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Potential for Anaerobic Biodegradation of Linear Alkylbenzene Cable Oils: Literature Review and Preliminary Investigation

S.J. Johnson, D.A. Barry, N. Christofi and D. Patel

Abstract

Linear alkylbenzene (LAB) cable oils are used for the electrical insulation of high-voltage underground power cables. Due to thermal movement of the cables, leaks can occur at the joints, resulting in cable oil leaking into the surrounding environment. A review of the literature indicates that relatively little is known about the fate of LAB as a bulk pollutant in soil. To investigate this, a physical model of a cable joint bay was constructed and contaminated with cable oil. Fluorometry confirmed that the cable oil became localised to the upper regions of the saturated zone where dissolved oxygen, pH, and oxidation-reduction measurements indicated that conditions were predominately anaerobic, with evidence for sulphate reduction. Mathematical modelling indicates that these conditions were not due solely to the geochemistry of the system. Mesocosm experiments suggest that LAB may be degraded naturally under anaerobic conditions at rates high enough to justify the use of monitored natural attenuation as a remediation strategy. The mechanisms involved in anaerobic degradation are not currently well understood and more research is required to clarify these and identify the microorganisms involved.

Key words: natural attenuation, aerobic/anaerobic degradation, groundwater, bioremediation, physical model, geochemical model, PHREEQC, mesocosm experiment

INTRODUCTION

The electricity transmission system in England and Wales uses a combination of overhead power lines and underground power cables. The power cables consist of a copper conductor, surrounded by electrical insulation in the form of lapped paper tapes impregnated with insulating oil. This core is contained within a metallic sheath for oil containment, as the oil has to be maintained at a positive pressure. A polymeric oversheath is applied for mechanical protection. The original insulating medium was mineral oil. Since the 1970s, a mixture of linear alkylbenzenes (LABs), colloquially known as dodecylbenzene (DDB) has been used. In general, cable oil leaks can occur through one of two mechanisms: (1) deterioration of the joints through thermal movement over a long period of time, or (2) third-party damage. At present, cable oil leaks are detected, located and repaired if the leak rate is above 40 L month^{-1} (based on the detection/location limits using currently available methods). The backfill material surrounding the cable is removed and sent to a licensed landfill. The soil surrounding the immediate joint bay area typically cannot be excavated due to the nature of the sites, which are often in urban areas where access to the surrounding soil is restricted by other services, which could be damaged if the soil was excavated.

Characterisation of a batch of commercially available cable oil using gas chromatography – mass spec-

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Authors
S.J. Johnson[a]*, D.A. Barry[a], N. Christofi[ab] and D. Patel[c]
a. Contaminated Land Assessment and Remediation Research Centre (CLARRC), University of Edinburgh, Edinburgh, EH9 3JN, UK
b. Pollution Research Unit, Napier University, Edinburgh, EH10 5DT, UK
c. National Grid, Kelvin Avenue, Leatherhead, Surrey, KT22 7ST, UK
*Corresponding author, previously at Institute of BioScience and Technology, Cranfield University, Bedford MK43 0AL, UK

trometry (GC-MS) has shown it to contain 18 major components, ranging from decyl- to tridecyl-benzenes (Rowland 1996). However, there is some variation between batches. LABs are also present in small quantities in the environment as residues from the manufacture and use of linear alkylbenzene sulphonate detergents (Eganhouse 1986). Physical properties are summarised in Table 1. It is likely that the low aqueous solubility will be the ultimate limiting factor in the rate of biodegradation. The low density and poor aqueous solubility make it likely that the oil will partition at the water table.

Table 1. Physical properties of linear alkylbenzene (LAB) cable oil

Property	Value
Appearance	Clear colourless liquid (1)
Density at 20°C	0.86 kg L^{-1} (2)
Boiling point	≥ 260°C (1)
	726°C (2)
Kinematic viscosity at 20°C	7.5 – 8.5 mm^2 s^{-1} (1)
	8.1 mm^2 s^{-1} (2)
Flashpoint	≥ 130 ×C (1)
	150°C (2)
Aqueous solubility	Immiscible (1)
	< 0.01mg L^{-1} (2)
	0.41 mg L^{-1} (3)
	4 – 7 nmol L^{-1} (4)
Vapour pressure at 25°C	4.9×10^{-4} mmHg (3)
	0.038 – 0.067 Pa (4)
Henry's Law constant	7.1×10^2 torr L mol^{-1} (3)
Soil partition coefficient, K_{oc}	2.2×10^4 (3)
Log octanol:water partition coefficient, K_{ow}	5.72 – 5.75 (3)
	4.97 – 5.08 (4)

Sources:
1 – BICC Cables safety data sheet, July 1994
2 – Shell health, safety and environment data sheets
3 – Gledhill *et al.* (1991)
4 – Sherblom *et al.* (1992)

This paper reviews current knowledge of the occurrence and fate of LAB in the environment. While there is much literature on the occurrence of LAB in aquatic sediments, this has been directed mainly towards using LAB as a marker for LAS detergents and hence of domestic wastewater contamination. Relatively little has been published about the fate of bulk LAB in the terrestrial subsurface. Data is presented that suggests that anaerobic conditions may prevail in cable oil-contaminated subsoil, that biogenic sulphate reduction occurs, and that the cable oil is the probable carbon source for actively respiring anaerobes.

LITERATURE REVIEW

Linear alkylbenzenes (LABs) are produced synthetically for use in the manufacture of linear alkylbenzene sulphonate (LAS) detergents, varnishes and, significantly for this study, as electrical transmission cable oil. Recently, they have been demonstrated to occur naturally in crude oil (Dutta and Harayama 2001).

