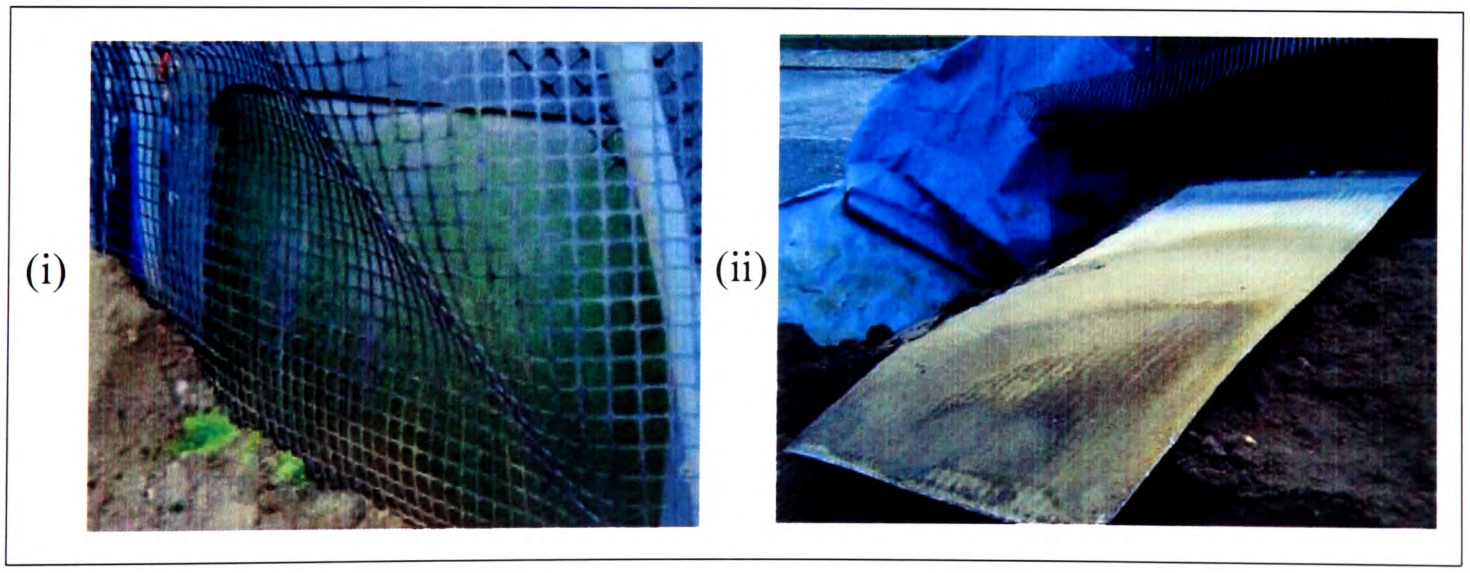
Item	No.	Supplier	Cost per item
Tarpaulin	2	Silverline Tools Ltd, Ebay	£8.00
Peristaltic pump	3	Masterflex	On loan
Power Supply Xantrex XHR150-7 (100V power supply)	1	Telonic Instruments Ltd.	£600.00
Wire	1	RS Components	£26.00
Daramic membrane	1	Grace GmbH, Germany	
Equal T-joint	4	Plumb Centre	£3.02
Elbow joint	2		£2.14
Bend 90 degrees and 32 mm	12		£6.59
Tee 90 degrees and 32 mm	10		£2.51
32 mm PVC piping			£32.00
L/S18 Norprene Tubing	6	Fisher Scientific, UK	£39.00
IP82 Norprene tubing	1		£126.00
Tubing attachments	20	VWR	£2.50
Decking	10	B & Q	£337.62
2 m overflow pipe (22 mm Ø)	8		
Geotextile	1		
Shovels	3		
Gloves	3		
Sealant	1		
PVC Sheets for electrode housing l	3		
Troughs for electrodes	6	Tesco	£1.50
Electrode housing	6	Capenhurst	On loan
3305 pH Controller and probe	1	Jenway Ltd., UK and BDH Ltd. UK	On loan
Hose	1	RS Components	£23.21
Ice box	1	Argos	£11.99

(The initial cost of electrode construction incurred by Capenhurst was ~ £110 [Stratton-Campbell *et al.*, 1999]).

Appendix 8.12 Schematic of the control cell.



Appendix 8.13 Photographs showing deterioration of the electrodes and electrode membranes. (i) Algae growth on the Daramic membrane and (ii) precipitation of cations (*e.g.* Ca^{2+}) on the surface of the cathode.



Appendix 8.14 Length Heterogeneity – PCR (LH-PCR).

