CRANFIELD UNIVERSITY

STEPHEN J. JOHNSON

MONITORING AND CONTROL OF *IN SITU* BIOREMEDIATION OF SOIL CONTAMINATED WITH CABLE OIL

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Stephen J. Johnson

"Monitoring and control of *in situ* bioremediation of soil contaminated with cable oil"

Supervisors:
Professor Naresh Magan, IBST, Cranfield University
Dr Daxaben Patel, National Grid Company plc
Mr David Crellin, Abington Partners

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Abstract

Linear alkylbenzene (LAB) cable oil is used to insulate underground electricity transmission cables in England and Wales. This investigation was intended to provide data on the potential for bioremediation of soil and groundwater contaminated with cable oil, and to assess the suitability of Control Cube datalogging and control technology as the basis for a potential bioremediation monitoring and control system.

A model of a cable joint bay was constructed, contaminated with cable oil and inoculated with microorganisms from a previously contaminated site. Dissolved oxygen, pH, and oxidation-reduction potential in recirculated ground water were monitored before and after the addition of a nutrient solution. A microbiological investigation of the soil used both selective and non-selective media. The distribution of cable oil in the model was studied by fluorometry.

It was found that the soil and ground water contained aerobic cable oil-degrading microorganisms (CDMs). Some of these were isolated and tentatively identified as Actinomycetes. Fluorometric investigation revealed that the cable oil was localised to the upper regions of the saturated zone. Conditions in the ground water were shown to be predominately anaerobic, with no evidence of significant removal of cable oil. Therefore, there was scope for the improvement of the rate of cable oil attenuation through biostimulation – manipulation of conditions by the addition of nutrients and appropriate terminal electron acceptors, specifically oxygen, to encourage the growth of known CDMs.

Control Cube and associated sensors and software were shown to have a number of features that would be desirable in a monitoring and control system for an active bioremediation effort. However, it was thought that the complexity of the interactions between the contaminant, the environment and CDMs was such that a simple feedback control system would not be appropriate and a predictive model would be required in order to allow feed-forward control.

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Nomenclature

Σ	Sum
λ	wavelength / m
ν	frequency / Hz
θ	Volumetric water content
ε	Dielectric constant
°C	Degrees Celsius
AU	Absorbance unit
ATP	Adenosine triphosphate
BTEX	Benzene, toluene, ethylbenzene, xylene
c	Velocity of light in a vacuum $\approx 3 \times 10^{8}$ m.s^{-1}
CDM	Cable oil degrading microorganism
CFU	Colony Forming Unit
DC	Direct current
DDB	Dodecylbenzene
dO_2	Dissolved oxygen concentration
E	Potential / V
E°	Standard potential / V
GMS	Glucose and mineral salts
h	Planck's Constant = 6.63×10^{-34} J
$H_2CO_3^*$	Sum of H_2CO_3 and $CO_{2(aq)}$
HPLC	High Performance (or Pressure) Liquid Chromatography
LAB	Linear alkylbenzene

LAS	Linear alkylbenzene sulphonate
M	Mole, Molar
MEA	Malt extract agar
n	Number of samples
NA	Nutrient agar
NAPL	Non aqueous phase liquids
NGC	National Grid Company plc
ORP	Oxidation-reduction potential
PAH	Polyaromatic hydrocarbons
PC	IBM compatible personal computer
PP	Pyrophosphate
ppm	Parts per million
PTFE	Poly(tetrafluoroethylene), “Teflon”
RO	Reverse osmosis
rpm	Revolutions per minute
SD	Standard Deviation
TDR	Time-domain reflectometry
TEA	Terminal electron acceptor
TMP	Trimetaphosphate
TPP	Tripolyphosphate

Chapter 1

Introduction

1.1 BACKGROUND

National Grid Company plc. (NGC) are responsible for the transmission of electricity in England and Wales, and interfacing with their counterparts in Scotland and France. Abington Partners are a small independent firm specialising in the design and development of data logging and control equipment and software. This project uses Abington technology to investigate an existing NGC problem – the occasional leakage of cable oil into soil and ground water.

Long distance transmission of electricity in the United Kingdom is by means of high-tension (up to 400 kV) overhead and underground cables. Where the cables are underground, a wrapping of paper impregnated with cable oil insulates them from the surrounding soil. Previously the oil was a mineral oil but more recently a well-defined mixture of C_{10}-C_{13} linear alkylbenzenes (LABs) has been employed (Rowlands, 1996). The mixture is dominated by isomers of C_{12} alkylbenzene and is thus generally referred to as dodecylbenzene (DDB).

The cable oil is fed to the cable from constant head tanks at a pressure of 0.3 to 0.5 bar. The topography of the system is such that transient pressures of up to 8 bar may occur. Once filled, the movement of oil within the cable is negligible (mainly due to thermal expansion and contraction) and so any significant change in the level of the header tanks is indicative of a leak. Hydraulic tests will detect leaks of 40 $L.month^{-1}$ or greater. Release of cable oil may be due to damage to the cable itself or, more often, a failure at the joint between two cables.

In the event of a leak, which may vary from as little as 20 $L.week^{-1}$ to as much as 100 $L.week^{-1}$ (Patel, 2000), cable oil is released into the surrounding matrix and distributes itself according to the hydrogeology of the location. Migration away from the point of release appears to be preferentially along the track of the cable, probably because of the increased porosity of the backfill. A proportion of the oil also makes its way into the surrounding soil, where it becomes partitioned between aqueous solution, adsorption to soil particles, evaporation to air spaces and, predominantly, free cable oil as non-aqueous phase liquid (NAPL).

Current techniques to deal with leaks involve excavation of the matrix to effect a repair, and removal of all the contaminated materials to controlled landfill sites. This approach is expensive since it requires the cable to be taken off-line, resulting in power outages or costly re-routing exercises. Also, since underground cables are often used in urban areas or environmentally sensitive locations where overhead cables are inappropriate, there may be difficulties in gaining access to the cable.

There will probably always be a need, with significant releases of cable oil, for the immediate area to be excavated to repair the source of the leak. An alternative method of dealing with smaller leaks, and remediating the residual oil where it has migrated away from the leak, is sought.

Much literature is available on the fate of LABs in the environment due to their use in the manufacture of many detergents in common use. Nichols (1996) found that

indigenous soil microorganisms readily break down DDB. This would make it a potential candidate for bioremediation.

Bioremediation is "the use of microorganisms or microbial processes to detoxify and degrade environmental contaminants" (Baker & Herson, 1994). It can be applied to a wide range of *in* and *ex situ* techniques.

A number of studies have been undertaken at the Institute of BioScience and Technology, starting in 1996. These have been aimed at the characterisation of cable oil in soil and ground water – its mobility, toxicity, bioavailability and fate. As part of these on-going studies, a scheme for the *in situ* remediation of cable oils through biostimulation has been suggested.

1.2 OBJECTIVES

This project was aimed at improving the efficiency of a proposed in situ bioremediation strategy for cable oil in soil and groundwater through the use of an integrated sensor and control system.

To achieve this, a large scale, contained model of a DDB contaminated site was constructed to investigate the process and to allow the assessment of Control Cube (a datalogging and control interface, developed by Abington Partners) and associated sensors, as the basis for the monitoring and control system of a bioremediation installation.

1.3 STRATEGY

The project involved several steps:

- Design and construction of a $1m^3$ model system for the study of the bioremediation process
- Use of the Control Cube to control a pump in response to water level in an extraction well
- Time Domain Reflectometry (TDR) to identify and monitor water distribution
- Use of the Control Cube as a data logger for temperature, pH, oxidation-reduction potential (ORP) and dissolved oxygen concentration (dO_2)
- Monitoring the effects of water recirculation on the logged parameters
- Microbiological analysis of soil and groundwater
- Fluorometric analysis of DDB distribution
- Addition of nutrients and further monitoring

Chapter 2

Literature review

2.1 INTRODUCTION

In 1995, it was estimated that there were between $5x10^4$ and $1x10^5$ hydrocarbon-contaminated sites in the UK alone (Shevah & Waldman, 1995). NGC cables account for a tiny minority of these, but lessons learnt in finding a strategy for dealing with cable oil may well have applications in the treatment of other hydrocarbon pollutants.

Decontamination of oil-polluted soil is the sum of abiotic and biotic losses. The significance of abiotic losses is not to be underestimated, especially in recently contaminated sites (Margesin & Schinner, 1999). This chapter is intended to justify the choice of a remediation strategy for cable oils by reference to related work, and to identify the requirements of any control system.

Due to the inaccessible nature of likely cable oil releases, a number of studies have been carried out into the susceptibility of cable oil to *in situ* bioremediation. In general, bioremediation cannot reduce contamination to zero (Margesin and Schinner, 1999) but, properly applied, it ought to be possible to economically reduce pollutants to acceptable levels.

A number of strategies are available for the bioremediation of xenobiotic contaminants. The choice of which to use depends on a wide range of factors, including:

- The chemistry of the contaminant
- The hydrogeology of the site

- The physical transport characteristics of the contaminant
- The availability of microorganisms able to degrade the contaminant
- Accessibility and cost of remediating the site by other means.

2.2 STANDARDS AND LEGISLATION

Environmental legislation in England and Wales is centred on the Environment Protection Act (HMSO, 1990), which provides for the formation of the Environment Agency as a centralised enforcement agency (Part VII) and, significantly for this study, forms the basis for subsequent contaminated land legislation (Part IIA).

Section 57 of the Environment Act (1995) added sections on the identification and remediation of contaminated land, advocating a risk-based approach and defining contaminated land as:

"…any land which appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in, 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." (HMSO, 1995).

Most recently, the Contaminated Land (England) Regulations (HMSO, 2000) came into force on 1 April 2000. The Regulations require Local Authorities to identify and maintain a register of "Special Sites" by virtue of land use and proximity to controlled

waters. Schedule 1 defines materials that are considered to constitute polluting materials (including mineral oil and other hydrocarbons) and lists vulnerable, aquifer-bearing rock formations.

The policy of the Environment Agency (Crowcroft, 2000) is "to encourage voluntary action by polluters or other appropriate persons" and "to encourage the use of remedial technologies which are effective in changing, fixing or transforming contaminants into less harmful substances, or reducing their volume or availability, thus reducing pressure on disposal to landfill". It is in this spirit that National Grid is funding research into the environmental fate and potential bioremediation strategies for cable oil.

There are no recommended environmental levels specifically for linear alkylbenzenes (LABs), but the Dutch Standard target and intervention values for oil in soil are 50 $mg.kg^{-1}$ and 5000 $mg.kg^{-1}$, respectively (Dutch Intervention Values, 1994) and these are the standards most often used for cable oil.

2.3 CHEMISTRY

2.3.1 Structure

Linear alkylbenzenes (LABs) are used as cable oils in electrical cables in England and Wales. Previously, mineral oil was used and this is still the case in many countries. Branched alkylbenzenes were used for a time and, like mineral oil, are still found in older, undamaged cables but by far the most common oil found in NGC cables, and hence the most significant potential pollutant from this source is dodecylbenzene (DDB).

In fact, what is often colloquially referred to as dodecylbenzene was characterised by Rowland (1996) as being a mixture containing 99% C_{10}-C_{13} phenylalkanes. A number of isomers exist, differing in the position of the benzene ring on the carbon backbone. C_5 to C_2 isomers of decylbenzene and C_6 to C_2 isomers of un-, do- and tri-decylbenzenes were found. C_1 isomers were entirely absent. The general term dodecylbenzene will be used to refer to this commercial mixture (Fig 2.1).

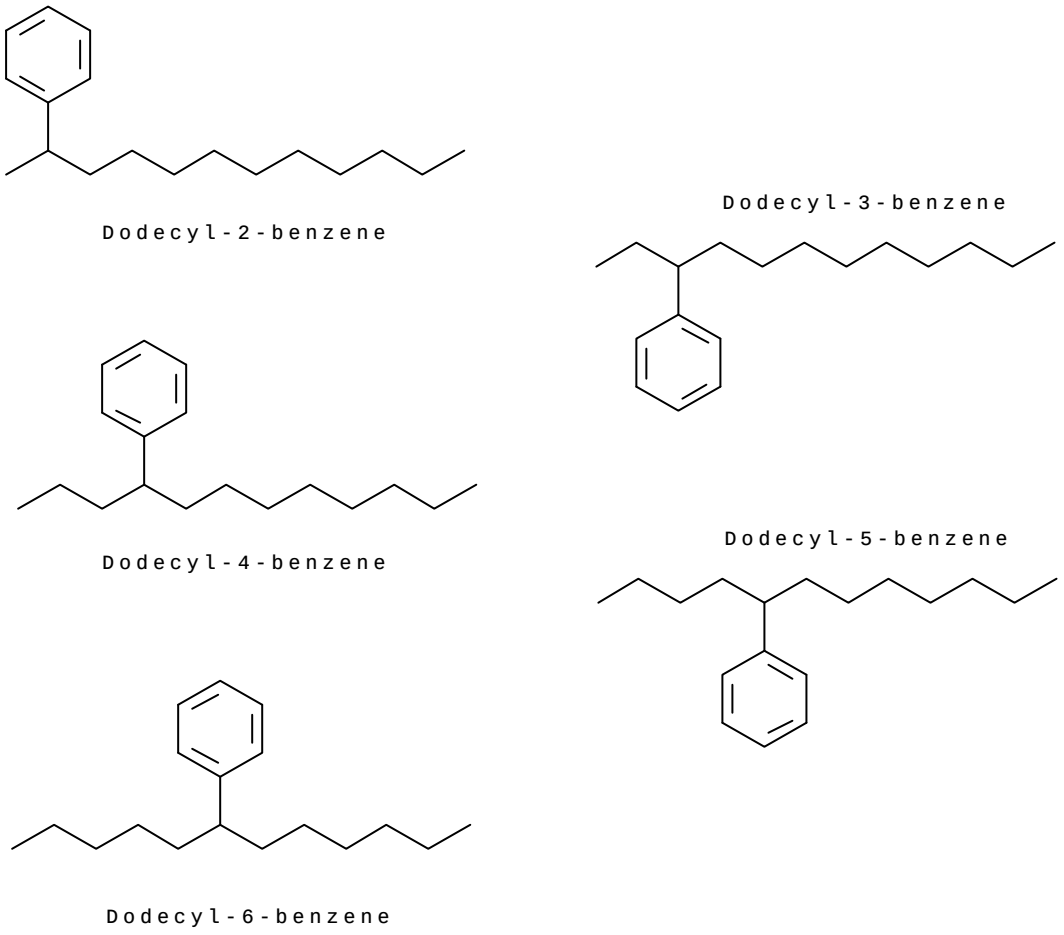

FIGURE 2.1 – Isomers of C_{12} LAB in commercial dodecylbenzene (C_{10} to C_{13} LABs not shown)

That the phenyl group is not found on the terminal carbon atom in synthetic alkylbenzenes had previously been noted. The lack of C_1 isomers is thought to be due to the requirement for an unstable primary carbonium ion intermediate, which would make the formation of this isomer energetically unlikely (Eganhouse, 1986).

With the exception of all terminal isomers and the central isomers of C_{odd} LABs, all the isomers of linear alkylbenzenes are chiral (Figure 2.2). The carbon atom to which the phenyl group is attached forms a chiral centre with each of its tetrahedral bonds being with a different group. In the case of the terminal isomer, the carbon atom is bonded to two hydrogen atoms. This is not an issue in cable oil since we have already seen that the terminal isomer is absent. In the case of the highest isomer of a C_{odd} LAB such as undecyl-5-benzene or tridecyl-7-benzene, both of which are found in DDB, the molecule is not chiral. Since cable oil is synthesised chemically, it is likely that both chiral forms of each isomer are present in equal amounts. This may have implications for enzymatic breakdown.

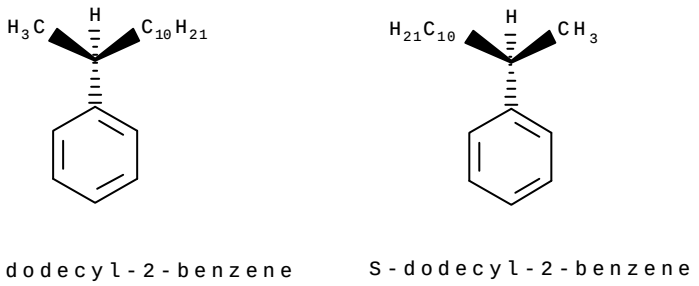

FIGURE 2.2 – Chirality of a linear alkylbenzene

2.3.2 Properties

Physical properties of the LAB are summarised in Table 2.1. Figures for a C_{12} LAB are given where possible. Where a range of figures is given, this reflects the varying isomeric properties:

TABLE 2.1 Physical properties of linear alkylbenzenes (LAB)

PROPERTY	**VALUE**
Appearance	Clear liquid (1)
Density at 20 °C	0.86 $kg.L^{-1}$ (1)
Boiling point	726 °C (1)
Kinematic viscosity at 20°C	8.1 $mm^2.s^{-1}$ (1)
Flashpoint	150 °C (1)
Aqueous solubility	Immiscible, <0.01$mg.l^{-1}$ (1) 0.41 $mg.l^{-1}$ (2) 4-7 $nmol.l^{-1}$ (3)
Vapour pressure at 25°C	4.9 x 10^{-4} mmHg (2) 0.038 – 0.067 Pa (3)
Henry's Law constant	7.1 x 10^2 $torr.L.mol^{-1}$ (2)
Soil partition coefficient, K_{oc}	2.2 x 10^4 (2)
Log octanol:water partition coefficient, K_{ow}	5.72 – 5.75 (2) 4.97 – 5.08 (3)
Source: *1 – Shell health, safety and environment data sheets* *2 – Gledhill, 1991* *3 – Sherblom et al., 1992*	

It is possible to make some predictions about the partitioning of cable oil between phases when it is in the soil. The low vapour pressure indicates that there is little

movement from the free liquid phase to air. The low Henry's Law constant indicates that there will be little movement from aqueous phase to air. Together, these mean that a very small proportion of the LAB will be found in air-filled voids. The high Log octanol:water partition coefficient (measure of lipophilicity/hydrophobicity) and Soil partition coefficient indicates little movement from free liquid to water. In terms of the amounts of material in each phase, [Free LAB] > [aqueous phase] > [gas phase], i.e. there will be a strong tendency for cable oil to persist as free LAB in soil; relatively little will be in solution and only a very small proportion will evaporate into gas-filled pores.

Since biodegradation relies on the material being available to microorganisms – either dissolved in water or as very small particles – it is likely that the rate of dissolution of LAB will be the ultimate limiting factor in the rate of biodegradation.

2.4 HYDROGEOLOGY

The hydrogeology of a site is the main influencing factor on the spatial extent of contamination by cable oils (Nichols, 1996). Hydrogeology will also play a major part in the choice of treatment system to be employed. It is thus useful to summarise some of the key concepts.