A variety of nomenclatures have been applied to LABs. Some authors name them according to the length of the longest aliphatic chain, while others treat them as fundamentally aromatic molecules with a single functional substitution consisting of unbranched (in the case of terminal isomers) or single-branched (at the carbon nearest the attachment to the benzene ring) alkyl chains. Thus, the isomer of $C_{18}H_{30}$ depicted in Figure 1 may be referred to in the literature as dodecyl-2-benzene, 2-phenyldodecane or (1-methyl-undecyl)-benzene. The latter, indicating a branched molecule, is the current IUPAC name. Nevertheless, the term LAB is still in common usage to distinguish these molecules from more highly-branched species. Some authors reserve the title LAB for terminal isomers, including toluene and ethylbenzene. It is common to use the shorthand *n*C*m* where *n* = position of the benzene ring and *m* = number of carbon atoms in the longest aliphatic chain. The isomer in Figure 1 would be referred to as 2C12 in this nomenclature. This leads to a situation in which each of the ~20 isomers may have as many as four designators. There is also variable use of, e.g. dodecylbenzene (DDB) to mean all $C_{18}H_{30}$ monoalkylated phenyls; only the terminal substitution of *n*-dodecane with a phenyl group or, colloquially, the entire mixture of synthetic LAB. Since there is ambiguity in the use of some of the terminologies, it is difficult to identify all the literature on the subject.

LABs used as precursors in the manufacture of LAS detergents are present in small amounts in the finished product. LABs are more persistent than LAS in riverine, estuary and marine sediments, which are often the receptors for waste effluents containing these detergents and so are a more conservative marker of long-term exposure to domestic wastewater. Sites at which this has been investigated include Tokyo Bay (Bayona *et al.* 1986), the Mersey Estuary (Preston and Raymundo 1993) and Santa Monica Bay (Zeng and Yu 1996; Zeng *et al.* 1997).

Existing studies of the biochemistry of LAB have largely been on terminal isomers (commercially available monoisomeric standards are all terminal isomers). It is unclear what the relevance of these studies is to

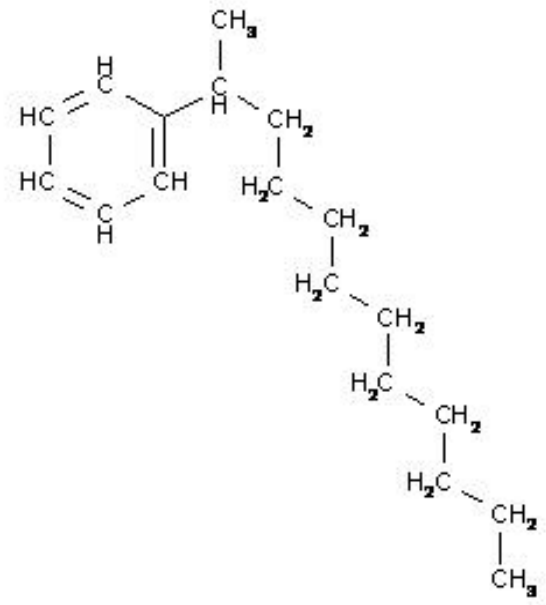

Figure 1. Single isomer of $C_{18}H_{30}$ linear alkylbenzene

LABs in the environment since commercially synthesised LABs do not include these isomers (Eganhouse 1986).

Aerobic biodegradation of 1-phenylalkanes has been demonstrated in *Nocardia* (Sariaslani *et al.* 1974), *Acinetobacter* (Amund and Higgins 1985) and *Pseudomonas* (Bayona *et al.* 1986; Smith and Ratledge 1989). Dodecylbenzene is considered to be readily biodegradable according to OECD guidelines 301D (1992). Indeed, cable oil is thought to act as a carbon source in contaminated soil (Reid *et al.* 2000). Studies on biodegradation rates of hydrocarbons indicate a hierarchy of ease of biodegradation in which *n*-alkanes > branched alkanes > low molecular weight aromatics > cycloalkanes (Leahy and Colwell 1990). The existence of many different isomers means that the trend is not so obvious for higher molecular weight aromatic compounds (Huesmann 1995). Nevertheless, this hierarchy is visible in features of LAB degradation that have been noted in the literature: there have been a number of studies of the isomeric composition of LAB in sediments from Tokyo Bay (Takada and Ishiwatari 1991; Takada and Ishiwatari 1990; Chalaux *et al.* 1995). These indicated preferential degradation of certain isomers. Other studies, of LAB in soils amended with anaerobically digested sewage sludge (Holt and Bernstein 1992; Mangas *et al.* 1998) and of LAB exposed to *Nocardia amarae* isolated from crude oil- and petroleum-contaminated soils (Bhatia and Singh 1996), found similar patterns of isomeric enrichment. They all found that external isomers of LABs are degraded in preference to internal isomers. This was also observed by Bayona *et al*. (1986) and Angley *et al*. (1992), both of whom found better biodegradation if the chain was long. This suggests that access to a free end of the alkane chain is significant in the initial degradation of LABs. Smith and Ratledge (1989) and Smith (1990) elucidated aerobic biodegradation routes for C1-C7 terminal alkylbenzenes, 1-phenyldodecane and 1-phenyltridecane by *Pseudomonas* sp. In the compounds with shorter chain lengths (up to seven carbons) the initial attack on the molecule was an oxidative cleavage of the benzyl ring. With longer chain lengths initial attack was via ω- or β-oxidation of the methyl terminus of the alkyl chain. It is possible that both methyl termini are attacked in non-terminal isomers.