Nucleic acid extractions were performed for the soil samples by adding approximately 0.5 g of soil into a microcentrifuge tube. Total nucleic acids were extracted from all samples according to the protocol of Griffiths et al. (2000). Total nucleic acid was extracted from sub samples by placing the sample into a "Multi mix" bead beating tube (BIO-101 lysing matrix E) with phenol: chloroform: isoamyl alcohol (25:24:1), lysing for 30 seconds at 5.5 m/s and subsequent chloroform: isoamyl alcohol (24:1) purification. Total nucleic acids were precipitated from the extracted aqueous layer with two volumes of 30% (w/v) polyethylene glycol 6000 (Fluka) in a 1.6 M NaCl solution for 2 h at room temperature (~25°C), washed in ice cold 70% (v/v) ethanol and finally re-suspended in 100 µl Tris-EDTA buffer, pH 7.4.

LH-PCR analyses were carried out according to the method of Whiteley *et al.* (2003). The oligonucleotides used were BF1 and 530R (refer to 4.3.1.2.2 for oligonucleotide sequences), BF1 was labelled with the D4 blue fluorescent dye (Proligo, France) so that the resulting amplicons could also be used in tRFLP analysis. The following PCR programme was followed using a Peltier Thermo Cycler:

1. 94°C for 3 min
2. 94°C for 30 s ← Repeat 28 times
3. 52°C for 30 s
4. 72°C for 1 min
5. 72°C for 5 min

The resulting amplicons were then purified using the QIAGEN QIAquick PCR Purification Kit. 3 µl of the cleaned amplified 16S rRNA gene products were used in the analysis on the Beckman Coulter CEQ2000XL capillary sequencer. Fragment analyses for LH-PCR were undertaken using a Beckman Coulter CEQ2000XL capillary sequencer and the CEQ Size Standard Kit-600 (Beckman Coulter Inc.) according to the manufacturer's instructions. The subsequent trace file data were analysed using the Beckman Coulter CEQ8000 software to produce a 0/1, absence/presence, binary matrix for peak sizes.