Figure 2.3 shows the main hydrogeological zones in a stylised area of soil and rock. The bedrock is a consolidated mass and is more or less impermeable. The overlying regolith is composed of a matrix of boulders, rocks, gravel, sand, silt, clay and organic material. The ground water is distributed such that there is a saturated zone, where all the pore

spaces are completely filled. The top of this zone, where the water level in a well dug through it would lie, is called the water table. Above the water table is a region, the capillary fringe, where the soil is saturated with water drawn up from the water table by capillary action.

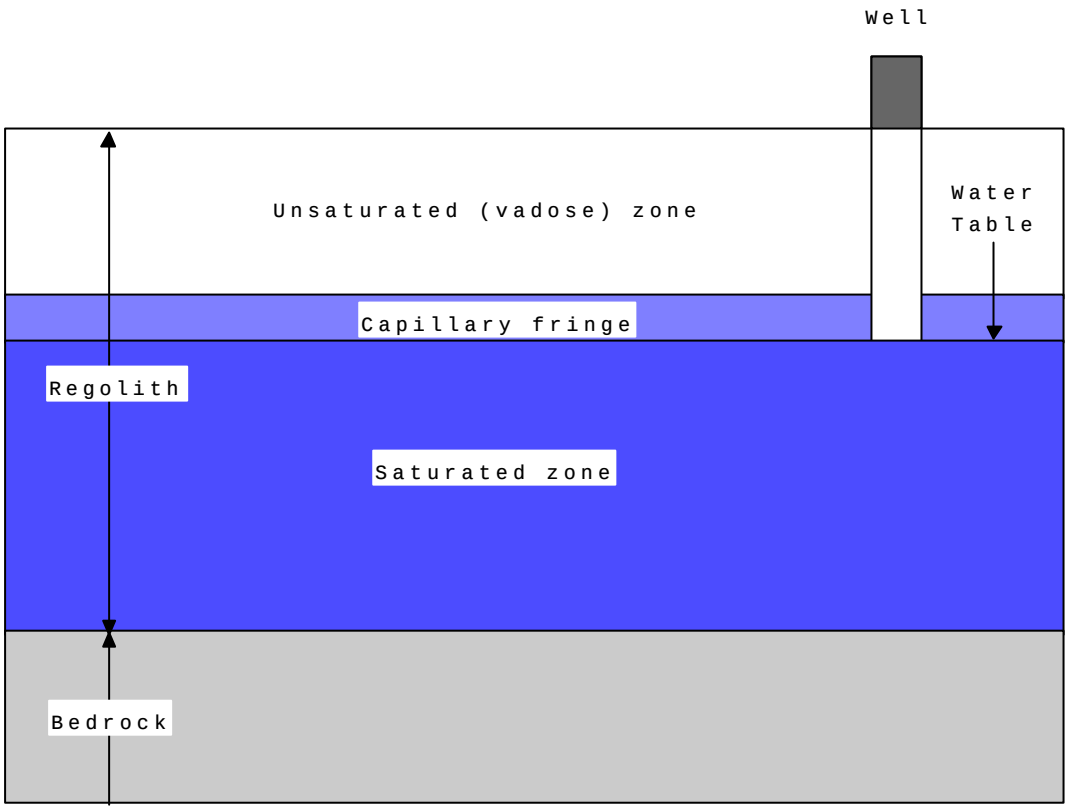

Figure 2.3 – Hydrological zones in soil

The water table is not always horizontal, but will reflect the topography of the bedrock and the overlying surface, the permeability of the regolith (which may not be uniform) and any points of abstraction or infiltration. Bodies of surface water may be permanent – where the water table is higher than the local surface level, or the area is underlain by an impermeable regolith structure such as a clay lens; or temporary – present only until recent precipitation percolates down to the water table. The water table is usually depressed around an extraction point and raised around a point of injection– the extent

of this depends on the rate of extraction and the permeability of the soil. Water in the soil will flow in response to a hydrodynamic gradient – a pressure difference due to gravitational or other forces. Above the capillary fringe, water is found adhering to the surface of the soil particles but the pores around the solid material are filled with gas.

If a non-aqueous phase liquid (NAPL) such as DDB is released into the ground it will flow according to the hydrodynamic conditions. Once it reaches the water table it will sink or float, depending on its density. DDB is less dense than water and it is relatively imiscible and so it will tend to float on the water table. Other factors that affect the mobility and distribution of DDB after a leak include its viscosity, and the degree to which it is adsorbed onto the surface of clay and organic particles in the soil.

The hydrogeological characteristics of a site will affect not only the distribution of contaminants, but also the ability of treatment agents (nutrients, electron acceptors, microorganisms) to reach the affected volume. Andreoni & Baggi (1996) suggested that bioremediation of soils using indigenous bacteria or bioaugmentation was a viable option if the intrinsic soil permeability and hydraulic conductivity were $\geq 10^{-9}$ cm^2 and $\geq 10^{-4}$ $cm.s^{-1}$, respectively.

The backfill around underground cables and joint bays is likely to be less densely packed than the surrounding, undisturbed regolith. Nichols (1996) noted that this artificially high permeability of soil around joint bays allows access for bioremediation. This supports the finding by Aelion (1996) that sand, with its larger grain size, is more suited than clay soils to bioremediation.

In studies on composts, Das & Keener (1997) found that the addition of water allowed greater compaction than simply compressing the compost manually. This effect also works with soil (Herbath, 2000) and can be used to more quickly simulate the settling of backfill material with time.

2.5 TRANSPORT

The behaviour of NAPLs in soil is highly dependant on the water content. There is a tendency for NAPLs to form discrete blobs in pore spaces, or to adhere to soil particles, especially clays and organic material. Water will flow over and around the NAPL without carrying it through the soil. This reduces the mobility of NAPL in water-saturated soil.

Surfactants can help in the formation of emulsions of NAPL in water. This has implications for the flow characteristics. However, surfactants rely for their activity on their effect on the charge of molecules with which they are in contact. Because of this, they can inhibit the enzymes even as they make the NAPL more available. Rouse *et al*. (1994) found that anionic and cationic surfactants inhibit bacterial growth but that non-ionic surfactants (e.g. Tween 80) have no effect on bacterial growth rates. Hayworth & Burris (1995) confirmed that the choice of any surfactant used needs to be tailored to the contaminant.

Some bacteria produce surfactants that improve mobility of oily contaminants but are compatible with cell membranes and enzyme systems (Ouyang *et al.,* 1995). It is likely

that these "biosurfactants" play a role in attenuation of NAPLs in soil. In order to avoid adversely affecting the process we would wish to encourage, it is probably better not to add surfactants, but rather to rely on *in situ* secretion of biosurfactants by indigenous bacteria.

In work on PAHs, Weissenfels *et al.* (1992) found that adsorption of the contaminant onto soil particles (clays or humic material) reduced biodegradation but simultaneously reduced toxicity. It is probable that both of these effects are due to reduced bioavailability. A number of processes can result in the plugging of pore spaces in the soil. This prevents the flow of water containing nutrients, oxygen and microorganisms so it is important that plugging is minimised in order to maintain the efficiency of an *in situ* remediation effort.

It has been shown (Aggarwal *et al.,* 1991) that orthophosphate, often added to allow microbial growth and reduce rate of H_2O_2 breakdown, where hydrogen peroxide was added to supply oxygen, was likely to cause clogging of soil pores through precipitation of insoluble products. A maximum of 10 $mg.L^{-1}$ is needed to avoid the plugging but higher levels would need be injected because adsorption to soil particles reduces the concentration available away from the injection point. Pyrophosphate (PP) and tripolyphosphate (TPP) break down to orthophosphate too quickly and so are unsuitable. Trimetaphosphate (TMP) breaks down more slowly and so is suitable for the application. However, in this study, orthophosphate was used to maintain comparability with parallel studies undertaken by Koussia (1999) and Herbath (2000).

While it may be necessary to provide nutrients lacking in the immediate environment, biofouling in the vicinity of the injection well can adversely affect efficiency so there is a need to keep bacteria at sub-optimal nutritional levels (Shevah & Waldman, 1995).

2.6 MICROBIAL BREAKDOWN

2.6.1 Toxicity

Linear alkylbenzenes (LABs) are relatively non-toxic. Gledhill *et al. (1991)* found that sewage plants removed them effectively from domestic wastewater. Toxicity testing showed that fish were unaffected at levels of LAB in excess of the water solubility of the material – with a layer of LAB floating on the surface. They are however acutely and chronically toxic to the water flea, *Daphnia magna*. This may impact on freshwater communities.

Herbath (2000) found that cable-oil degrading microorganisms (CDM) were able to grow in soils containing up to 10000 ppm DDB, without observable ill effects.

2.6.2 Biodegradability

There is a strong tendency for LABs to be preferentially partitioned to soil particles. This reduction in bioavailability of LABs both reduces its toxicity and makes it resistant to microbial attack (Gledhill *et al.* 1991). This may result in a higher observed rate of removal since there will be an immediate reduction in the amount of detectable LAB.

In general, hydrocarbons are readily degraded in soil (Chaineau *et al.*, 1995). However, most work of this kind has been done on soils contaminated with mixtures of hydrocarbons such as diesel fuel. In such mixtures, co-metabolism of components that would be persistent if present by themselves may result in an accelerated removal rate.

There are two basic approaches to bioremediation, based on the principles of bioaugmentation and biostimulation (Balba *et al.*, 1998). However, the two are not mutually exclusive:

2.6.2.1 Bioaugmentation

Bioaugmentation involves the addition of organisms that are known to be able to degrade the target contaminant. These may be native flora, enriched in the laboratory or field by exposure to the contaminant, or they may be organisms from elsewhere. The intention is usually to avoid the lag phase that occurs while indigenous bacteria grow in numbers to a point where they can deal with the pollutant effectively, or to add organisms that have metabolic abilities not found in the resident flora. In practice there is usually a microbial consortium already in place with a suite of metabolic pathways capable of dealing with most hydrocarbons. These quickly out-compete any foreign microorganisms. The literature does not show much agreement over the efficacy of this approach and it would appear that it is of limited use. There may be an advantage if a freshly contaminated site is treated very quickly, but this relies on very rapid detection and identification of a contamination incident and the leaks encountered by NGC tend to be non-obvious. If it were to be at all effective, it would need to be combined with

biostimulation techniques.

2.6.2.2 Biostimulation

Biostimulation is the manipulation of abiotic factors to optimise conditions for microbial remediation of a contaminant. Activities include the addition of inorganic nutrients such a nitrate and phosphate, and electron acceptors (e.g. oxygen as molecular O_2 in solution or indirectly as H_2O_2).

Perhaps the first engineered bioremediation efforts were those pioneered by Raymond (1974) and described in a review article by Tursman & Cork (1992).

Ritter & Scarborough (1995) consider that bioaugmentation is appropriate only for *ex situ* remediation where conditions can be closely controlled (e.g. by land farming or in a bioreactor), while *in situ* remediation is best tackled by biostimulation.

2.6.3 Natural attenuation

While there has been a great deal of work published on the remediation of hydrocarbons in general, there has been very little specific to linear alkylbenzenes. It is worth looking at the more general literature to gain an idea of the general principles.

Hydrocarbons are found in a wide range of environmental settings and anthropogenic sources account for only a small proportion of the overall environmental load. Large volumes of hydrocarbon deposits are found in nature – they are, after all, organic molecules in all senses of the word. It should come as no surprise, therefore, that

organisms capable of utilising these materials as both carbon and energy sources should exist.

There is a wealth of evidence that hydrocarbons are removed from the environment by biological systems. In studies on soils contaminated with BTEX compounds (benzene, toluene, ethylbenzene and xylene), which are structurally similar to LABs, Weidemeier *et al.* (1996) found that volumes of soil known to be contaminated exhibited low oxidation-reduction potential (ORP) and depletion of dO_2, NO_3^- and NH_4^+. All are indicative of aerobic microbial activity. More recently it was shown that the addition of a large amount of carbon source leads to depletion of inorganic nutrients (Margesin & Schinner, 1999).

Weissemann & Kunze (1994) had earlier shown that hydrocarbon contamination of soil led to depletion of nitrogen and that this could be a limiting factor in the rate of removal. A number of studies have demonstrated that rates of removal can be enhanced by the addition of nitrogen (Haigh, 1995); nitrate, oxygen, phosphate and ammonia (Bregnard *et al.,* 1996); water, air and nutrients (Phelps *et al.,* 1994).

The addition of sewage sludge to contaminated soil increases the removal rate of hydrocarbons (Holt & Bernstein, 1992). This may be due to the added nutrients and water in the sludge, the incorporation of air during the mixing process, the bacterial community in the sludge, or a combination of all three. The fact that later studies have shown improvements solely through the addition of nutrients, water and air suggests that the indigenous microbial community possesses the requisite suite of metabolic

pathways.

In studies on C_{12} LAB (dodecylbenzene) in Tokyo Bay, Takada & Ishiwatari (1990 & 1991) found that overall, about 50% of the LAB was removed in aquatic sediments. The isomeric composition of the material remaining was different from the original contaminant. External isomers, that is, ones where the phenyl group is near the end of the alkyl chain, were more readily degraded than internal isomers, with the phenyl group nearer the middle of the chain. This preferential breakdown is a hallmark of biological activity. Purely chemical breakdown would be expected to affect all the isomers equally.

While a variety of organisms have been demonstrated to degrade hydrocarbons, remediation of diesel has been shown to be mainly bacterial rather than fungal (Harrison & Betts, 1996). Organisms shown to degrade LABs include *Nocardia amerae* (Bhatia & Singh, 1996) and *Pseudomonas* sp. (Smith and Ratledge, 1989).

Although pure cultures of specific microorganisms have been shown to degrade LABs, it is very rare to find a habitat that contains a single species. Bacteria in the environment occur as mixed populations and even under culture conditions, mixed consortia of microorganisms have been shown to be more efficient than any single type (Dave *et al.,* 1994; Singleton, 1994). Even when bacteria that have been selected for their ability to catabolise a particular material are added to a contaminated soil, there is often no discernible effect on bioremediation rates (Phelps *et al.,* 1994; Prince, 1992). A healthy soil community will in all probability possess biochemical pathways to utilise most

hydrocarbon contaminants and will be better adapted to prevailing conditions than any introduced organism. However, bioaugmentation with *indigenous* microorganisms that have been cultured with elevated levels of the contaminant may enhance *in situ* biodegradation rates (Weber & Corseuil, 1994).

Some recent studies on DDB at Cranfield University have produced promising results for DDB remediation. Cheston (1997) and Tebbutt (1998) found that uncontaminated soils contained organisms that could degrade DDB at low concentrations. Koussia (1999) found removal rates of 0.15 ìL.mL^{-1}.week^{-1} in unamended soil, with a 50% increase in rate when nutrients were added. Herbath (2000) saw significant removal in both aerobic and anaerobic conditions.

2.6.4 Mechanisms of LAB degradation

Work on biodegradation rates of hydrocarbons indicate a hierarchy of ease of biodegradation in which n-alkanes > branched alkanes > low molecular weight aromatics > cycloalkanes (Leahy & Colwell, 1990). Saturated straight chains are readily biodegraded, saturated rings less so. The existence of many different isomers means that the trend was not so obvious for aromatic compounds (Huesmann, 1995). This hierarchy is visible in features of LAB degradation that have been noted in the literature: Takada & Ishiwatari (1991) and Holt & Bernstein (1992) and Manga *et al* (1998) all found that external isomers of LABs (i.e. those in which the phenyl group is towards the end of the alkane chain) are degraded in preference to internal isomers in aquatic sediments.

Bayona *et al.* (1986) found better biodegradation where the phenyl group was at the end of a chain, or where the chain was long; and Bhatia & Singh (1996) also reported that degradation occurred more readily if the phenyl group was near one end of the alkyl chain.

These findings would suggest that access to a free end of the alkyl chain is significant in the initial degradation of LABs. This is supported by Smith & Ratledge (1989) and Smith (1990), who demonstrated that the phenyl group is attacked before the alkane chain if the side chain is short (7C).

2.6.5 Conditions

As with any biochemical reaction, biodegradation rates are heavily dependent on chemical and physical conditions such as pH and temperature, as well as availability of materials.

Table 2.2 - Ideal conditions for bioremediation

	pH	Temp/°C	Water content/%	C:N ratio	C:P ratio
Dibble and Bartha (1979)	7.8		30-90	60:1	800:1
Kiser (1995)			22-45		
O'Leary *et al.* (1995)				5-15:1	
Ritter & Scarborough (1995)	6.5-8.5	27-35			

Johnston et al (1996) reported that BTEX compounds were recalcitrant under anaerobic conditions. Earlier, Holt & Bernstein (1992) found that LABs were not degraded anaerobically. More specifically, Nichols (1996) found that cable oil degrading microorganisms (CDMs) are aerobic. However, Herbath (2000) suggested that anaerobic degradation of commercial cable oil does in fact occur. Significant numbers of anaerobic CDMs were demonstrated in microbiological investigations.

While there are indigenous CDMs in at least some soils, their activity is not generally high enough for natural attenuation to occur at a rate sufficient to offset a typical cable oil release (Cheston, 1997). On the other hand, biofouling in the vicinity of a nutrient injection well due to excessive levels or poor choice of nutrients can adversely affect the efficiency of active measures. It has been shown that it is necessary to keep bacteria at sub-optimal nutritional levels (Shevah & Waldman, 1995). Lackey *et al.* (1994) also found that keeping nutrient levels at below optimum levels gives improved rates of biodegradation and suggested that the poor availability of nutrients encourages the use of alternate carbon sources. This will have implications for the dosing strategy adopted in any biostimulation effort.

Enrichment of hydrocarbon degrading microbial communities occurs soon after contamination. Margesin & Schinner (1999) found oil-degrading organisms increased from 0.001% to 10% of the microbial population of a freshly diesel-contaminated site in 30 weeks.

Contrary to the recommendation of Ritter and Scarborough (1995), there was little or no increase in hydrocarbon degradation above 20°C (Margesin and Schinner, 1999). It was suggested that this might be due to increased toxicity at higher temperatures.

2.6.6 Treatment

There are a large number of factors that will affect the rate of biodegradation. Some are more significant than others and for simplicity it is useful to choose factors that can be measured to provide a picture of what processes are occurring. Singleton (1994) suggested manipulation of pH, temperature, and aerobic/anaerobic conditions. These are all easily measured with relatively inexpensive instruments.

Margesin & Schinner (1999) found that, while adding nutrients altered the rate of biodegradation, addition of low levels of nutrients (100 mg N and 45mg P /kg soil) gives greater stimulation than higher levels. Since these nutrients will be used up during biodegradation, it suggests that adding small amounts of nutrients continuously or at intervals to maintain an appropriate level would allow optimal conditions to be maintained.