Bhatia and Singh (1996) examined the aerobic biodegradation of commercial LAB by *Nocardia amarae*. They found that the position of the benzene ring affected ease of degradation, with external isomers being more readily degraded than internal ones. They examined the breakdown products and found that the *cis, cis*-muconic acid pathway was most significant, with the phenyl acetic acid pathway also being significant where the alkyl chain was an odd number of carbons long and the phenyl substitution was at an even carbon. In these cases *trans*-cinnamic acid formation provided a minor pathway.

Recently, work has been undertaken into the fate of cable oil in soil. Terrestrial subsoils are generally composed of chemically weathered minerals and thus have a lower organic carbon content than most aquatic sediments. Redox conditions vary over longer distances and the microbial flora is significantly different, especially when compared to marine environments. However, studies have produced promising results for cable oil bioremediation. Cheston (1997) and Tebbutt (1998) found that uncontaminated soils contained aerobic organisms that could degrade cable oil at low concentrations. Koussia (1999) found removal rates of 0.15 $\mu L\ ml^{-1}\ week^{-1}$ in unamended soil, with a 50% increase in rate when nutrients were added.

To date, much of the work done assumes that attenuation of LAB in the environment is due to a combination of physical process and aerobic biodegradation. However, oxygen is not always available and other terminal electron acceptors may be available in soil. For instance, electron acceptors are used preferentially by microbial communities according to availability in a BTEX plume ($O_2 > NO_3^- > Fe(III) > SO_4^{2-}$). Hence, it is important to consider potential degradation routes under a variety of redox regimes to understand whether degradation will continue to occur as each electron acceptor is depleted, or whether it is necessary to manipulate conditions to maintain a particular set of conditions.

Little is known about anaerobic degradation of the oil. However, where information on the degradation of a specific compound is unavailable, it may be possible to predict the likelihood that it will be degraded under particular conditions by referring to known degradation of other compounds with similar structures (Wackett and Ellis 1999). LABs share a number of structural features with BTEX compounds and with *n*-alkanes so it is useful to review research on a variety of compounds and electron acceptors. Until about a decade ago, it was generally considered that hydrocarbons were recalcitrant under anaerobic conditions. This is now changing and anaerobic biodegradation of hydrocarbons is beginning to be considered an accepted remedial option in some cases (Coates and Anderson 2000). Unlike aerobic metabolism, where there are relatively few pathways available for degradation of a compound, the exact pathways of anaerobic breakdown of hydrocarbons are usually novel. There is a general pattern, however (see Figure 2). Anaerobic degradation of BTEX compounds often proceeds via the formation of benzoyl-CoA.

Anaerobic degradation is generally slower than aerobic degradation of the same compound. Ethylbenzene degradation has been shown to be slow in anaerobic conditions (Borden *et al.* 1995). Degradation of some compounds may be inhibited if other, more readily degraded carbon/energy sources are available: for instance, benzene degradation is thought to be inhibited in the presence of other hydrocarbons (Krumholz *et al.* 1996). Residual LAB in sediments has been attributed to the anaerobic conditions found there (Takada and Ishiwatari 1990). However, Herbath (2001) has demonstrated anaerobic degradation of cable oil with evidence of sulphate reduction in anaerobic soil mesocosm experiments, and cultured anaerobic cable-oil degrading microorganisms.

Nitrate-, sulphate- and iron-reduction have all been shown to play a part in degradation of numerous hydrocarbons (Aronson and Howard 1997). Long-chain alkanes seem to be most often degraded in sulphate reducing conditions while, on the whole, BTEX is more susceptible to nitrate reducing conditions. Fe(III) reduction has been shown to be significant in the degradation of a number of BTEX compounds (Lovley and Anderson 2000).

Aerobic degradation of alkane chains can be likened to fatty acid degradation by β-oxidation. However, β-oxidation requires molecular oxygen and so is unlikely to occur in strictly anaerobic conditions. Aekersberg *et al*. (1991) were the first to show that hexadecane and other long chain alkanes could be degraded to CO_2 by a bacterial strain under sulphate-reducing conditions. There was some evidence that this strain pro-