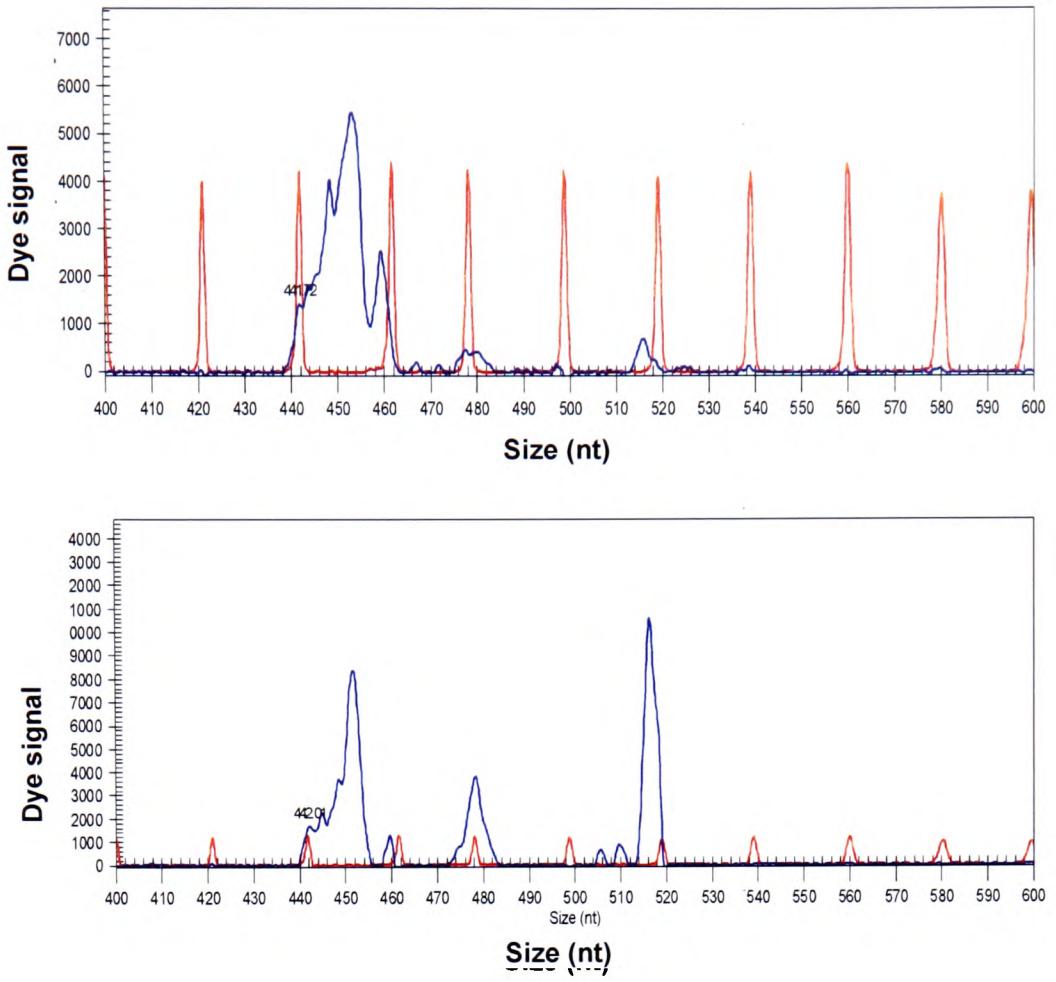
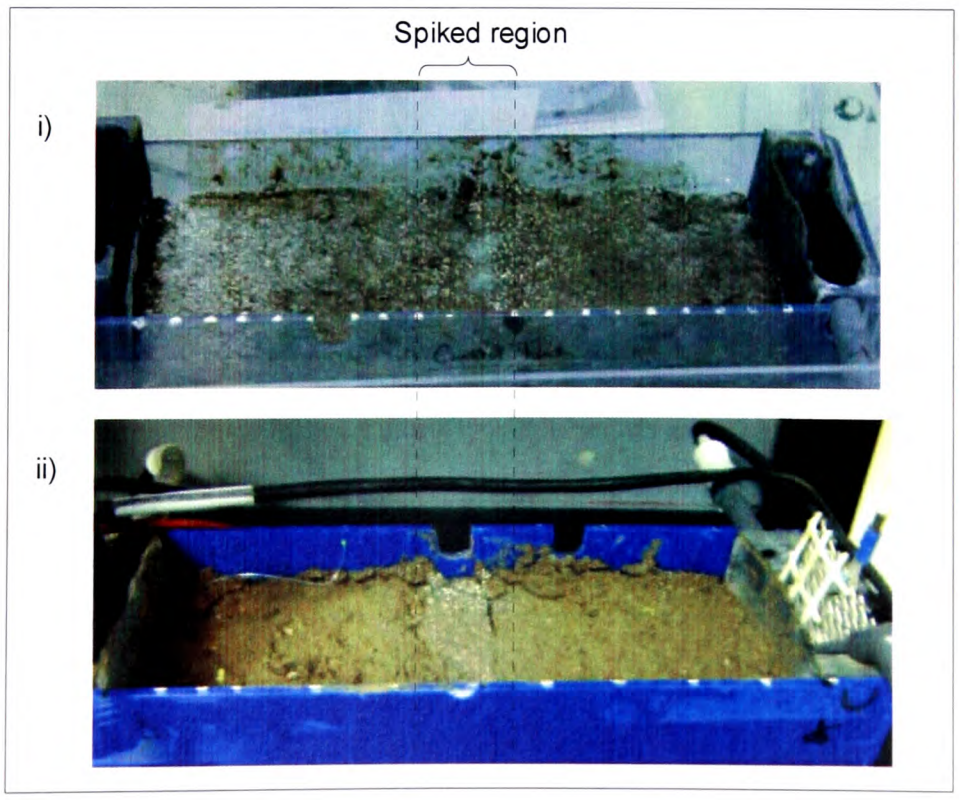


Figure i a) LH-PCR output for Reading soil (Data is based on one soil sample), b) LH-PCR output for Reading soil after electrokinetics for 14 days (60 V, ~ 10 mA) (Data is based on one soil sample).

Appendix 8.15 Evidence of fungal involvement on the degradation of the target contaminants.



Appendix 8.16 Rapid assay for assessing hydrocarbon-degrading capabilities of indigenous microorganisms.

Approximately 0.3 mg of the target hydrocarbons were added to each well of a Biolog® MT2 plate (Biolog, Oxoid, Hampshire, UK) (Figure i). As bacteria utilise the different carbon sources, tetrazolium acts as a hydrogen carrier, and is reduced. The reduction of terazolium results in colour development; from clear to violet, giving the wells a greater light absorbance at 620 nm.

0.5 g of soil was placed into a 15 ml centrifuge tube (Bibby Sterilin Ltd., Staffordshire, UK) and to this, 10 ml of phosphate buffered saline (PBS) (Oxoid, Hampshire, UK) (Na_2HPO_4, 1.15 g l^{-1}; NaCl, 8 g l^{-1}; KH_2PO_4 1.15 g l^{-1}; KCl, 0.2 g l^{-1}, pH 7.3) was added together with 5 mm glass beads to a total volume of 12.5 ml. The mixture was vortexed (3,000 rpm, 1 min) and centrifuged (4000 rpm, 10 min). 1 ml of the supernatant was then combined with a further 14 ml PBS, and again vortexed and centrifuged. An extra 5 ml PBS was combined with the supernatant before 100 µl of the solution was added to each well of the 96 well plate. The method has been optimised to provide 10^8 bacterial cells ml^{-1} (Haack, *et al.*, 1995; Lear, 2003 Pers. Comm.). The plates were incubated at 28°C and absorbance measured at 620 nm using an optical density microplate luminometer (Anthos Lucy 1 (Software v1.5), Labtech International Ltd., Ringmer, East Sussex, UK) every 24 h for 10 days.