McCarty & Semprini (1993) reported that injection of nutrients into soil might simply displace contaminated water without mixing effectively. This would suggest that much of the mixing could be done on the surface – extracting contaminated ground water, adding and mixing nutrients and re-injecting into the contaminated volume.

If the water table is shallow it is possible to use a sprinkler system to apply pumped water directly to the soil surface. This reduces the costs that would be associated with digging an injection well or infiltration galleries (Sweed *et al.* 1996). This may be of use in rural areas, but since much underground cables pass under roads and buildings, we will assume the use of injection wells.

Li *et al.* (1993, 1995) used a mathematical model of bioremediation of a petroleum-contaminated soil and found that if the water content of the soil was 40-50% of the maximum field capacity, dissolution of oil into water became rate limiting. Yang *et al.* (1995) came to a similar conclusion. This confirms the predictions made from examining the physical properties of LABs in Table 2.1.

Where the degrading organism is aerobic, dissolved oxygen may be limiting. There are a number of ways in which oxygen may be delivered. Possibilities include direct injection of air or oxygen into the soil, aeration or oxygenation of the injected water, or injecting substances that evolve oxygen *in situ*, such as hydrogen peroxide, which breaks down to oxygen and water. Work has also been published on the use of slow-release oxygen sources. Vesper *et al.* (1994) found that sodium percarbonate encapsulated in poly(vinylidene) chloride released oxygen over a period of about two months. It is however difficult to deliver to the site without mechanically (the authors have developed a hydraulic method) fracturing the matrix. This may make it inappropriate for use around subsurface structures such as electrical cables.

2.7 CONTROL AND MONITORING

There seems to be rather little in the literature specifically to do with the application of control technology to bioremediation. Describing a pump and treat system, Minsker & Shoemaker (1998) noted that cost benefits may be made if the pumping rate is varied at intervals through the treatment. This relied on discrete measurements of the state of the system with a conscious decision made on the part of the operator to alter the pumping rate. In order to design a self-regulating treatment system, automatic data collection would need to be combined with a decision-making algorithm and actuators.

Feedback control is not an entirely unknown technique in bioremediation. Mountain *et al*. (1998) reported the use of sensors and computers to monitor and regulate conditions in biopiles used to remediate excavated soil after an oil well blowout near Milan. The *ex situ* nature of this work meant that there was far greater control over moisture content, oxygen availability and mixing than in an in situ operation.

Shouche *et al*. (1993) modelled the degradation of carbon tetrachloride (CCl_4) in soil and found that by supplying acetate as an electron donor in timed pulses, the maximum biomass concentration (read biofouling) could be reduced by an order of magnitude. This illustrates the importance of a full characterisation of the dynamics of a contaminated site.

Chawla *et al*. (2000) also published a paper in which they developed a mathematical model of CCl_4 degradation to find the optimum pattern of nutrient addition to maximise biodegradation and minimise biofouling near the injection wells. Even assuming that

there was only one each of electron donor, electron acceptor, contaminant and microbial population; and assuming that the contamination and biomass remained in the same place, the model contained five partial and four ordinary differential equations. The mathematics is beyond the current study, but could form the basis of further work.

While there is a large literature on Control Theory, this study is a preliminary investigation into the application of Control Cube technology to the problem and so the detailed theory lies outside the scope of this work. To control a system, first the system must be characterised, understood and modelled. Generally, this is not the case in contaminated environments.

Chapter 3

Equipment, materials and methods

3.1 INTRODUCTION

The original intention was to carry out work on the model joint bays situated at Cranfield University Sewage Treatment Works, which were developed and constructed for a PhD research project by Herbath (2000). However, due to technical difficulties it was decided to fabricate a smaller analogue system of about 1 m^3 for this project. Since the full-scale bays were referred to as "the pit", this smaller analogue quickly became known as "the mini pit".

3.2 MATERIALS

3.2.1 Soil

The soil used in this study was chosen to allow comparison with other studies (Koussia, 1999; Herbath, 2000). Designated "Soil A" in Herbath (2000) and characterised in Table 3.1. The original choice of this soil for a range of related studies was based on its common occurrence in association with NGC underground cables, the speed with which it settles into its *in situ* structure and, perhaps most significantly, its availability in sufficient quantity to fill the original pits.

Table 3.1 Analysis of soil (from Andrews, 1999 except * from Lovelace, 1999)

Dry matter content of air dried soil	98.5 % (m/m)
Water content of air-dried soil	1.6 % (m/m)
Particle Size distribution	
600 μm - 2 mm	16.97 %
212 μm – 600 μm	31.86 %
106 μm – 212 μm	4.27 %
63 μm – 106 μm	26.52 %
20 μm – 63 μm	5.19 %
2 μm – 20 μm	7.44 %
< 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)	
WHC_{max}	30.7 % (m/m)
$WHC_{0.05\ bar}$	17.2 % (m/m)
$WHC_{01.\ bar}$	14.0 % (m/m)
$WHC_{0.4\ bar}$	11.3 % (m/m)
$WHC_{2\ bar}$	9.2 % (m/m)
$WHC_{15\ bar}$	5.8 % (m/m)
Hydraulic conductivity *	1.54 $m.day^{-1}$
Gravimetric moisture content at saturation *	23.39 %
Porosity *	42.83 %
Volumetric moisture content at saturation *	35.30 %

3.2.2 Contamination

The mini pit was contaminated with 2 L of cable oil, added as a single bolus through the injection well on the 9th July, 1999. The volume was chosen to give the same volumetric contamination as that in the main pit (Herbath, 2000).

3.2.3 Inoculum

The mini pit was inoculated on 16th July 1999 with 1 L of a liquid culture of microorganisms grown in dodecylbenzene (DDB) + mineral salts. The culture was provided by Koussia (1999) and was grown using an original inoculum of soil from the main pit, which contained the same soil as the mini pit, and which had been contaminated with cable oil some seven months previously (Herbath, 2000). It was hoped that by augmenting the mini pit with indigenous organisms from an almost identically contaminated site, bioremediation would begin at a faster rate than would otherwise be the case.

3.2.4 Nutrients

Nutrients were added to the mini pit on the 6th January, 2000 (Table 3.2). The nutrients were selected in order to maintain comparability between results from different studies (Koussia 1999; Herbath, 2000).

Table 3.2 Nutrients added to mini pit

NaH_2PO_4	48.08 g
Na_2HPO_4	4.95 g
$NaNO_3$	182.10 g

The nutrients were calculated to provide soil nutrients of 100 $mgN.kg^{-1}$ soil and 45 $mgP.kg^{-1}$ soil (Margesin & Schinner, 1999). For simplicity, it was assumed that approximately 300 kg of soil was affected since the water table in the mini pit was maintained at approximately one third of the way from the base of the container. It was also assumed that the nitrogen (N) and phosphorous (P) content of the soil were unchanged from the analysis performed by Silsoe Soil and Land Research Centre (Andrews, 1999).

Mineral salts were added in the same proportion as in the parallel studies by Koussia (1999) and Herbath (2000). Koussia (1999) had used a mixture in which 92% of the P was supplied as sodium dihydrogen orthophosphate (NaH_2PO_4), and 8% as disodium hydrogen orthophosphate (Na_2HPO_4). N was supplied as sodium nitrate ($NaNO_3$). Aggarwal *et al.* (1991) had cautioned against the use of orthophosphate as a soil amendment because of the potential for clogging of soil voids, but the formulation was retained for comparison.

The mineral salts were weighed to 0.01 g on an Oertling HC22 balance (Serial No. 833159). In order to deliver the nutrients to the soil block, approximately 10 L of water was siphoned from the settlement tank into a plastic container. The mineral salts were added to this water and the container sealed and shaken well. The resulting nutrient solution was added to the mini pit via the injection well, pouring slowly over about ten minutes to minimise the risk of breakthrough at the surface.

It was expected that there would be an immediate effect on readings from the settlement tank as the probes were not immersed in water for the time it took for the 10 L bolus of mineral salts to move through the soil block and refill the tank.

3.3 APPARATUS

3.3.1 Mini pit construction

The mini pit was based on a high-density poly(ethylene) (HDPE) container, sold commercially for the transport of bulk materials (Figure 3.1,C1400 4-way entry tank from Mailbox Mouldings International, Bayley Street, Stalybridge, Cheshire SK15 1QQ. Telephone 0161 330 6511).

The internal dimensions were 1150 x 990 x 1090 mm (length x width x height) , with an internal volume of approximately 1 m^3. It had a ribbed, moulded construction and was mechanically very strong. The mechanical strength was important since, unlike the large pits, the mini pit was not submerged in the ground, but freestanding (Figure 3.2).

Injection and extraction wells were fabricated from 50mm diameter PVC drainpipe as shown in Figs 3.3 and 3.4. The pipe was cut to length with a hacksaw. Holes were drilled with an electric drill fitted with a 6mm bit. The wells were assembled with PVC cement, which forms an almost immediate bond, allowing the wells to be installed straight away. To prevent ingress of soil particles, nylon sheaths (15 denier "knee high" ladies stockings) were fitted over the T-pieces of the well assemblies and held in place with nylon cable ties.

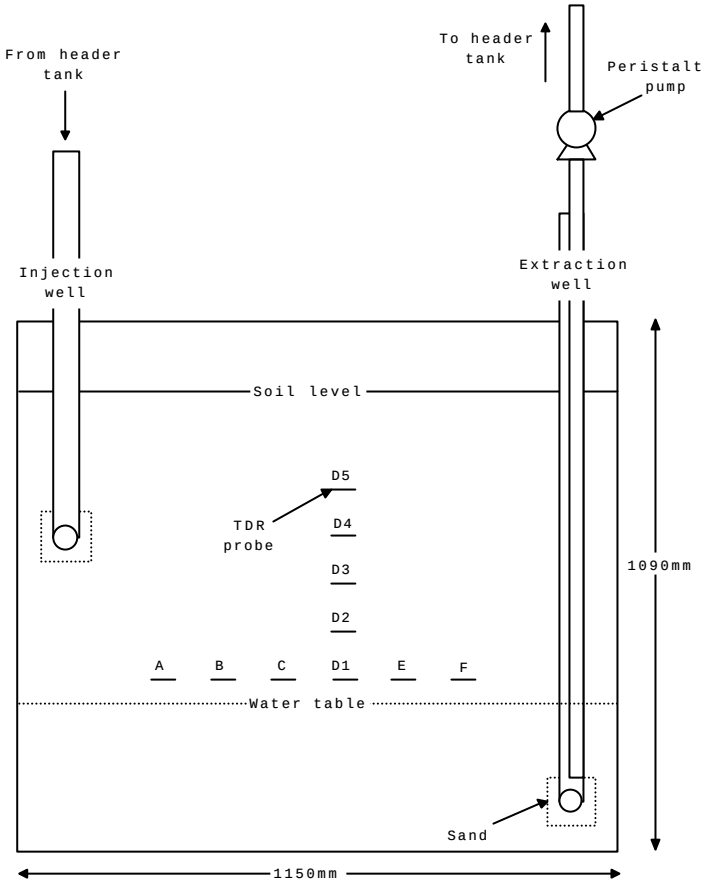

Figure 3.1 Mini pit – side elevation

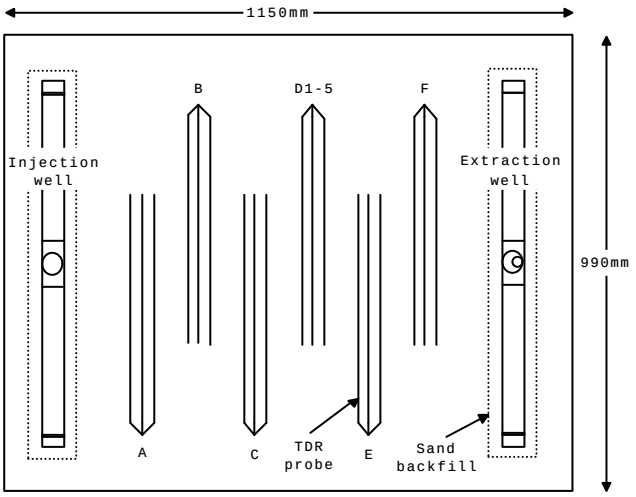

Figure 3.2 Mini pit – plan

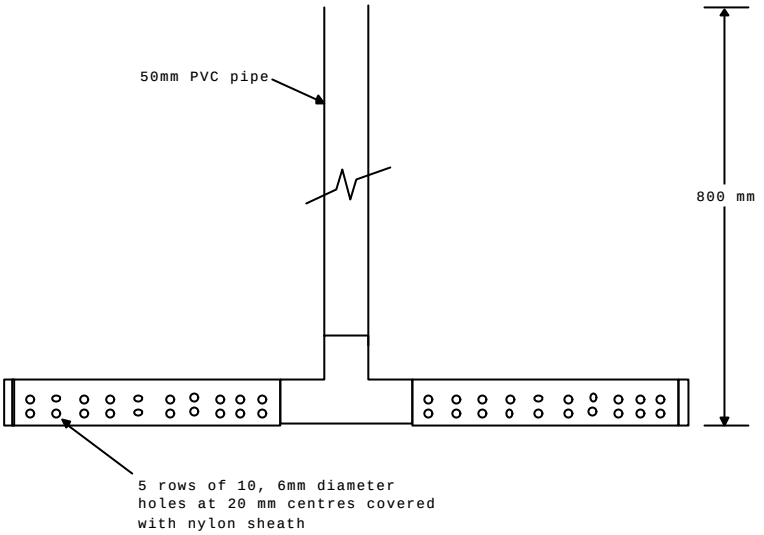

Figure 3.3 Detail of mini pit injection well

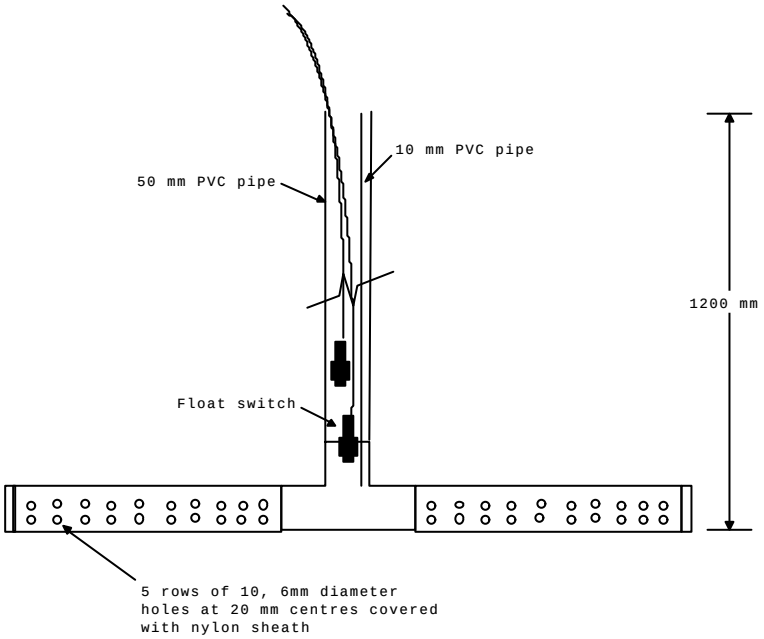

Figure 3.4 Detail of mini pit extraction well

The inside of the mini pit was marked at 100 mm intervals with an indelible marker to aid soil and probe placement (Plate 3.1). Soil was loaded into the mini pit in 150 mm layers. Each layer was consolidated by treading down, and the surface scored to provide a key and prevent the formation of discrete horizontal pathways for water flow before the next layer was loaded.

Plate 3.1 Placement of extraction well

The injection and extraction wells were fitted at opposite ends of the tank to maximise the effective length of the soil block. The extraction well was installed 150 mm from the floor of the tank to minimise the dead volume and the injection wells was installed at approximately 300 mm below the soil surface to maximise the vertical distance between the two wells, while reducing the likelihood that injected water would break out at the

surface. The drilled portions of both wells were surrounded on all sides by 50mm of Builder's Sand to form a diffuser.

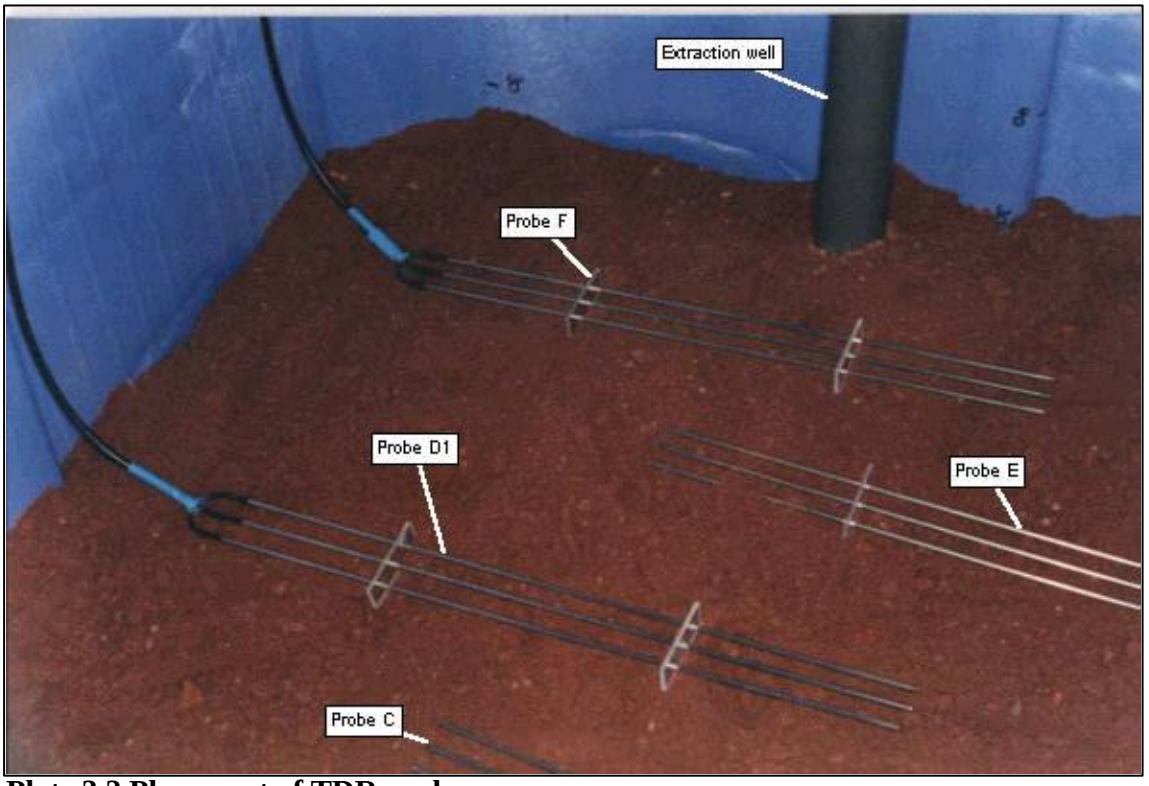

Plate 3.2 Placement of TDR probes

Time domain reflectometry (TDR) probes were placed in the soil as it was loaded, and the cables taped to the inside wall of the container. The BNC connectors were labelled prior to installation for easy identification. A horizontal row of six TDR probes (A B, C, D_1, E, F) was placed at a height of 300 mm above the floor of the tank, just above the intended water table, as shown in Plate 3.2. A second, vertical column of TDR probes $D_2 - D_5$) were placed at 100 mm vertical intervals above probe D_1 in the horizontal array.