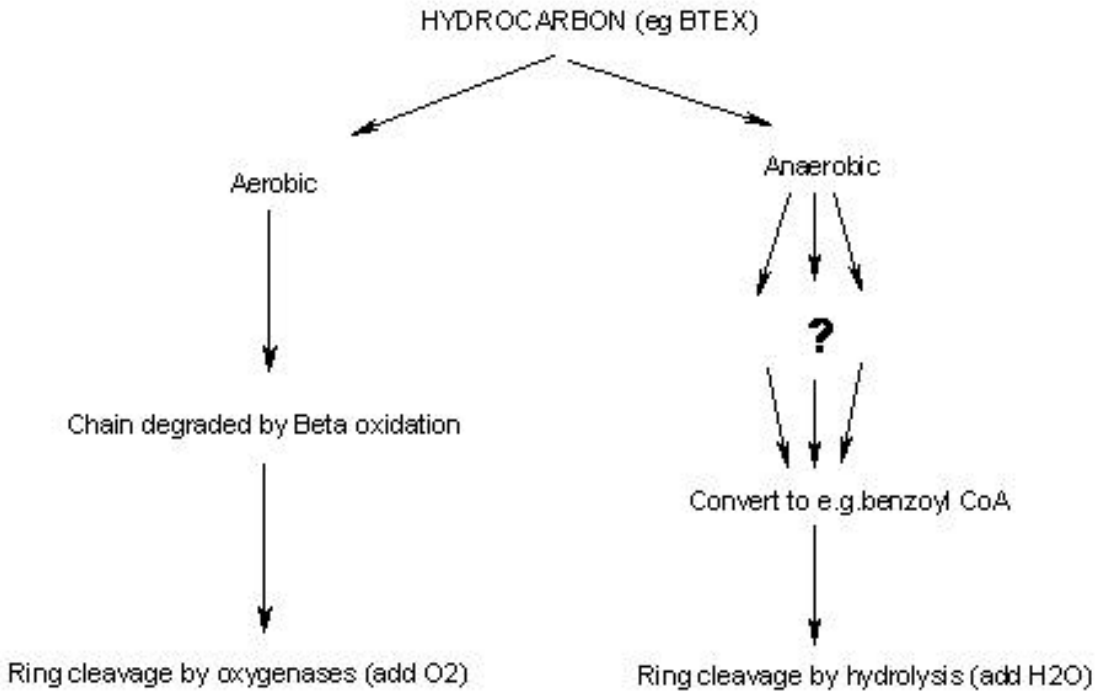

Figure 2. Routes to hydrocarbon biodegradation

duced membrane lipids with an odd number of carbon atoms when fed alkanes with an even number of carbon atoms. This suggests that the alkane chain undergoes the removal or addition of an odd number of carbon atoms in this organism, which contrasts with the strictly even removal of β-oxidation. This even-to-odd transformation has not been seen in subsequently identified alkane-degrading anaerobes and each species is specific to a limited range of chain lengths, indicating that a range of novel pathways are used (Heider *et al.* 1999).

There is evidence that short-chain alkylbenzenes are degraded anaerobically: Toluene (methylbenzene) is degraded under methanogenic conditions by organisms isolated from landfill sites (Wang and Barlaz 1998). A review article by Heider *et al*. (1999) describes catabolism of toluene to benzoyl CoA (a central intermediate in anaerobic catabolism of aromatic compounds) under sulphate-reducing, denitrifying and iron-reducing conditions. They describe catabolism of ethylbenzene by denitrifying bacteria, but note that the pathways for toluene and ethylbenzene are different, and indeed that most anaerobic hydrocarbon degradation pathways are quite novel. Catabolism of propylbenzene by sulphate-reducing bacteria was also reported. Denitrifying bacteria have been shown to possess the CoA ligases required for the formation of acyl-CoA (Villemur 1995). A number of alternative pathways for toluene have been proposed (Chee-Sanford *et al.* 1996; Heider *et al.* 1999) and toluene has been shown to be attacked by hydrolysis of the benzene ring (Grbic-Galic 1991). Anderson and Lovley (2000) showed that benzene can be degraded under sulphate-reducing conditions. Recent work on toluene, ethylbenzene and xylenes in aquifers (Elshahed *et al.* 2001) indicate that all are degraded to benzoate, with the production of methane, under sulphate-reducing conditions. It was suggested that the presence of intermediates from these pathways in the environment might be used to confirm that the contaminants are being degraded *in situ*. Other studies have recently led to the isolation of enzymes from denitrifying bacteria capable of further conversion of benzoyl CoA under anaerobic conditions (Boll *et al.* 2000). It has been shown that oxygenases involved in toluene degradation may be expressed, and function, at low DO (Costura and Alvarez 2000). A minimum DO is required for the oxygenation reaction itself, but it does indicate that at least some enzymes associated with aerobic degradation may be functional in an environment where (aerotolerant) anaerobic respiration prevails. At lower redox values, oxygenases are unlikely to function, and breakdown is more likely to be via hydrolysis. Studies on the degradation of benzoate by the phototropic bacterium *Rhodopseudomonas palustris* and the denitrifiers *Thauera aromatica* (formerly *Pseudomonas* sp strain K172) and *Azoarcus evansii* (formerly *Pseudomonas* sp strain K740) have led to the partial elucidation of a number of pathways (Harwood and Gibson 1997; Koch *et al.* 1993). The studies, using nuclear magnetic resonance (NMR) have demonstrated the sequence of breakdown products, though the enzymes involved have yet to be confirmed. There have not been any reports of higher alkylbenzenes being degraded anaerobically prior to Herbath (2001).

PRELIMINARY INVESTIGATION

A model of an underground cable joint bay (‘mini pit’) was constructed to provide data on the conditions that develop following a leak, for comparison with other studies (Cheston 1997; Tebbutt 1998; Koussia 1999; Herbath 2001). It also allowed the assessment of commercially available sensors and control technology (Johnson 2000).

The soil used in this study was a Horizon B soil of the DeBathe series, as used in related studies (Koussia 1999; Herbath 2001). It is characterised in Table 2. The original choice of this soil was based on its common occurrence in association with underground cables and its easy availability. The low organic carbon content was an advantage since any appreciable biological activity would probably be due to the added carbon source in the presence of adequate nitrogen and other nutrients. The soil was packed in the ‘mini pit’ (see below) according to a protocol devised for a larger model system (Macdonald 2000) such that the structure of the soil would be retained and the creation of preferential (‘short-circuit’) pathways avoided.

The mini pit (Johnson 2000) was constructed from a high-density poly(ethylene) (HDPE) container (C1400 4-way entry tank from Mailbox Mouldings International), with an internal volume of approximately 1 m^3. Injection and extraction wells were assembled from drilled PVC drainpipe. Time domain reflectometry (TDR) probes were placed in the soil as it was loaded to allow monitoring of water content. TDR has become a recognised method of measuring water content of soil (Davis and Chudobiak 1975; Davis and Annan 1977; Topp *et al.* 1980). The experimental facility had no temperature control. The mini pit was contaminated with 5 L of cable oil, added as a single bolus through the injection well. It was inoculated with microorganisms cultured with cable oil as a sole carbon source, taken from a larger model system which had been contaminated with cable oil some seven months previously (Herbath 2001). Nutrients were added to provide final concentrations of 100 mg N kg^{-1} soil and 45 mg P kg^{-1} soil (Margesin and Schinner 1999).