The following equation was used to translate the optical density reading to a figure that relates to carbon utilisation:

$$\frac{(S-C)}{B}\times 10^9$$

where S is the sample well OD_{620nm}, C is the control well OD_{620nm} and B is bacterial CFU's g^{-1} dry soil.

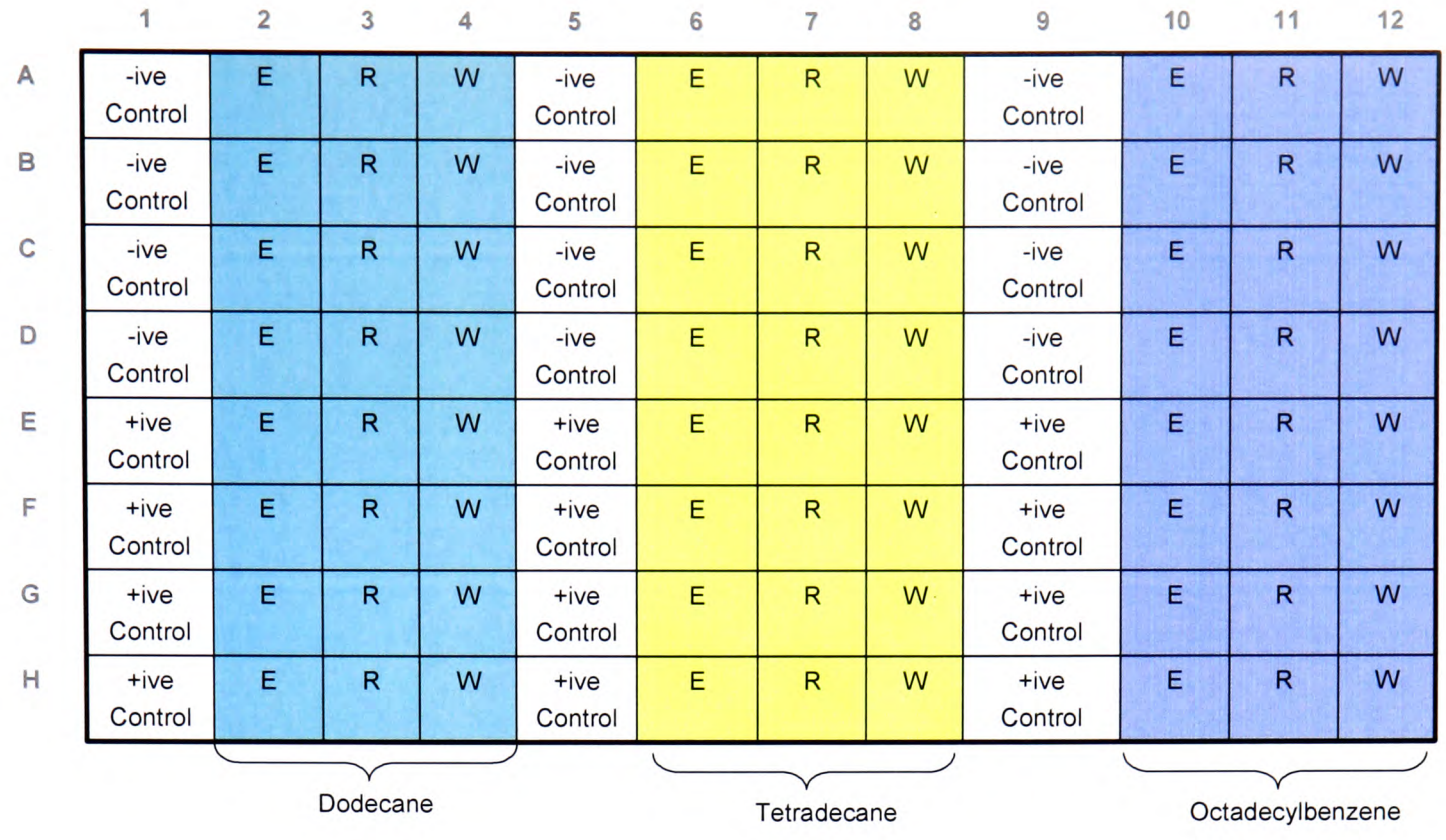


Figure i Layout of MT2 Microplate, where E = EDF Soil, R = Reading Soil and W = Wytham Soil.