Once loaded, the soil was saturated with water. Initial attempts to add the water through the injection well resulted in water breaking through the soil surface around the well. To avoid the formation of preferential flow paths, this approach was abandoned and water was added from a hosepipe onto a plastic sheet on the soil surface. Water was added until it pooled on the surface, then the pit was left for eight days to allow the soil to settle. At the end of this time, the water was pumped out to storage vessels for future use. The soil level dropped by about 50 mm, indicating that further compaction had occurred.

3.3.2 Settlement Tank

Once drained down to the desired level the remaining water was diverted to a settlement tank (Figure 3.5) mounted on an angle-iron framework about 1 m above the soil surface (Plate 3.3).

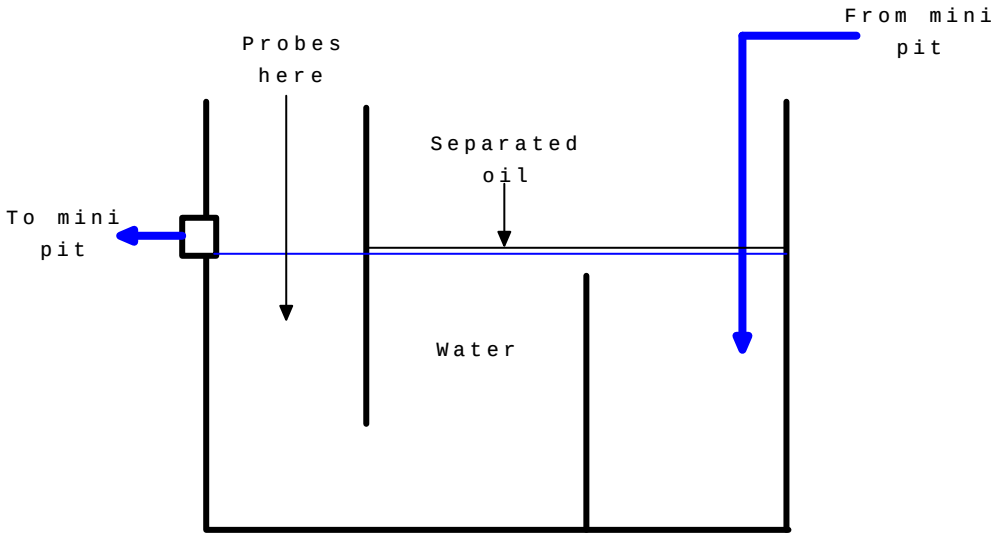

Figure 3.5 Settlement tank

The settlement tank was fabricated from a domestic central heating header tank. It was fitted with two transverse baffles and overflowed through an outlet to a plastic pipe that emptied into the injection well. The main baffle intersected the water surface and prevented cable oil on the surface entering the overflow. Another baffle was situated at the bottom of the tank and served to prevent water from passing under the left-hand baffle before any entrained cable oil had time to separate out.

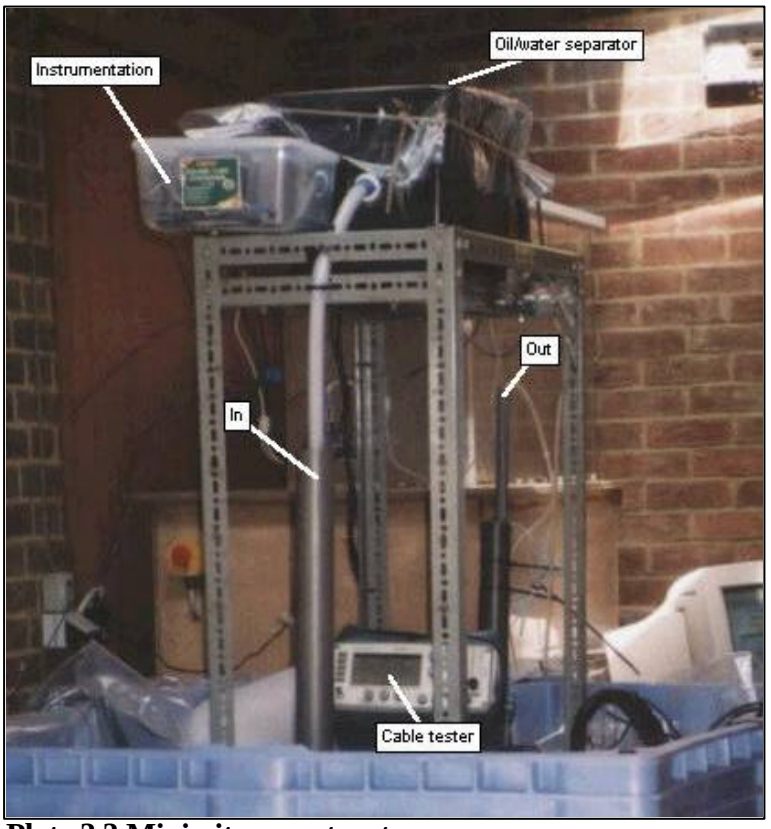

Plate 3.3 Mini pit superstructure

The settlement tank served several functions (Plate 3.4): it allowed access to the water at the point just prior to reinjection to the soil block for measurement of physico-chemical parameters, the baffles separated free light non-aqueous phase liquid (NAPL) that was entrained with water pumped from the mini pit, and the free surface of the

water and the slow flow rate allowed oxygen to diffuse into the water prior to reinjection.

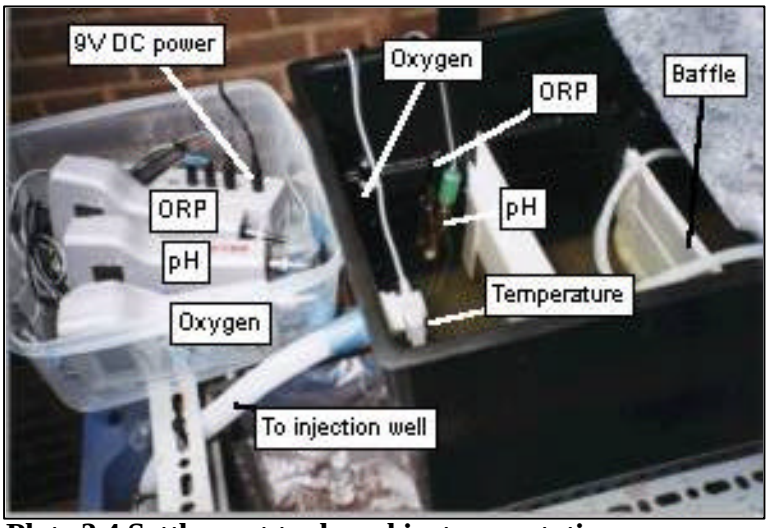

Plate 3.4 Settlement tank and instrumentation

3.4 CONTROL CUBE

The Control Cube is an 8-channel datalogger/control interface available from Unilab (Catalogue Number 800.008). For convenience, two units were used with the mini pit (Plate 3.5). One to control the water circulation using two float switches to sense water level and a mains controller to power a peristaltic pump. The other to record pH, dissolved O_2, oxidation-reduction potential (ORP) and temperature of the injection and extraction streams.

Plate 3.5 Control cubes and pump

3.5 PUMPING

Water recirculation was by means of a peristaltic pump controlled in response to two vertical float switches (SH43W from Maplin Electronics) installed in the extraction well

The pump was switched on when the upper float was in the raised (ON) position, and allowed to run at full speed until the lower switch was in the lowered (OFF) position. In this way the water level in the extraction well was controlled within a 100mm vertical range. The rate of pumping was monitored and more water added as required to achieve a pumping rate of about 1-3 cycles per hour.

The control script incorporated a counter and was run from the computer rather than being downloaded to the Control Cube to allow a continuous display of elapsed time and number of pump cycles. The volume pumped per cycle, measured by diverting the outlet from the flow-though cell to a measuring cylinder, was 200 ml.

3.6 MONITORING

A number of physical and chemical parameters were measured, either manually at intervals or logged semi-continuously using Control Cube.

3.6.1 Time Domain Reflectometry (TDR)

TDR is a technique used in the power transmission and telecommunications industries to locate breaks in cables by detecting the reflection of an applied potential pulse from the broken end. The time it takes for the pulse to make the round trip is proportional to the length of unbroken cable and is dependent on the resistivity of the surrounding material. In the case of an insulated cable this is constant but, by fixing the length of a specially designed, uninsulated probe, information regarding the resistance of the matrix can be obtained. When properly calibrated and in a more or less homogeneous matrix, water content can be calculated. This was exploited in the mini pit to monitor the distribution of water in the soil block.

Ten probes were acquired from Plymouth University. Each consisted of three parallel stainless steel rods (5 mm diameter, 500 mm long) held at 25 mm centres by insulating spacers. The rods were connected to one end of a 2 m length of 50 Ω coaxial cable, the other end of which terminated in a BNC connector to allow connection to the cable

tester. The outer rods were connected to the shielding and the central rod to the core of the cable. All connections were soldered and insulated with a thick layer of hot-melt glue.

TDR readings were taken at intervals using a Tektronix 1502B Metallic TDR Cable Tester (Serial number R010188) using the following procedure:

1. Connect cable tester to mains
2. Switch on (Pull power switch out) and wait for tester to initialise
3. Press [Menu]
4. Turn vertical position knob to place highlight over SETUP MENU
5. Press [Menu]
6. Turn vertical position knob to place highlight over DISTANCE/DIV
7. Press [Menu] to change from ft/div to m/div
8. Press [Menu] twice to return to graph display
9. Connect a 2m coaxial cable with a BNC connector to zero the tester
10. Rotate noise filter knob to “horizontal”
11. Use horizontal position knob to move cursor to approximately 2m
12. Adjust vertical scale to clearly show peak due to end of cable
13. Place cursor on peak (Plate 3.6)
14. Press [Store] to set the zero point
15. Set noise filter control to 16 average
16. Set distance/div to 0.25m
17. Disconnect 2m coaxial cable

18. Connect TDR probe
19. Adjust vertical scale to show peak
20. Place cursor on the point of intersection, L' (Fig 3.5; Plate 3.7) the and note distance and identity of probe
21. Repeat 18-20 for each probe

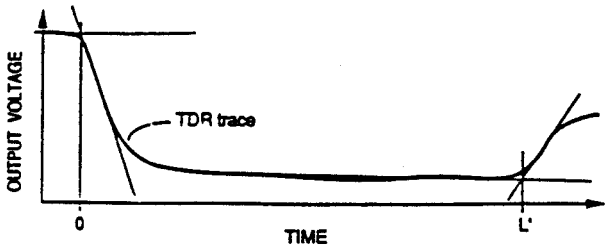

Figure 3.6 TDR trace showing intersection point at L' (from Herbath, 2000)

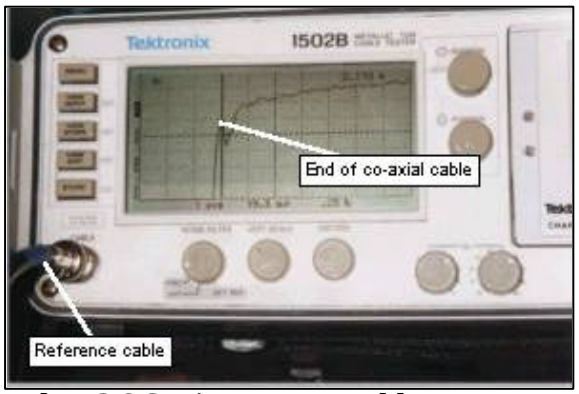

Plate 3.6 Setting zero on cable tester

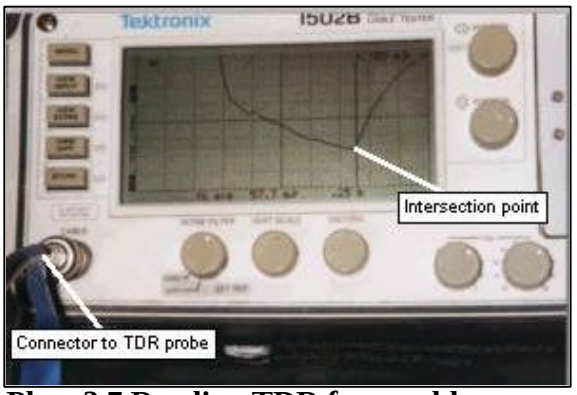

Plate 3.7 Reading TDR from cable tester

The dielectric constant (ε) and thus the volumetric water content (θ) of the soil at each probe was calculated in Microsoft® Excel using a spreadsheet supplied by Herbath (2000).

3.6.2 Software

3.6.2.1 Control Disc

The Control Cubes were programmed using Control Disc software (D800.000 from Unilab), running on a Windows PC and connected to a serial port. Control Disc routines are text files but are created using a graphical interface by dragging flow chart components from an on-screen "toolbox" and completing dialogue boxes as required. The routines can be run from the PC, which remains connected to the Control Cube, or downloaded to the Control Cube and run remotely.

The pump controller routine (Appendix 1) was run from a PC since a Microsoft® Windows 3.1 machine was available to dedicate to the task. This gave the advantage that an on screen display of time since the routine was initialised and a counter that tracked the number of times the pump had been activated could be on constant display.

The Control Disc routine for collecting data was extremely simple (Appendix 2). Its function was to poll all the attached sensors once per hour (= 3600 seconds, the longest available recording interval). The routine was downloaded to the Control Cube from Control Disc, running on a notebook PC running Windows 98.

3.6.2.2 Datadisc 32

Data was downloaded from the Control Cube to a notebook computer every few weeks using Datadisc 32 from Abington Partners, running under Microsoft® Windows 98. The data was saved in DDW (Datadisc for Windows) format using a file name based on the date the recording was started, in the form ddmmyy.DDW. So for instance a recording

started on 21 July 1999 would be saved as 210799.DDW. Original Datadisc files were archived and the data exported to Excel for further analysis.

The logging routine was restarted, using Control Disc, as soon as the data had been downloaded to ensure that there was minimal interruption to the data record.

3.6.2.3 Excel

The bulk of the data analysis was carried out using Microsoft® Excel 2000, running under Microsoft® Windows 98. Excel allowed data files to be concatenated to yield a more or less continuous data stream covering the life of the mini pit.

3.6.2.4 Visi*Store*

Plates were electronically scanned, catalogued, calibrated and annotated using Visi*Store* software from Abington Partners.

3.6.3 Sensors

3.6.3.1 Sensor placement

Eight channels of sensor data were logged continuously from the mini pit. Temperature, pH, ORP and dO_2 were recorded from the points of water input and output.

The water in the settlement tank was taken to be the input to the soil and sensors for pH, temperature, ORP and dO_2 were placed in the tank, adjacent to the overflow. Shortly after the start of the experiment the top of the tank was covered loosely with polythene sheeting to reduce evaporation.

In order to measure conditions in the water being extracted from the mini pit, a sealed flow-through cell (Fig. 3.7) was installed between the peristaltic pump and the overflow to the settlement tank. The cell was originally fabricated for use at the main pit (Herbath, 2000) but was available for use with the mini pit. The original idea for the cell came from Silsoe; it was designed by Yolande Herbath and fabricated at British Hydrodynamic Research Group (BHRGroup Ltd) at Cranfield.

The flow-through cell consisted of an acrylic cylinder, capped with circular acrylic plates. The end plates were machined, drilled and tapped to accept brass nipples onto which the polythene tubing from the pump was fitted. It was mounted on the angle-iron frame, next to the settlement tank (Plate 3.8). The top plate incorporated holes drilled to accept watertight ports into which probes for pH, temperature, ORP and dO_2 were inserted.

Data from sensors in the settlement tank were designated pH IN, ORP IN, Oxygen IN and Temperature IN. Data from sensors in the flow-through cell were designated pH OUT, ORP OUT, Oxygen OUT and Temperature OUT.