Table 2. Analysis of soil prior to contamination, from Andrews (1999) except* (Lovelace 1999)

Parameter	Value
Dry matter content of air dried soil	98.5%
Water content of air-dried soil	1.6%
Particle size distribution:	
Sand (63 μm – 2 mm)	79.62%
Silt (2 μm – 63 μm)	12.63%
Clay (< 2 μm)	7.75%
Organic carbon	0.1 mg kg^{-1}
$pH_{1:5\ soil:water\ extract}$	5.7
$Manganese_{1:5\ soil:water\ extract}$	0.3 mg kg^{-1}
$Calcium_{1:5\ soil:water\ extract}$	0.5 mg kg^{-1}
$Copper_{1:5\ soil:water\ extract}$	< 0.05 mg kg^{-1}
$Nickel_{1:5\ soil:water\ extract}$	0.3 mg kg^{-1}
$Ammonium_{1:5\ soil:water\ extract}$	65.9 mg kg^{-1}
$Nitrate_{1:5\ soil:water\ extract}$	34.1 mg kg^{-1}
$Phosphate_{1:5\ soil:water\ extract}$	< 0.05 mg kg^{-1}
$Sulphate_{1:5\ soil:water\ extract}$	3.8 mg kg^{-1}
$Carbonate_{1:5\ soil:water\ extract}$	< 0.05 mg kg^{-1}
$Bicarbonate_{1:5\ soil:water\ extract}$	164.8 mg kg^{-1}
Bulk density	1.3 g cm^{-3}, 1.51 g cm^{-3}*
Particle density	2.7 g cm^{-3}
Water holding capacity (WHC):	
Maximum	30.7%
0.05 bar	17.2%
01. bar	14.0%
0.4 bar	11.3%
2 bar	9.2%
15 bar	5.8%
Saturated hydraulic conductivity *	1.54 m day^{-1}
Gravimetric moisture content at saturation *	23.39%
Porosity *	42.83%
Volumetric moisture content at saturation *	35.30%

Groundwater was recirculated at a rate of about 5 L day^{-1} via an overhead reservoir, which allowed access for measurement of physico-chemical parameters. The reservoir was open to the atmosphere to allow oxygen to diffuse into the water prior to reinjection. Temperature, pH, oxidation-reduction potential (ORP) and dissolved oxygen (DO) were recorded from the points of water input and extraction. In order to measure conditions in the water being extracted from the mini pit without exposure to atmosphere, a sealed flow-through cell was used (Herbath 2001). For comparison, a mathematical model of the soil and groundwater geochemistry was constructed using PHREEQC (Parkhurst and Appelo 1999), a computer modelling application provided by the United States Geological Survey.

Samples of soil from the mini pit were cultured on nutrient agar and on a selective agar containing Bushnell-Haas salts (Atlas 1993) with 0.05% cable oil as the sole carbon source (DDB agar). A fluorometric technique (Fu *et al.* 2000) was used to assess the level and distribution of cable oil in the soil.

RESULTS AND DISCUSSION

Following contamination of the soil in the mini pit with cable oil, the oil became localised to the upper regions of the saturated zone. In the field, it is likely that this region can alternate between aerobic and anaerobic conditions and hence LAB may be exposed to a range of redox states. The mini pit system exhibited anaerobic conditions in the upper saturated zone, as evidenced by pH, oxidation-reduction potential and dissolved oxygen (see below). The monitored data show three major perturbations. These were caused by pump failures and the removal of water from the reservoir to aid nutrient addition.

The pH of water in the reservoir (IN) and flow-through cell (OUT) are shown in Figure 3. The pH of the water being pumped from the soil was consistently lower, at approximately 6.0, compared to that in the reservoir (6.5 – 7.0). The groundwater pH was thus slightly lower than the suggested optimum levels for bioremediation of 7.8 (Dibble and Bartha 1979) or 6.5 – 8.5 (Ritter and Scarborough 1995). An increase in pH upon exposure to the atmosphere was noted. This may be due to the loss of the buffering effect of dissolved bicarbonate, which escaped into the atmosphere from the water surface as CO_2, or it may be that aerobic microorganisms in the reservoir removed acidic products of anaerobic microbial metabolism.

The variation in oxidation-reduction potential (ORP) is shown in Figure 4. The instruments used to record ORP were only able to read down to about +5 mV, which translated to a Standard Potential of about –200 mV when corrected for the Ag/AgCl reference electrode. This gives an artificial 'plateau' effect in the trace of ORP OUT. To account partially for this, occasional spot checks were made using a bench pH meter connected to the same ORP electrode. These are plotted as single points (filled triangles in Figure 4) and indicate that the ORP in the groundwater was approximately -600 mV for most of the investigation. The soil analysis (Table 2) indicated that nitrate, sulphate and bicarbonate were present and thus potentially available for use as terminal electron acceptors by soil microor-

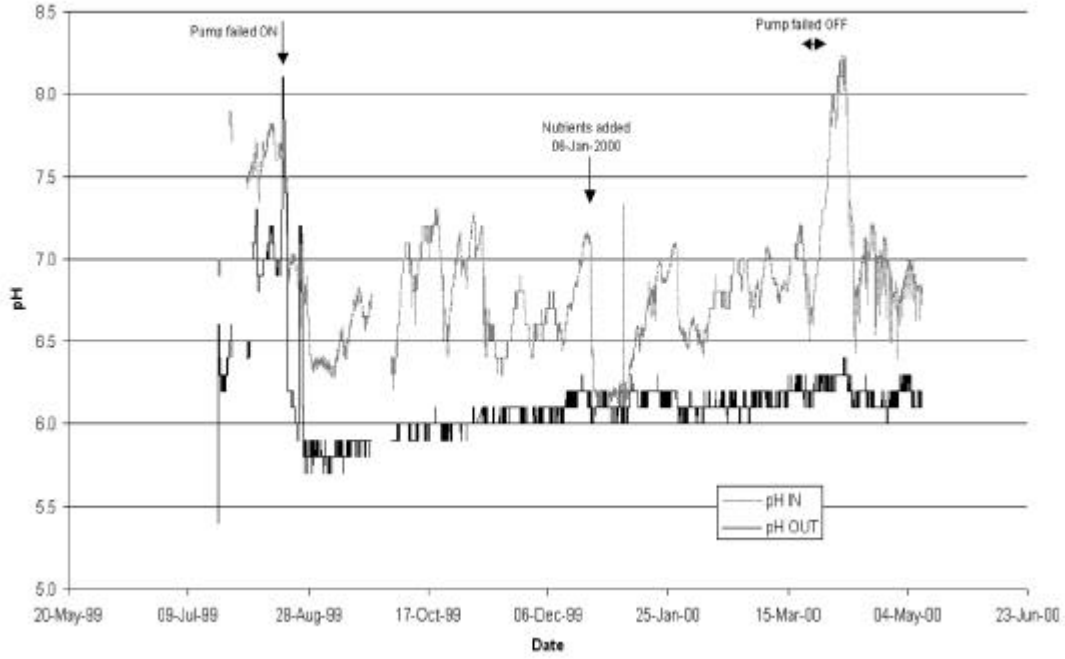

Figure 3. Variation of groundwater pH

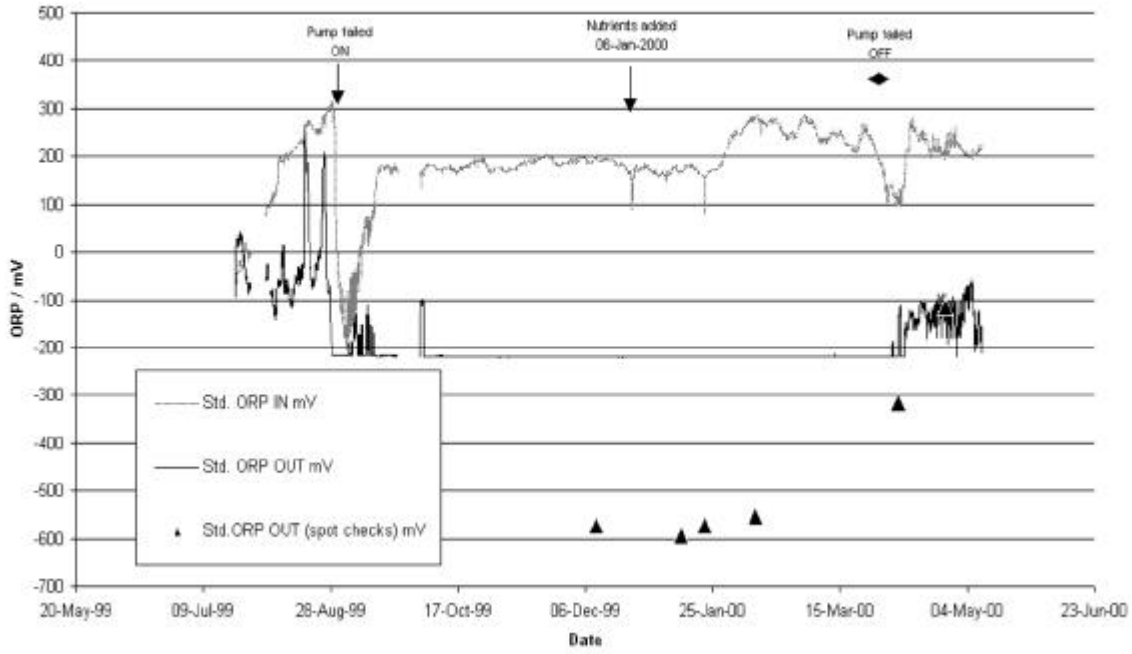

Figure 4. Variation of groundwater oxidation-reduction potential

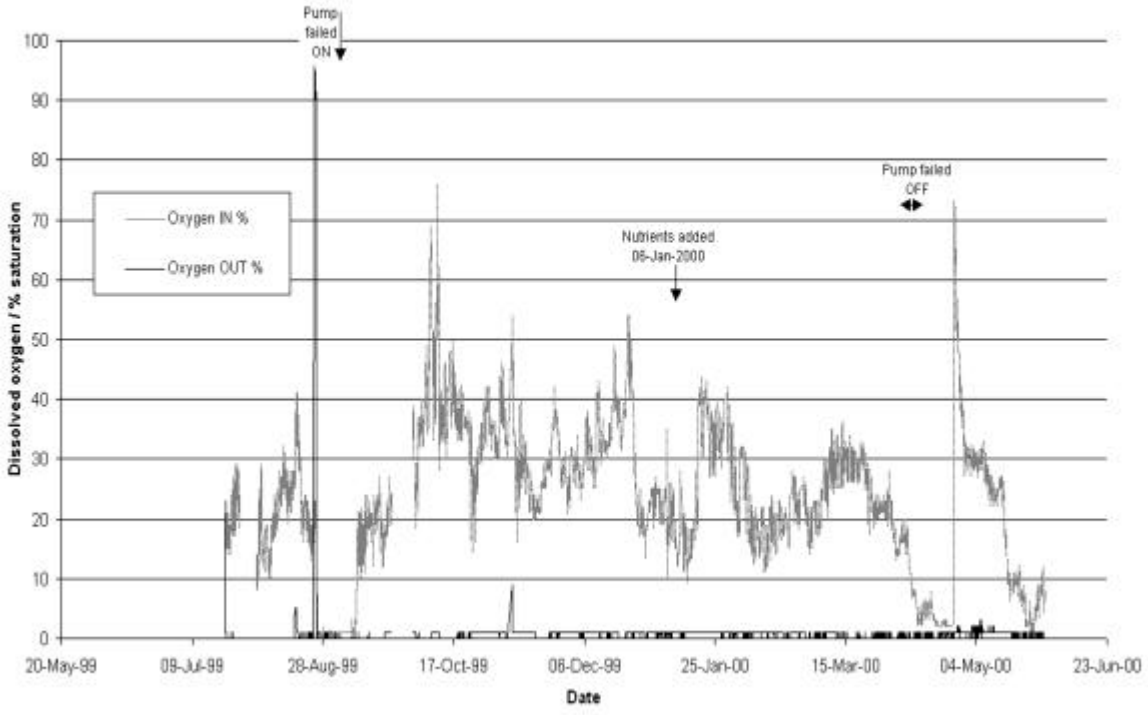

Figure 5. Variation of groundwater dissolved oxygen

ganisms. The low ORP observed in the groundwater is consistent with reduction of sulphate to sulphide. Further evidence of this reduction was the odour of hydrogen sulphide, noted when the settlement tank became anaerobic after the pumping system failed in the ON position. These signs suggest that microbial flora in the groundwater included sulphate-reducing organisms. A similar odour was reported by Herbath (2001) on dismantling anaerobic tanks used to investigate the removal of cable oil from soil under a variety of nutritional and an/aerobic regimes. In summary, the combination of low ORP, known availability of sulphate in the soil and the distinctive odour indicate that sulphate reduction played a significant part in the chemistry of the mini pit. It is highly likely that this was due to anaerobic biological activity, probably associated with cable oil degradation, since this was the only significant carbon source.

Figure 5 shows the temporal changes in dissolved oxygen (DO) in the groundwater. The events that were observed to perturb the pH also affected these readings. With the exception of the first pump failure, the DO reading from the flow-through cell did not depart