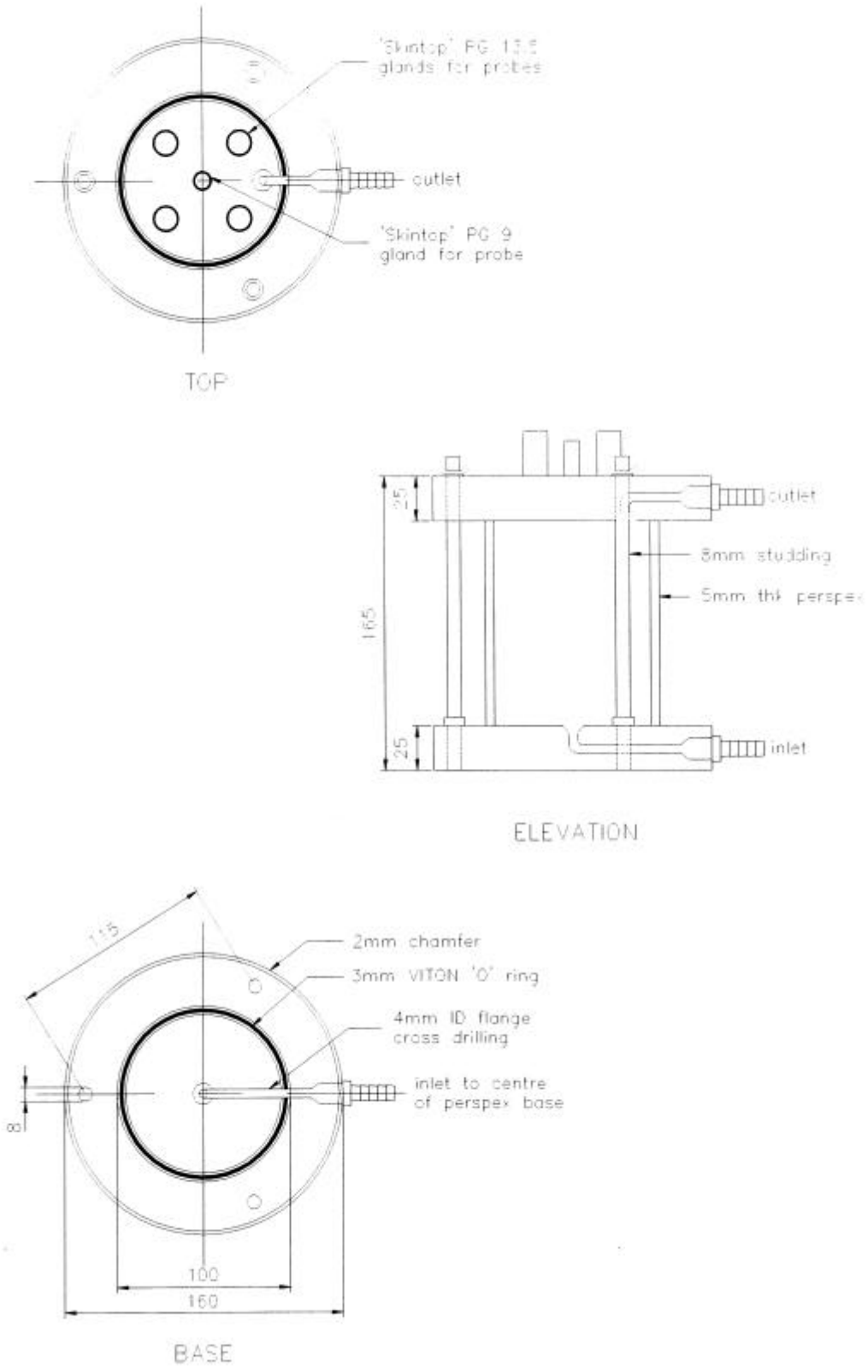

Figure 3.7 Detail drawings of flow-through cell (Herbath, 2000)

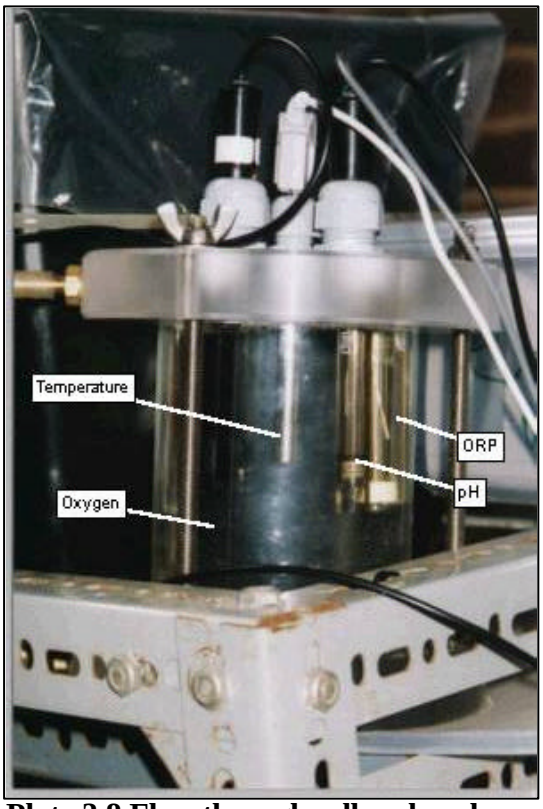

Plate 3.8 Flow-through cell and probes

3.6.3.2 pH

pH was measured using Philip Harris pH SensorMeters (E30390/8) connected to a Control Cube. The pH electrodes were commercially available polymer-bodied combination pH electrodes (Y37220/4 from Philip Harris). The reference portion of the cell was a Calomel half-cell filled with saturated KCl solution and the measuring electrode a silver/silver chloride (Ag/AgCl) half-cell filled with 4 M potassium chloride/silver chloride (KCl/AgCl) solution.

The pH SensorMeter has a single point calibration. The electrode was placed in fresh pH 7 buffer and the SensorMeter switched on. The "Set pH 7" key was pressed and the reading was allowed to stabilise. After calibration, the SensorMeter was checked using

PH 4 and pH 9 buffer solutions to ensure that the error in the reading was no more than 0.1 pH unit.

The pH SensorMeter was automatically recognised by the Control Cube. There were some problems with the recognition of the pH IN sensor but, since the sensor recognition was independent of the data, it was possible to convert raw analogue/digital data from the SensorMeter to pH by applying the appropriate calibration in an Excel spreadsheet. The calibration was:

$$pH = \frac{Rawdatavalue \times 14}{54560}$$

(Crellin, 1999).

3.6.3.3 Dissolved Oxygen

Dissolved oxygen was measured using Dissolved Oxygen SensorMeters (Philip Harris Catalogue Number E30320/9) and polargraphic oxygen electrode (Philip Harris Catalogue Number E30365/9). The electrode is a commercially available unit based on a Clark electrode. The SensorMeter was calibrated to read 100% with the electrode in air.

3.6.3.4 Oxidation-reduction Potential (ORP)

ORP was measured using a Philip Harris pH SensorMeter, using its mV output. The electrodes are commercial polymer bodied, sealed reference combination electrodes. The reference electrode is a silver/silver chloride (Ag/AgCl) half-cell, and the electrode is filled with a 3.5 M potassium chloride (KCl) gel.

This is a popular design for general use in aqueous solution because of its simplicity, good stability and ease of use compared with (for instance) a mercury/mercury chloride or a Standard Hydrogen Electrode

ORP potentials are defined relative to the potential of a Standard Hydrogen Electrode, which by definition has an ORP of zero. Figure 3.8 shows the variation, with temperature, of the Standard Potential of an Ag/AgCl electrode, filled with 3.5 M KCl at 298 K.

In order to convert the reading from the SensorMeter to the Standard Potential of the environment being tested, it is necessary to subtract the Standard Potential for the reference electrode:

$$E^o = E - (0.0007x + 0.2228)$$

Where:

$E°$ = Standard Potential / V

E = Displayed ORP / V

x = temperature / °C

(Sawyer *et al.*, 1995)

The temperature correction (calculated from the Nernst equation) is small in this case, but is included for completeness. In addition to this correction, the SensorMeter is limited to positive potentials and will only reliably indicate E of 5 mV and above. This will have the effect of causing any graph to "bottom out" at a Standard Potential of

about –0.2 V. To mitigate for this limitation, occasional spot checks of the potential from the ORP electrode were taken using a Hanna model H18519N bench pH meter (serial number 136041), set to mV.

3.6.3.5 Temperature

Temperature was recorded using Control Cube Water Temperature Sensors (D800.025 from Unilab). These were powered from the Control Cube and no batteries or external power supply was required.

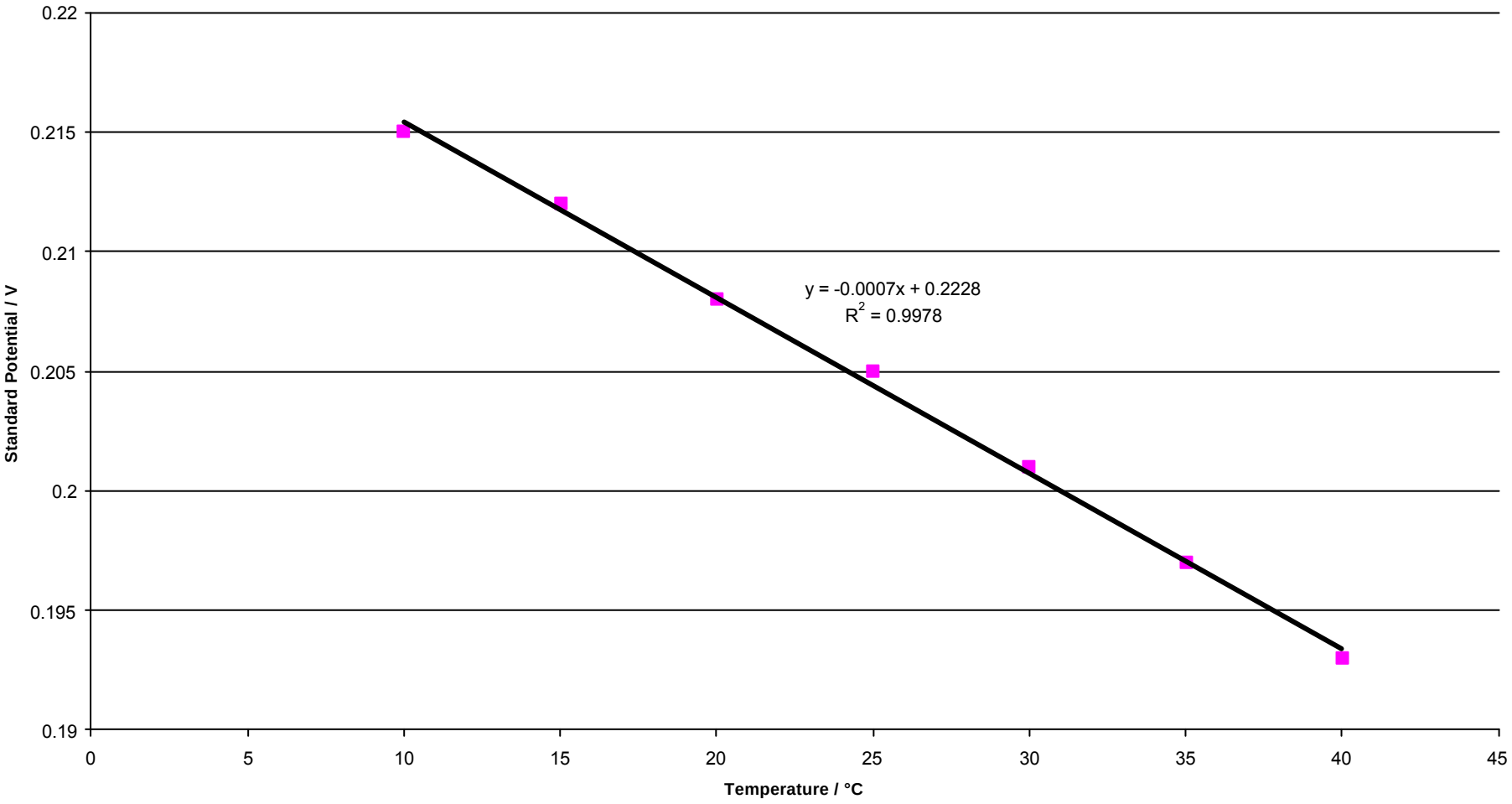

Figure 3.8 Variation of Standard Potential of Ag/AgCl electrode with temperature (data from Sawyer *et al*., 1995)

3.7 SAMPLING

Occasional samples of soil were taken from the mini pit to allow microbiological investigation and fluorometric analysis.

Soil samples were taken using a clean, but non-sterile 20 mm diameter auger (04.02.01.20.B from Van Walt Limited, Prestwick Lane, Grayswood, Haslemere, Surrey GU27 2DU. Tel: 01428 661660). A core was obtained from close to the centre of the soil block, extending down to 700 – 800 mm. It was not possible to obtain a cohesive core from below this level using this design of auger because the soil at this depth was too wet.

The core was subdivided into 100 mm subsamples from the surface down and each subsample was placed in a new resealable poly(ethylene) sample bag, marked with the date and the range of depths covered by the sample. Samples were stored at –80°C until they were analysed.

3.8 MICROBIOLOGY

Microbiological analysis was undertaken of groundwater and soil from the mini pit to determine whether DDB-degrading organisms were present, their distribution in the soil and an initial attempt to identify them.

3.8.1 Sample preparation

Samples were weighed to the nearest 0.01 g using an Oertling HC22 balance (Serial No. 833159). All subsequent preparation was carried out using aseptic technique. Where Universal bottles were used, the neck was flamed briefly in a Bunsen burner flame immediately upon opening and again before closing . Serial dilutions were prepared to ensure that cultures suitable for counting were produced.

A 1 g soil sample was added to 9 ml sterile saline in a Universal bottle. This diluted the soil to 10^{-1}. The cap was replaced and the bottle shaken well to suspend the soil microorganisms. Then 100 µl of the liquor was removed using a micropipettor with a new, sterile tip and added to 9.9 ml sterile saline. This was mixed by filling and refilling the micropipettor tip several times. The resulting suspension was further diluted by adding 100 µl to 9.9 ml sterile saline. This resulted in dilutions of 10^{-1}, 10^{-3} and 10^{-5}.

More complicated methods of sample preparation were rejected in order to maintain comparability with the work of Herbath (2000) and because the method of extraction has not been shown to affect the community profile of extracted organisms (Mayr *et al*, 1999).

Spread plates were prepared using 100 µl of each dilution (see 3.7.3.1). This gave final "dilution factors" of 10^{-2}, 10^{-4} and 10^{-6} (multiply counts by 10^2, 10^4 and 10^6, respectively to give CFU.g^{-1} soil).

3.8.2 Media preparation

All culture media were made up using reverse osmosis water. The medium was mixed and autoclaved for 15 minutes at 121°C. The medium was allowed to cool to approximately 50°C before being poured into sterile, 90 mm plastic Petri dishes. To allow easy identification of media, the edge of each plate was marked with 0, 1, 2 or 3 lines, using an indelible marker.

3.8.2.1 Malt extract agar (MEA)

Used to check for the presence of fungi. Oxoid malt extract agar made up according to the instructions, with the addition of 0.01% w/v chloramphenicol to inhibit bacterial growth. Edge of dish left unmarked.

3.8.2.2 Nutrient agar (NA)

For general cultures. Oxoid nutrient agar made up according to the instructions. Marked with single line (/) on the edge, marking both base and lid of the dish.

3.8.2.3 Dodecylbenzene agar (DDB)

For the identification and culture of cable oil-degrading microorganisms (CDMs). Prepared by autoclaving Bushnell-Haas agar (Table 3.3) and adding 0.05% v/v of filter-sterilised DDB by running slowly down the side of Soniprep 150 sonicator probe (Serial number JM450), with probe just touching surface of medium. Plates marked with a double line (//) line on the edge, marking both base and lid of the dish.

3.8.2.4 Glucose and mineral salts agar (GMS)

1% w/v Glucose + Bushnell-Haas agar salts. Marked with triple line (///) on the edge, marking both base and lid of the dish.

Table 3.3 Bushnell-Haas agar (from Atlas, 1993)

Agar (No 2)	15.0 g
KH_2PO_4	1.0 g
K_2HPO_4	1.0 g
NH_4NO_3	1.0 g
$MgSO_4$.7H2O	0.2 g
$FeCl_3$	0.05 g
$CaCl_2.2H_2O$	0.02 g
Reverse osmosis (RO) water.	To 1.0 L

3.8.3 Inoculation and incubation

All work was done using strict aseptic technique. Prior to beginning work the bench was swabbed with isopropanol. The work was done next to a lit Bunsen burner. Spread plates were prepared for general cultures. To isolate organisms for closer examination, streak plates were used.

3.8.3.1 Spread plates

Three plates were prepared at each dilution to allow for limited statistical analysis.

100 µl of inoculum was placed on the surface of the culture medium using a micropipettor fitted with a sterile tip. Micropipettor tips were used for one dilution only

and were discarded after use. The inoculum was spread evenly over the whole surface of the plate using a glass spreader, which was dipped in isopropanol and flamed in a Bunsen flame before each use. Plates were marked on the underside with the date, source of inoculum and dilution factor, then inverted and left to dry before placing in an incubator. After an appropriate incubation period at 30ºC (3 days, except for DDB plates, which were incubated for 5 days) the cultures were removed to a 4°C cold room for storage before counting.

3.8.3.2 Streak plates

To further investigate microorganisms cultured on spread plates, individual colonies were picked off using a flamed and cooled nichrome wire loop. They were streaked onto the surface of a nutrient agar plate and incubated at 30ºC for 3 days.

3.8.4 Counting

Plates were examined to assess the extent of growth and to select appropriate plates for counting. The plates chosen for counting were those with the greatest number of non-confluent colonies (typically 30-300).

Selected plates were inverted on a Gallenkamp colony counter (Cat No. CNW325010X, App No. 5A0441) and colonies marked with a fibre-tipped pen on the underside of the Petri dish. The number of colonies and the dilution factor were noted, along with the date, culture medium, source of inoculum, and incubation time.

To covert the counts per plate to CFU.g^{-1} soil, the number of colonies was multiplied by the dilution factor. The dilution factor was reached by dividing the actual dilution of the inoculum by 10 to account for the fact that only 100 µl (i.e. 0.1 ml) of inoculum was used.

3.8.5 Description and photography

After incubating the first plates, a number of cable oil-degrading organisms were described and isolated. Representative culture plates were also photographed using a single lens reflex camera loaded with 400 ASA/ISO colour print film. Photography was from above, with the Petri dish lid removed.

3.8.6 Identification

After photographing the original plates, individual colonies of CDMs were isolated on nutrient agar streak plates (see 3.7.3.2) in order to allow further identification by Gram staining and microscopy

3.8.6.1 Slide preparation and Gram staining

The microorganisms were mounted on microscope slides and Gram stained to aid identification using the following procedure:

1. Mark the upper surface of a clean microscope slide with a wax pencil
2. Flame a loop and use it to place a loopful of RO water on the slide.
3. Select an isolated colony of the microorganism to be examined and pick off with a flamed needle or loop

4. Mix bacteria in water
5. Heat fix by passing over a Bunsen flame several times until the water has evaporated. Do not allow the slide to get too hot (test on back of hand)
6. Flood the slide with crystal violet (methyl violet) for 20 seconds
7. Wash with water
8. Flood with Lugol's Iodine (iodine in saturated potassium iodide solution)
9. Immediately wash with water
10. Decolourise by flooding briefly with alcohol
11. Wash with water
12. Counter stain by flooding with safranin solution for two minutes
13. Wash with water
14. Blot excess water with a paper towel and leave to dry

3.8.6.2 Microscopy and photomicrography

The prepared microscope slides were examined under an Olympus BH-2 binocular microscope fitted with x4, x10, x20, x40 standard objectives and x100 oil-immersion objective.

Photographs were taken of some slides under the x100 oil immersion objective, using a single lens reflex camera attached to a vertical camera tube fitted to the microscope. A stage micrometer was not available so the images were not calibrated.

3.9 FLUOROMETRY

Samples of soil from the mini pit were analysed fluorometrically to assess the distribution of DDB through the soil column. The method used is that described by Herbath (2000).

The technique exploits the fact that molecules in solution can absorb photons of incident light and in the process reach an excited state. Relaxation of the molecule to the original, or ground state may be by non-radiative means or it may be accompanied by emission of a photon of light. This emission is termed fluorescence. Any molecule is capable of fluorescence but most do not because their non-radiative relaxation paths occur at a faster rate than radiative paths. Fluorescence is most likely to occur in compounds with a rigid molecular structure. Aromatic rings fluoresce intensely and substituted groups modify the wavelength and strength of the absorption maximum.