appreciably from zero. The water being pumped out of the soil contained no measurable oxygen, this suggests that there were aerobic processes occurring. These may have been biological but not necessarily so. If conditions in the soil were already strongly reduced, the oxygen could be consumed by chemical oxidation of the reduced products of anaerobic respiration. Since there were only two points of DO monitoring, there is no way of knowing how quickly the oxygen was used up but it is likely that any available oxygen was depleted near the injection well.

Figure 6 shows the distribution of aerobically cultured soil microorganisms within a vertical soil core from the mini pit. There are enhanced numbers of microorganisms near the water table (~ 600 mm) and below, where there is more water and a carbon source in the form of cable oil. Microorganisms capable of using cable oil as a sole carbon source (cable oil-degrading microorganisms, CDMs) were found to grow under aerobic conditions. This had previously been found by Cheston (1997) and Tebbutt (1998). The organisms isolated from DDB agar were all Gram positive and some were observed to form hyphae and spore-like structures. These are all features of the actinomycetes. Members of this group include the genus

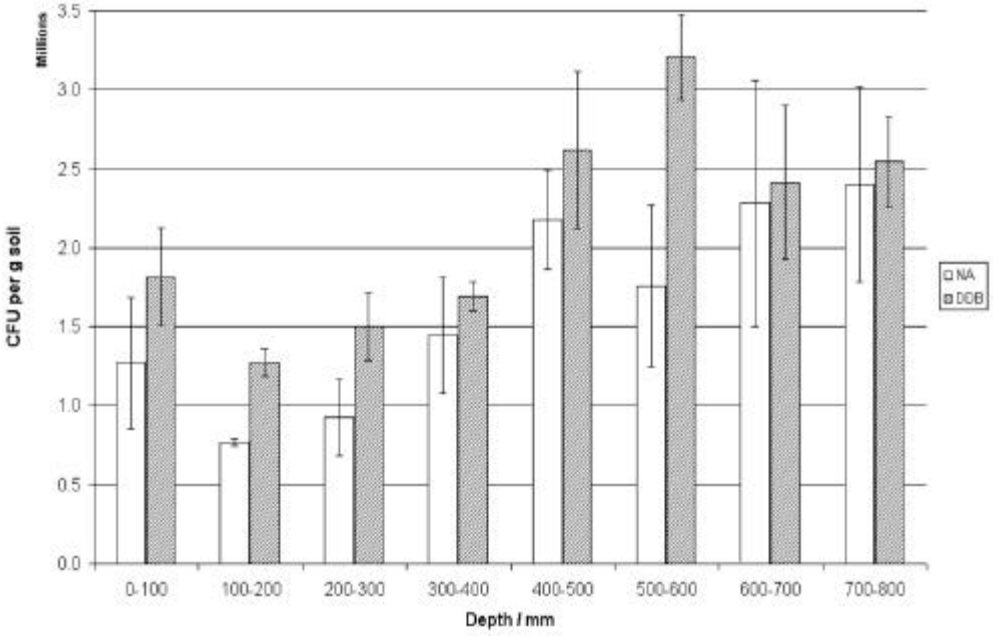

Figure 6. Vertical distribution of aerobic soil microorganisms (Error bars = 1 Std. dev., n = 3, NA = Nutrient agar, DDB = 0.5% cable oil (dodecylbenzene) agar)