The energy of a photon of light is related to the wavelength:

$$Energy = h\frac{c}{l} = hn$$

Where:

Energy = energy / J

h = Planck's Constant = 6.63×10^{-34} J

c = velocity of light in a vacuum ≈ 3×10^{8} $m.s^{-1}$

λ = wavelength / m

ν = frequency / Hz

Fluorescent molecules will absorb light at one wavelength, the excitation wavelength and shortly afterwards ($\leq 10^{-5}$ s), emit light either at the same, or a longer, emission wavelength. In resonant fluorescence, the molecule relaxes to the ground state in a single step and the light emitted is of the same energy and therefore the same wavelength as the incident light. More commonly, a combination of non-radiative relaxation and fluorescence means that the emission wavelength is longer than the excitation wavelength; the difference between the two wavelengths is called the Stokes shift.

In a fluorometer, the excitation wavelength is selected using an appropriate filter and the fluorescence is measured using a filter and detector placed at right angles to the incident light.

At low concentrations (Absorbance ≤ 0.05, transmittance $\geq 90\%$) the fluorescence power of a solution is proportional to the concentration of the fluorescent species. At higher concentrations, quenching effects mean that the response reaches a maximum and may even decrease.

3.9.1 Extraction

The choice of extractant was governed by the need for a solvent that gave good extraction efficiency, and which did not interfere with the fluorescence of the DDB, either by absorption or emission at the excitation or emission frequencies of DDB.

Hexane was selected as the most appropriate solvent for DDB fluorometry. It gives approximately 90% extraction of DDB from soil with typical moisture content. High Performance Liquid Chromatography (HPLC) grade hexane has a low background fluorescence and maximum absorbance at the relevant frequencies are 0.02 Absorbance Units (AU) at 254 nm and 0.005 AU at 280 nm.

1. Weigh out 7.5 g soil +/- 0.5 g
2. Add 15 ml hexane using a 10-30 ml Zipette dispenser in a fume cupboard
3. Shake using a Stuart Scientific wrist-action shaker @ 1000 rpm for 20 minutes

Herbath (2000) had used this method in preference to Soxhlet extraction due to time constraints. Schwab *et al* (1999) demonstrated that the more complex Soxhlet method does not result in a significantly more efficient extraction of petroleum hydrocarbons from soil.

3.9.2 Set-up and calibration

The analysis was carried out with a Turner Designs TD-700 fluorometer. The light source in this instrument is a clear quartz lamp. Excitation and detection wavelengths were selected using a 254 nm excitation filter and a 280 nm emission filter.

Since fluorescence involves excitation by ultra-violet (UV) wavelengths, glass cuvettes were unsuitable and so quartz cuvettes of 10 mm path length were used. Latex gloves were worn to prevent grease from fingertips from interfering with the light path, the

optical faces of the cuvette were polished with lens tissue before use and the cuvette was used in the same orientation each time.

The fluorometer was calibrated according to the manual to produce a calibration curve (Figure 3.9).

The quenching effects mean that for an indicated fluorescence value, there are often two possible DDB concentrations. In order to ensure that the sample lay on the left of the peak, samples were spiked with 100 $\mu l.ml^{-1}$ DDB in hexane solution. If the fluorescence reading did not increase after spiking, it was assumed that quenching had occurred and a diluted sample (1:30 with hexane) was used.

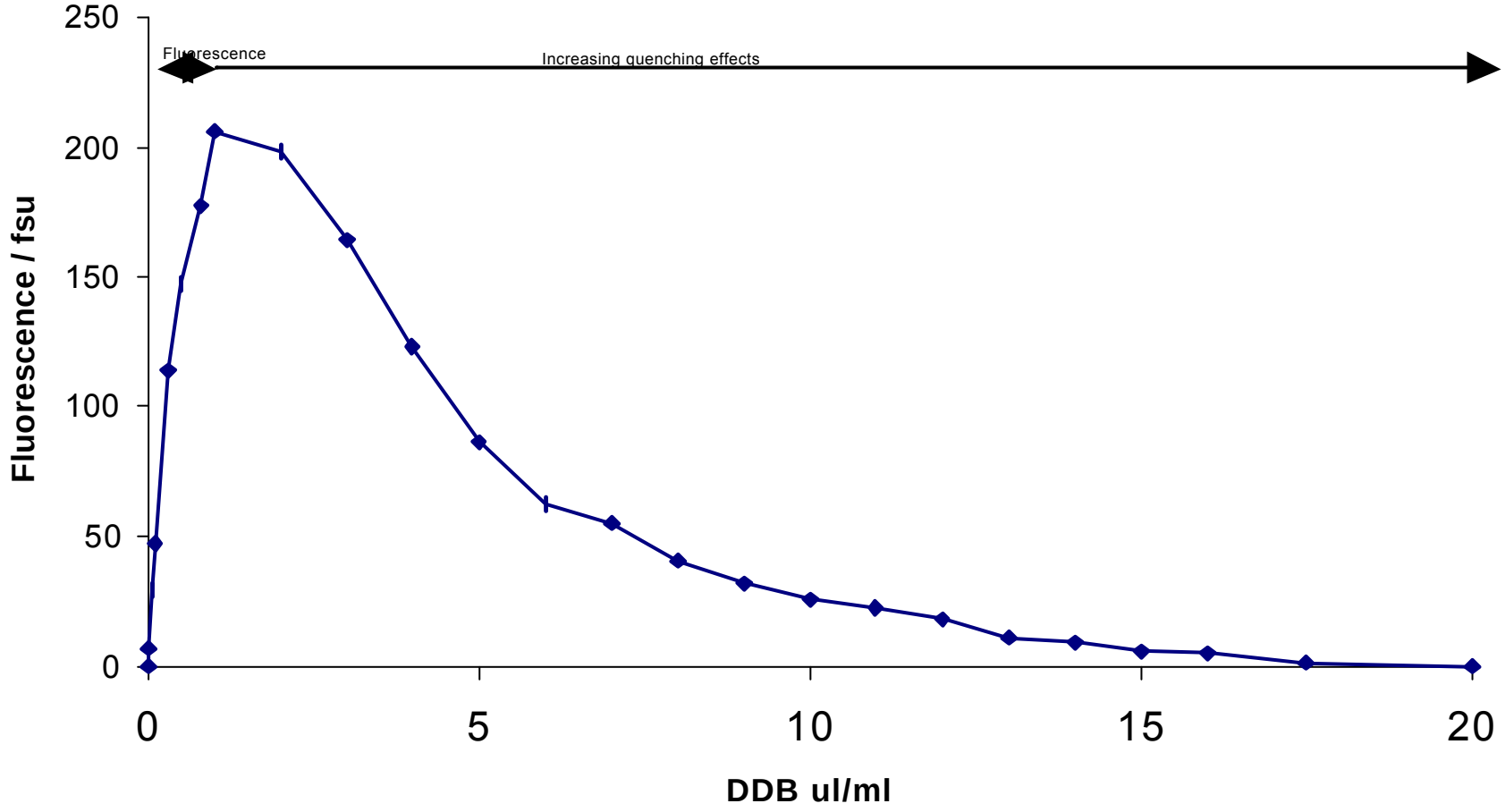

Figure 3.9 Fluorescence calibration curve

3.9.3 Analysis

The soil samples taken for microbiological analysis on 28 August 1999 and 24 November 1999 were stored at –70°C and it was decided that they should be analysed for DDB content. The small sample size precluded the use of replication of analyses and so particular care was taken. The following procedure was followed:

1. Rinse cuvette three times with hexane
2. Fit cuvette in holder, ensuring same orientation each time
3. Fill ¾ full with test solution and close with a Poly(tetrafluoroethylene) (PTFE) cap to reduce evaporation.
4. Place holder in fluorometer
5. Press * key to begin analysis and note fsu reading
6. Add 100 µl of 1 $\mu l.ml^{-1}$ DDB in hexane standard solution
7. Press * key to begin analysis and note fsu reading

If the reading in step 8 is higher than that in step 6, the sample lies on the left of the peak and the reading from step 6 can be assumed to be correct. If the reading from step 8 is lower, the sample lies to the right of the peak and will need to be diluted to place it in the linear portion of the curve.

Chapter 4

Results

4.1 ENVIRONMENTAL CONDITIONS

4.1.1 Temperature

The temperature was monitored more or less continuously over the period of the study. This allowed data to be corrected to account for the varying responses of the ORP electrodes with temperature, and allowed microbial activity to be related to external conditions. The data (Fig.4.1) shows a regular diurnal variation and an underlying seasonal cycle. The temperature measured in the flow-through cell (OUT) generally changed slightly faster than that measured in the settlement tank. This is probably due to the greater volume of water in the settlement tank acting as a thermal buffer. A number of gaps occur in the data series, which reflect failures in the data logging equipment.

4.1.2 Pumping rates

Pumping rates were calculated by multiplying the number of times the pump had cycled since the last time the pumping routine was reset, by the volume per pump cycle, and dividing by the elapsed time. This was not always possible since the counter would reset after approximately 3 weeks so if it were not checked during this time a gap would appear in the data series. This was a limitation of the counter routine in the Control Disc application. Nevertheless, a reasonably complete data series was obtained (Figure 4.2) The wide variation in pumping rate in the early weeks of the study was due to a combination of an early failure in the pump routine which resulted in the pump remaining on for several days, and high rates of evaporation from the settlement tank and the soil surface. This was minimised by covering the soil and free water surface with clear polythene sheeting.

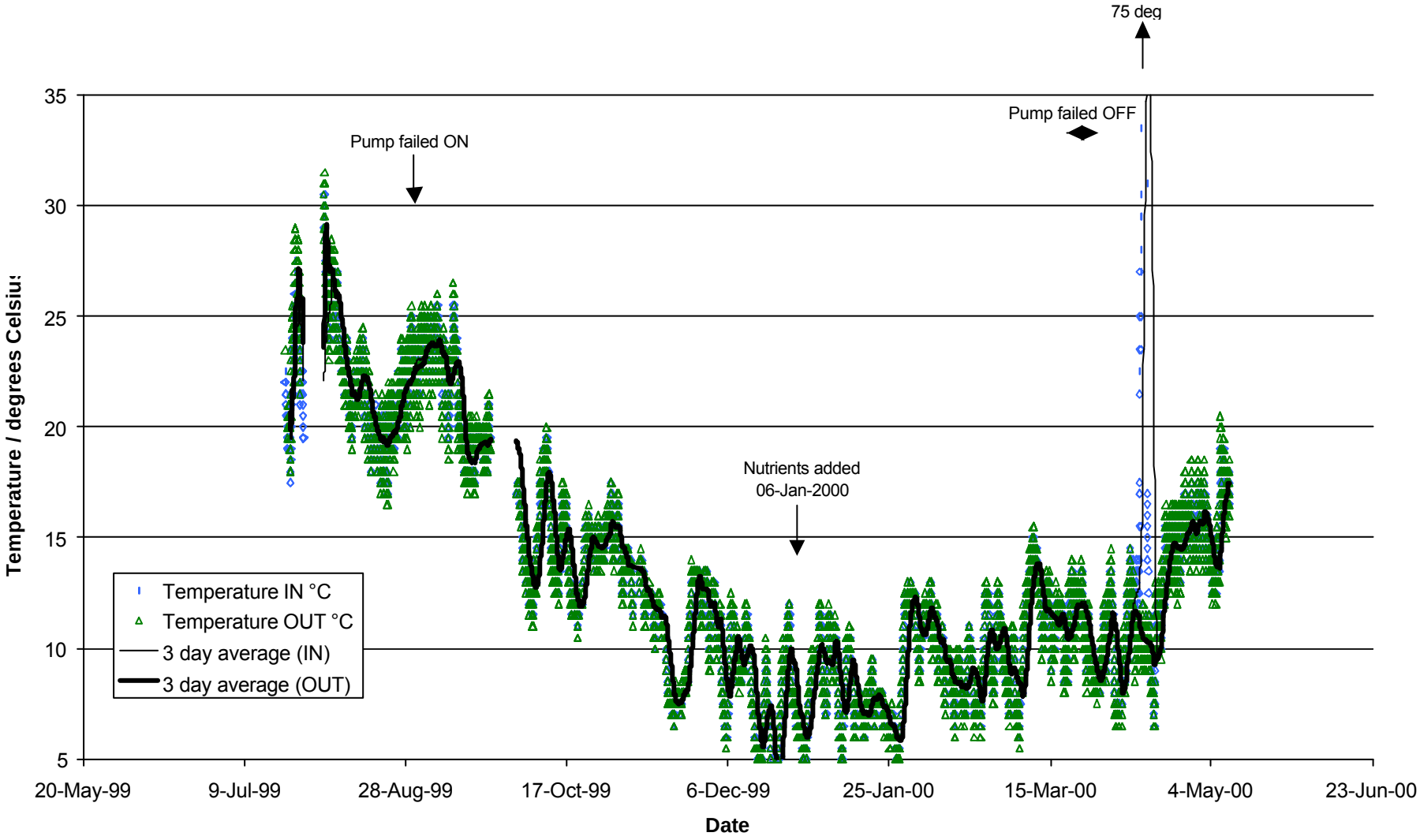

Figure 4.1 Variation of temperature with time during the mini pit investigation

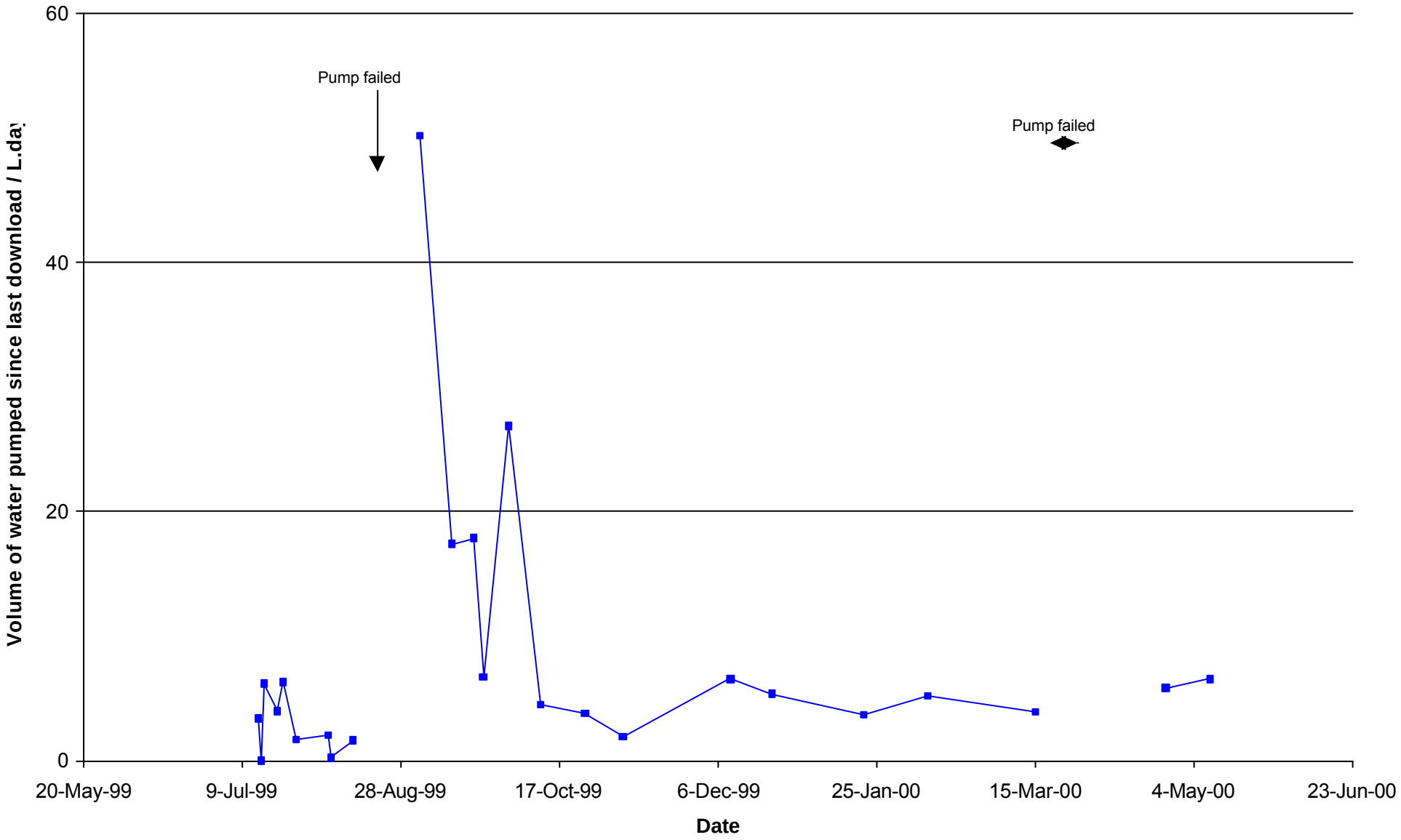

Figure 4.2 Variation of the rate of ground water pumping with time in the mini pit investigation

4.1.3 Water distribution

Time domain reflectometry (TDR) readings were taken at intervals to monitor the water distribution within the mini pit. The intention was to be sure that the water distribution did not change significantly over the period of the investigation.

In Figure 4.3, the moisture content of the soil measured along a horizontal line 300 mm above the floor of the tank, just above the intended water table, is shown plotted against time for each probe. The horizontal array of TDR probes were designated A, B, C, D_1 and E, with A being closest to the injection well.

Readings from individual probes were consistent throughout the study and most were in the range 30-35%. This suggests that the soil at this level was more or less saturated (35.30%; Lovelace, 1999) and that the probes are therefore within the capillary fringe, just above the water table.

The probe nearest the injection well (probe A) appeared to be appreciably drier than the other probes in the horizontal array. This may reflect inconsistencies in the fabrication of the probes, or may indicate a real variation in the water level.

Figure 4.4 shows similar data for a vertical array of TDR probes placed approximately at the mid point of the mini pit. D_1 was the same probe as that in the horizontal array. The other probes were placed at 100 mm vertical intervals above D_1 and designated D_2, D_3, D_4 and D_5.

At the start of the study, the mini pit was completely filled with water, before being drained down to the intended level. As a result, all of the probes indicated that the soil was saturated (30-35%) at this time.

As the water was drained, the readings from the vertical array reflected the changing moisture content. The lowest probe remained saturated, the two highest probes settled at about 25%, and the intermediate probes, D_2 and D_3, indicated intermediate moisture content

All of the probes seemed to indicate slowly rising moisture content, despite the fact that the pumping rate remained steady at about 5 $L.day^{-1}$. It is not clear whether this is a real change in the moisture content of the soil, or whether biofouling or other physico-chemical changes affected the probe characteristics.