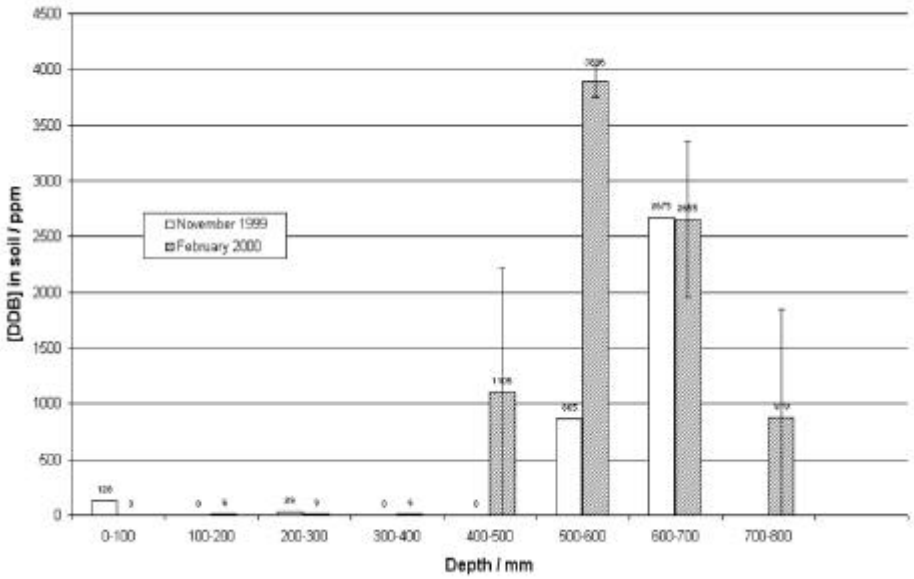

Figure 7. Cable oil distribution (Error bars on data from February 2000 = 1 Std. dev., n = 2, water table ~ 600 mm below soil surface)

Nocardia, which has been demonstrated to degrade LABs (Bhatia and Singh 1996). Many aerobic microorganisms are able to persist in a dormant form under anaerobic conditions. Hence, the existence of colony-forming units of cable oil-degrading aerobes in the soil, even where there is cable oil, is not evidence that biodegradation by the particular microorganisms isolated is taking place. It is also possible that a proportion of these organisms may be facultative anaerobes, able to grow under nitrate reducing (possibly denitrifying) conditions. Herbath (2001) demonstrated anaerobic soil organisms capable of survival on cable oil and mineral salts alone. She reported a significant reduction in cable oil concentrations within six months in soil under anaerobic conditions at 8°C, suggesting that natural attenuation of cable oil in anaerobic groundwater may be significant.

Soil cores were analysed for cable oil content (Figure 7). The data confirms that the cable oil was concentrated at or near the water table. This localisation of the oil was consistent with there being free oil partitioned at the water table (~ 600 mm depth, confirmed by TDR readings) and adsorbed onto soil particles, with only a very small proportion in solution. The data do not indicate that oil concentrations decreased with time, but since the sampling was destructive, the positions of each core were different. The apparent increase in oil concentrations was probably due to spatial inhomogeneity in the oil distribution (Macdonald 2000).

PHREEQC (Parkhurst and Appelo 1999) was used to construct a mathematical model of the soil and groundwater geochemistry using soil analysis data from Table 2 and including the added nutrients. The results of running the model (details not shown here) clearly indicate that the soil chemistry was unable to account for the highly reduced conditions and odour of hydrogen sulphide, suggesting that biologically-mediated mineralization of organic matter and nutrients must have occurred.

CONCLUSIONS

It appeared that the oil added to the soil was strongly localised to the upper saturated zone, where both aerobic and anaerobic conditions may occur. Previous investigations into cable oil degradation in soil assumed that conditions would be aerobic (Cheston 1997; Tebbutt 1998; Koussia 1999), and all of the degradative pathways so far described are aerobic. However, this study indicates that this is not necessarily the case. LABs are easily degraded under aerobic conditions, but appear to be less susceptible to anaerobic breakdown, possibly due to differences in the available degradative pathways of different isomers. However, there is strong evidence from this work that biological activity can occur in soil under anaerobic conditions, with cable oil as the only significant carbon source. This is in agreement with Herbath (2001), who found that anaerobic removal of cable oil in soil does occur. While anaerobic degradation of LAB has not been demonstrated previously, the last decade or so has seen great advances in the understanding of anaerobic catabolism of hydrocarbons. LAB shares structural features with *n*-alkanes and with BTEX compounds. Examples of these compounds have been shown to be degraded under a variety of redox regimes (Aronson and Howard 1997; Harwood and Gibson 1997; Heider *et al.* 1999). It is not unreasonable to surmise whether LABs may be vulnerable to the same processes. The most likely route for anaerobic degradation of LABs will probably be an initial attack on the alkyl chain(s) to form an acyl CoA, followed by a hydrolytic ring cleavage.

This suggests that, in the case of oil contamination in urban areas, places with restricted access or larger areas of low-level contamination where excavation of the soil would be difficult, monitored natural attenuation (MNA) may provide a practical solution to dealing with cable oil contamination. If degradation rates were demonstrated to be high enough, MNA would likely be the preferred method. This strategy would require a fuller understanding of the microbiology and biochemistry of anaerobic degradation since it may be necessary to use enhanced natural attenuation – adding appropriate nutrients and electron acceptors – in some scenarios. In addition, the critical degradation components would need to be identified to determine the rate of degradation and provide a measure of the level of contamination remaining. Aerobic cable oil degrading microorganisms have been demonstrated in anaerobic regions of the soil, possibly as inactive spores that are able to grow once oxygen is available. If natural attenuation in these conditions proves to be too slow, there remains the option, for localised areas of contamination, of manipulating conditions to provide an environment suitable for the faster metabolism of aerobic microorganisms.

Further investigations into the fate of cable oil under a range of redox conditions are under way, using GC-MS to examine isomeric composition of the oil and any associated degradation products.

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