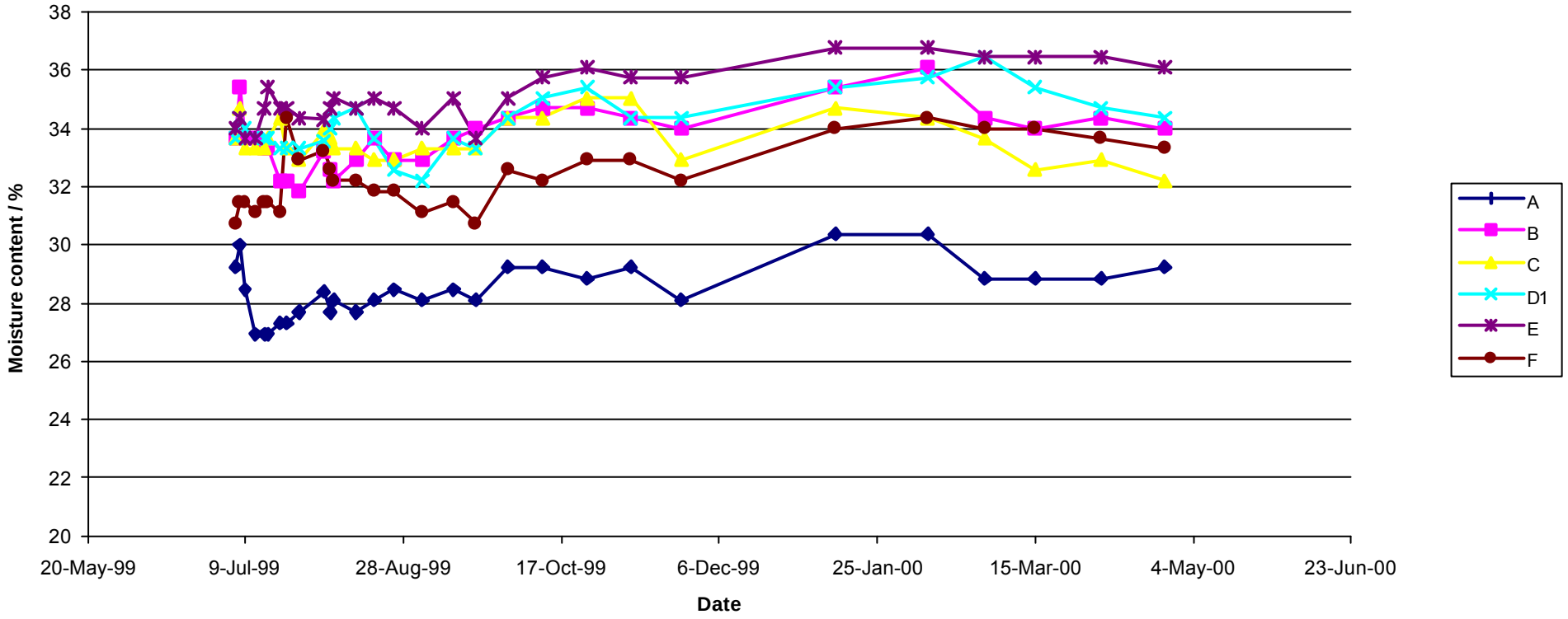

Figure 4.3 Soil water content in the mini pit (horizontal distribution)

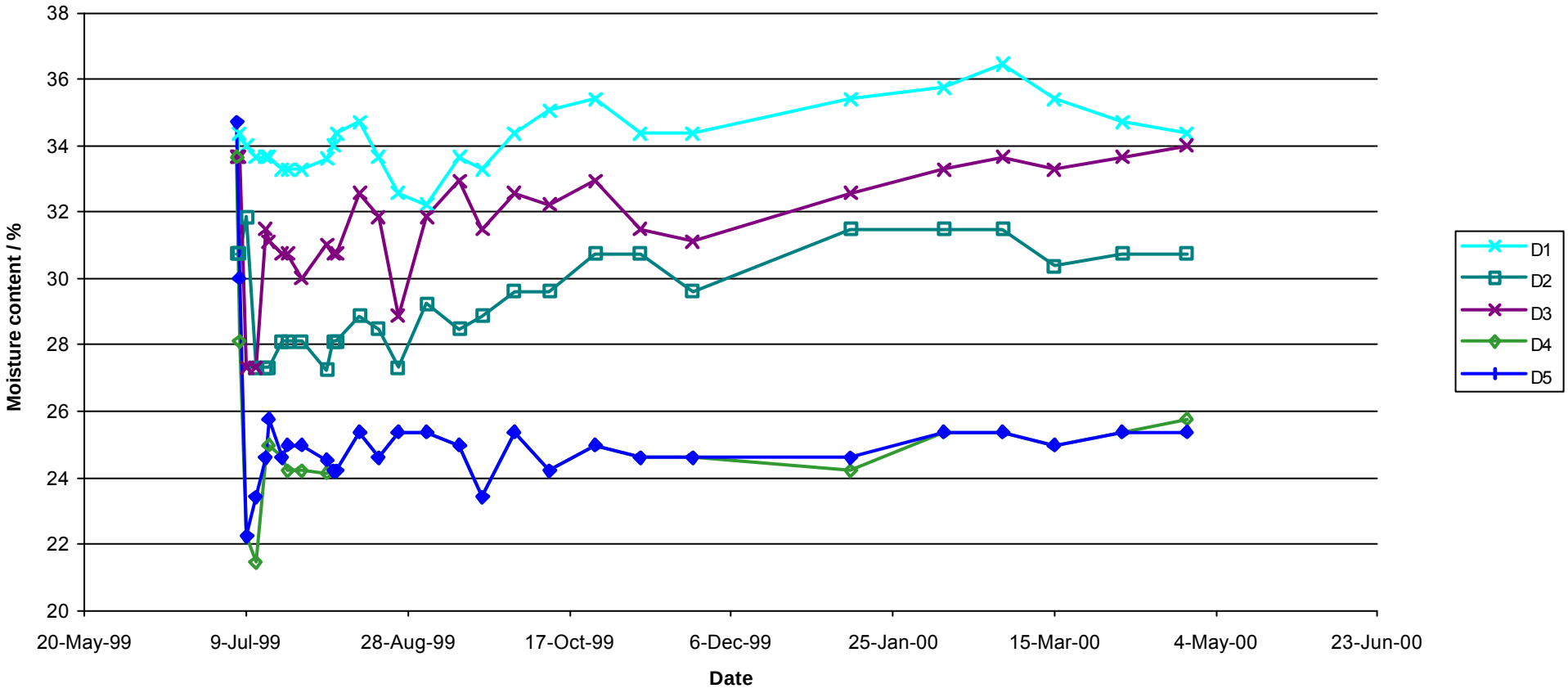

Figure 4.4 Soil water content in the mini pit (vertical distribution)

4.2 MONITORING

Dissolved oxygen, pH and oxidation-reduction potential (ORP) were monitored at the injection and extraction points.

4.2.1 pH

The pH of water in the settlement tank (IN) and flow-through cell (OUT) are shown in Figure 4.5. There were three major perturbations in the pH values, which may be related to identifiable events. In early August, the pumping system failed in the ON position for approximately three days. Since the hydraulic conductivity of the soil was too low to support this rate of pumping, the effect was to draw air through the flow-through cell and bubble it though the water in the settlement tank. The pH reading at both points fell sharply and upon reinstatement of the correct pumping regime the readings recovered over about 10 days.

The second identifiable perturbation occurred following the addition of nutrients to the mini pit on 6 January 2000. This was achieved by siphoning 10 L of water out of the settlement tank, mixing the nutrients with this water and then pouring the resulting solution into the injection well. This left the probes in the settlement tank uncovered until the water level in the tank recovered. It is likely that the probe was uncovered long enough for it to dry out appreciably (about two days, given a pumping rate of 5 $L.day^{-1}$) and so it took longer than two days for the pH IN reading to recover.

The final identifiable event was a second failure of the pumping system, this time in the OFF position in late March 2000. This time, the pH IN rose to more than pH 8, before once again returning to its normal range of approximately 6.5 – 7.0 upon reinstatement of the pumping routine.

Although these events were not planned, they were useful in that they demonstrated the robustness of the overall system. Conditions were not permanently changed as a result of any of the events and recovery began immediately upon reinstatement of the pumping regime.

Apart from these events there are several features of note:

1. pH IN was initially between 7.5 and 8.0, reflecting that of tap water
2. The pH IN was consistently about 0.5 to 1.0 pH units higher than the pH OUT.
3. pH OUT was approximately 6.5
4. pH OUT was more stable than pH IN
5. pH IN was influenced by pH OUT – this was demonstrated by the fact that pH IN returned to 8.0 during the time when the pump was not running. This suggests that the residence time of the water in the settlement tank was not long enough for the pH value to stabilise. A larger volume or agitation/aeration of the settlement tank might have affected this.

4.2.2 Dissolved oxygen

Figure 4.6 shows the amount of dissolved oxygen (dO_2) in the mini pit water over time. The events that were observed to perturb the pH readings also affected these readings.

During the early pump failure the apparent dO_2 level in the flow-through cell rose to 100%. This reflects the fact that the cell was filled with air during this episode and the oxygen sensor had been calibrated to read 100% in air. Conditions in the settlement tank simultaneously became anaerobic, with a strong "rotten egg" smell becoming apparent. It was not clear why this should have occurred but it is possible that the polythene cover over the settlement tank may have had an effect. This had been fitted shortly before the

pump failure with an elastic loop used to hold it onto the lip of the tank. The elastic was removed to allow improved gas exchange while still minimising evaporation.

The addition of nutrients on 6 January 2000 and the attendant drop in water level in the settlement tank resulted in a reduction of recorded dO_2 in the settlement tank. This was the opposite of what might have been expected since the probe was in air for up to two days.

Following the second pump failure, the recorded dO_2 in the settlement tank fell to zero. This was noticed when the data that indicated this fall was downloaded. Further investigation revealed that there was a hemispherical mass of translucent jelly over the PTFE membrane of the oxygen electrode. This was removed and, since it was also noticed that there was a greenish colouration to the water and visible strands of, presumably, cyanobacteria or algae, the settlement tank was covered with a sheet of expanded polystyrene foam to reduce the amount of light from the skylight immediately above reaching the water surface.

With the exception of the first pump failure, the dO_2 reading from the flow-through cell did not depart significantly from zero.

4.2.3 Oxidation-reduction potential

The variation in oxidation-reduction (Redox) potential (ORP) is shown in Figure 4.7.

The values are corrected for the temperature response of the electrode and expressed as Standard Potential (i.e. relative to a Standard Hydrogen Electrode).

The SensorMeters used to record the ORP were only able to read down to about +5 mV, which translated to a Standard Potential of about –200 mV. This gives an artificial "plateau" effect in the trace of ORP OUT. To partially mitigate for this, occasional spot checks were made using a bench pH meter connected to the same ORP electrode. These are plotted as single points (red triangles in Figure 4.7) and indicate that the ORP in the ground water was approximately -600 mV for most of the investigation.

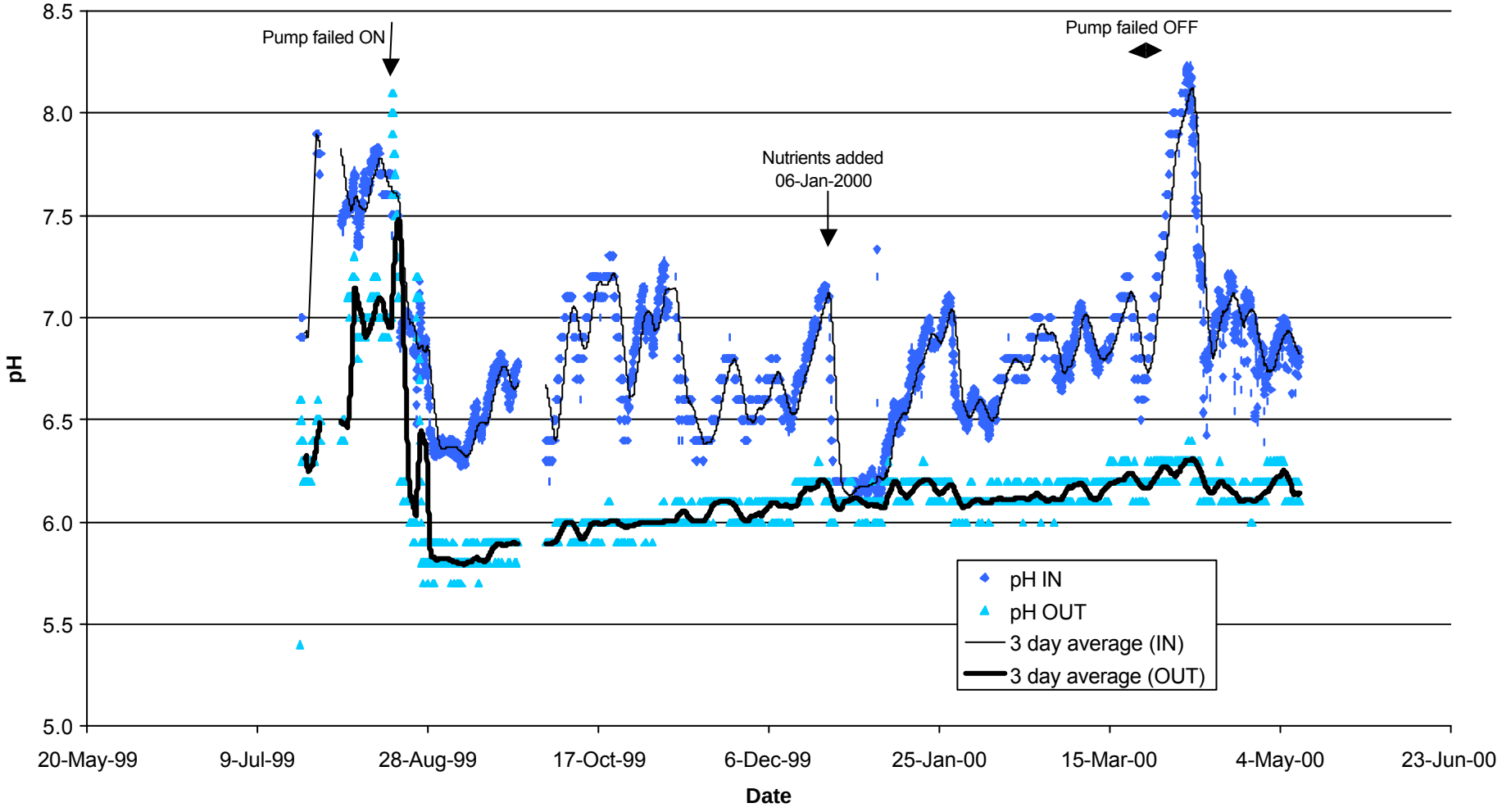

Figure 4.5 Variation of ground water pH with time in the mini pit investigation

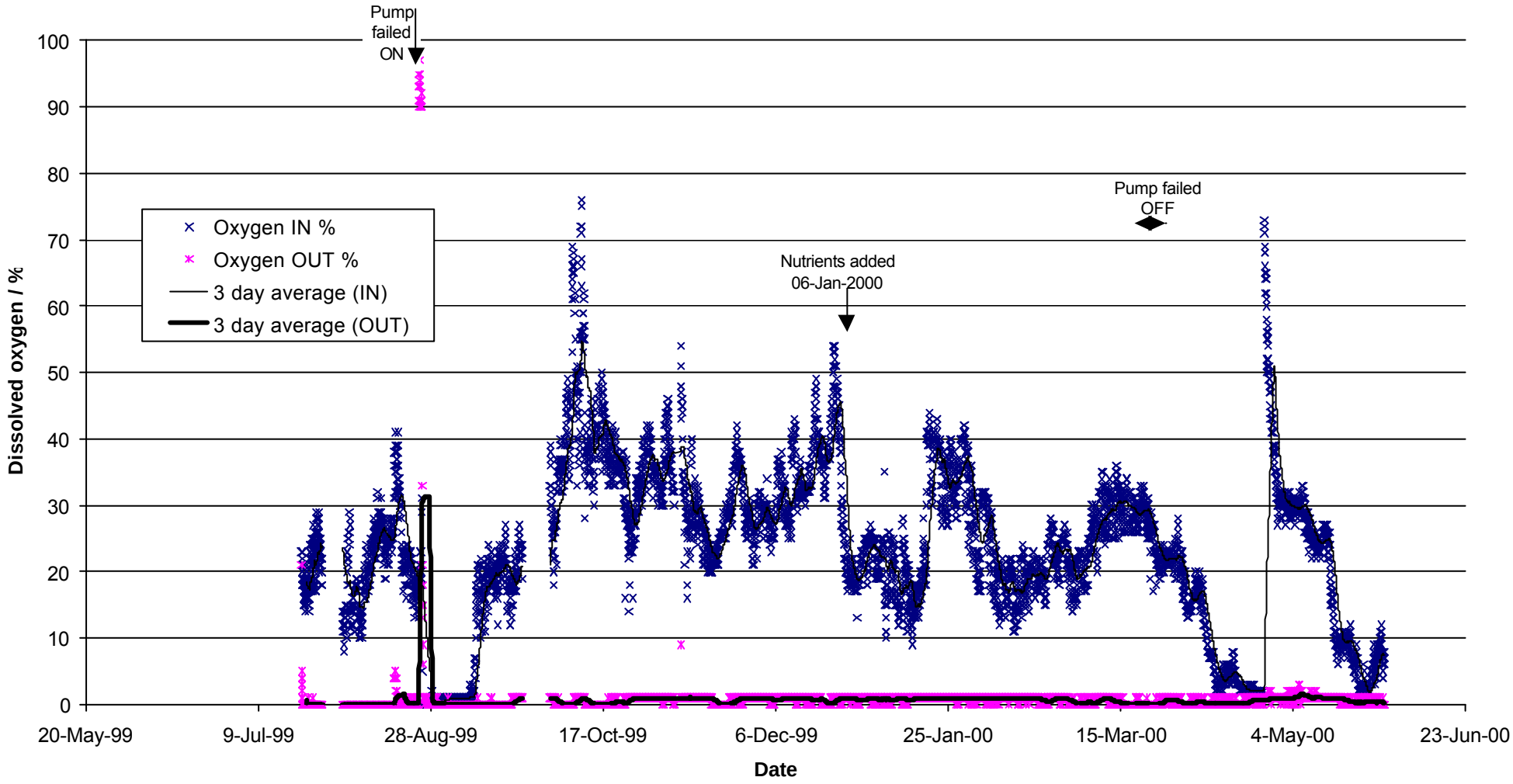

Figure 4.6 Variation of ground water dissolved oxygen with time in the mini pit investigation

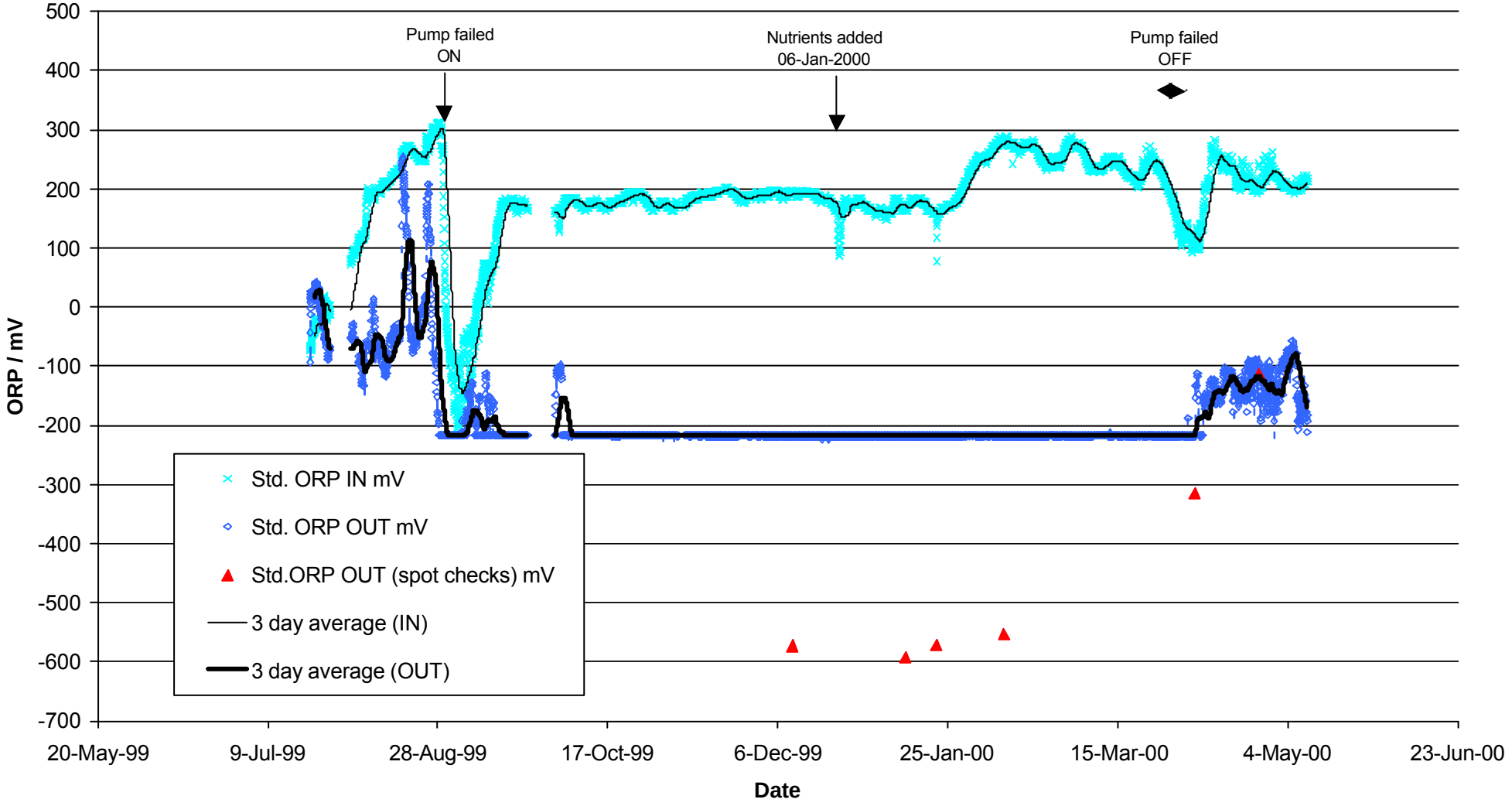

Figure 4.7 Variation of ground water redox potential (ORP) with time in the mini pit investigation (See 4.2.3)

4.3 MICROBIOLOGY

4.3.1 Identification

Work was done jointly with Herbath (2000) to identify some of the microorganisms found in the main pit, from where the inoculum for the mini pit was obtained.

Plates 4.1 and 4.2 are typical of spread plates produced by culturing on nutrient agar (NA) and DDB agar, respectively.

Plate 4.1 Typical nutrient agar plate

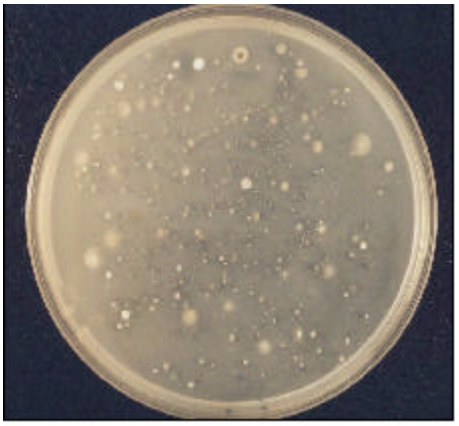

Plate 4.2 Typical DDB agar plate

A wide variety of colonies were observed on both plates. The only immediately recognisable colonies were the feathery mould-like colonies of *Bacillus cereus* var. *mycoides* on many of the NA plates.

Since the organisms of greatest interest were those that were able to utilise DDB as a carbon source, attention was focussed on the colonies on the DDB plates. A number of colonies were isolated before being Gram stained and examined under the microscope.

Isolation plates and micrographs are shown in Plates 4.3-4.10. All agar plates were 90 mm Petri dishes and were on DDB agar unless otherwise stated. The micrographs were taken under a x100 oil immersion objective.

Plate 4.3a DDB isolate 1 spread plate

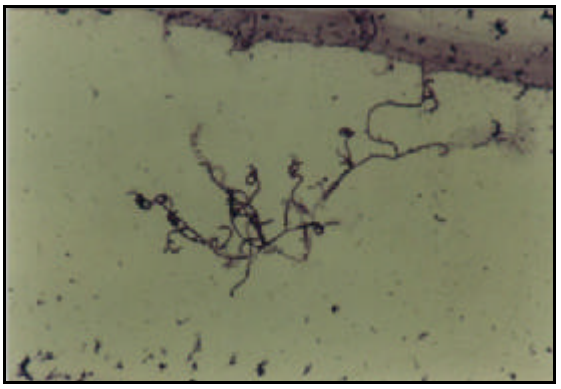

Plate 4.3b DDB isolate 1 Gram stain

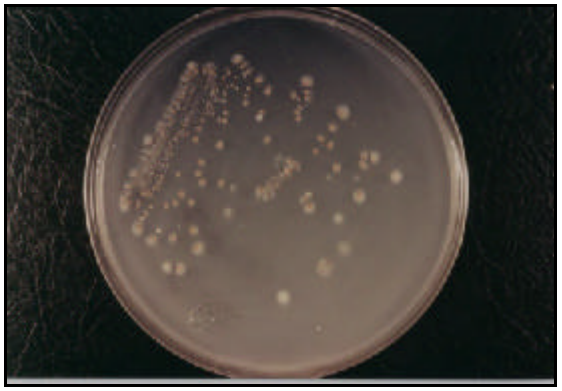

Plate 4.4a DDB isolate 2 spread plate

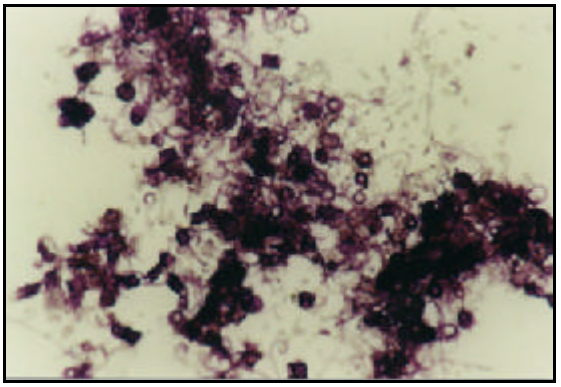

Plate 4.4b DDB isolate 2 Gram stain

Plate 4.5a DDB isolate 4 spread plate on NA (note diffusible pigment)

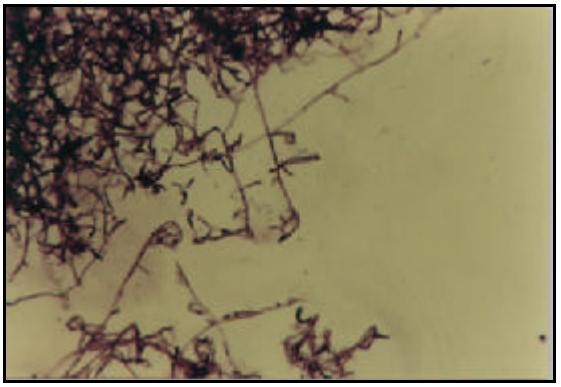

Plate 4.5b DDB isolate 4 Gram stain

Plate 4.6a DDB isolate 5 spread plate

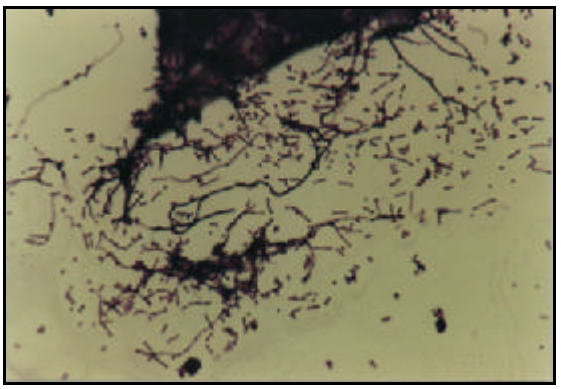

Plate 4.6b DDB isolate 5 Gram stain

All the organisms isolated from the DDB plates were aerobic, Gram-positive cells, which formed filamentous chains or hyphae. Some appeared to bear spore-like structures. At least one (Plate 4.5a) demonstrated the production of diffusible substances into the culture medium.

4.3.2 Plate counts

The following two graphs (Fig. 4.8 and 4.9) show the distribution of soil microorganisms within a vertical soil core from the mini pit. Samples were cultured on nutrient agar (NA) and DDB agar. Error bars are one Standard Deviation (SD, n = 3). The degree of confidence in the vertical spacing of microorganisms is not high since the soil became compacted during extraction. The lack of data for the 700-800 mm interval in November is due to the soil at this depth being too wet to allow removal with the available tools.

There were higher numbers of viable organisms in the soil from the first count. This is possibly due to the higher temperature in August. Numbers from both sets of samples were appreciably lower than those from the main pit and tanks (Herbath 2000).

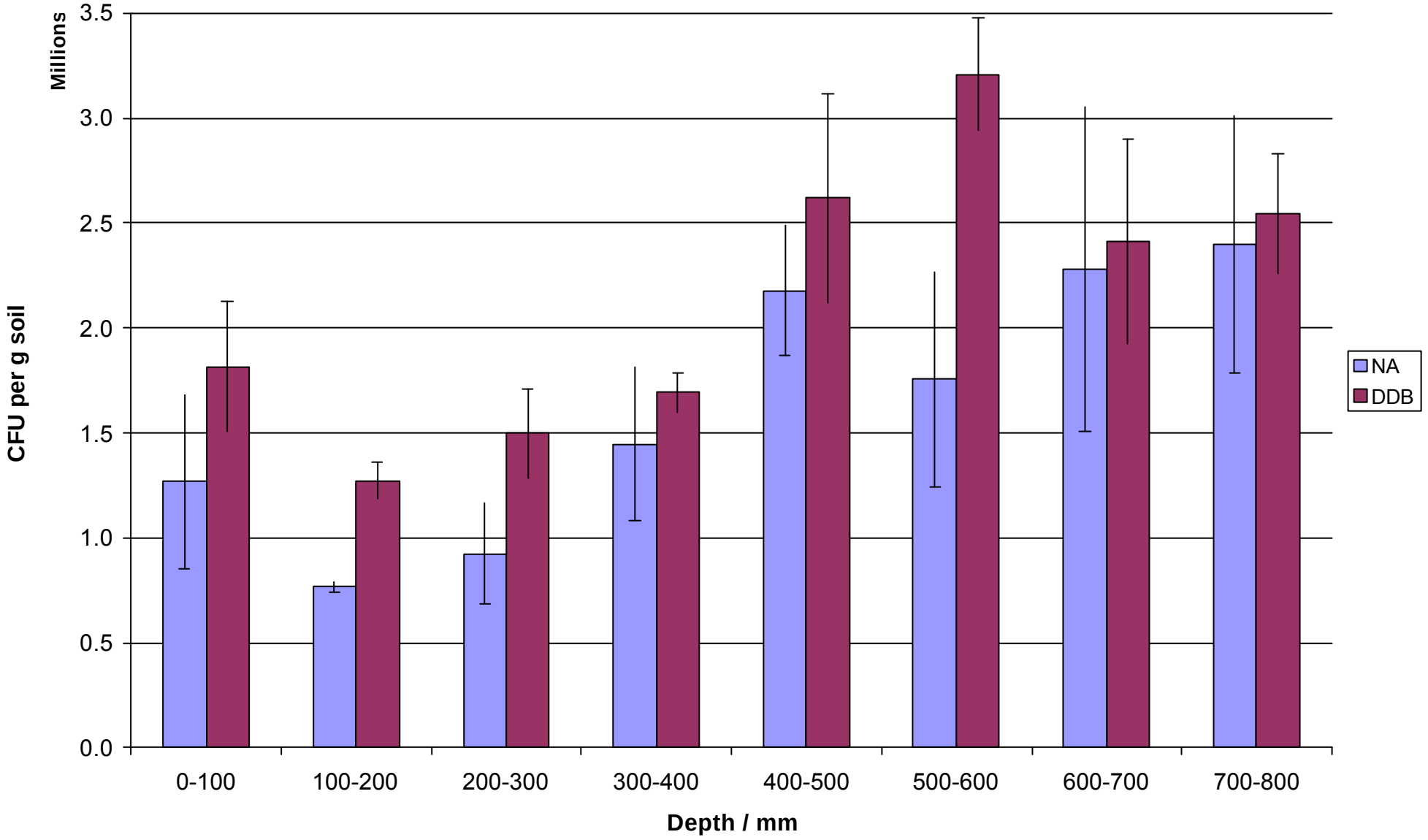

Figure 4.8 Microbiological counts from mini pit soil samples collected on 27 August 1999 (Error bars = 1 SD, n = 3)

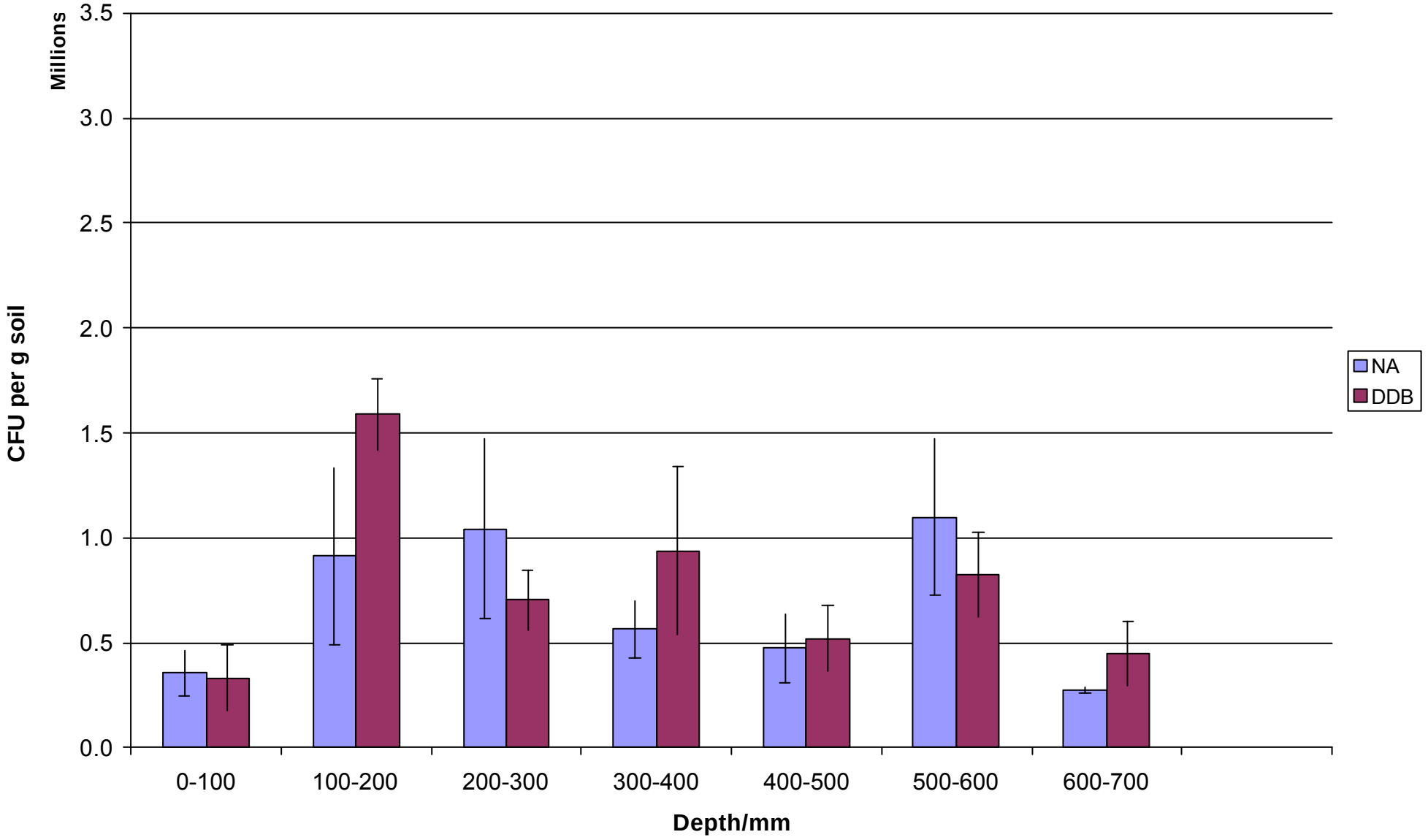

Figure 4.9 Microbiological counts from mini pit soil samples collected on 24 November 1999 (Error bars = 1 SD, n = 3)

4.4 FLUOROMETRY

Samples taken in November 1999 and February 2000 were analysed for DDB content.

The results are shown in Fig. 4.10. Due to a shortage of sample material, only a single sample from each 100 mm depth interval was analysed from the November sampling. There was more material available from the February sampling so the February analyses were duplicated and the arithmetic mean of the two readings plotted. The error bars indicate one standard deviation.

The greatest error appears to be just above the water table in the capillary fringe. This is the region where the greatest amount of "smearing" of DDB would be expected and fluctuations in the water level will have the most profound effect here.

The data do not show a significant change in DDB concentration at the water table (approximately 600 mm depth) so it is not possible to say that bioremediation took place. This may be the case, but the data sets are too small and too closely spaced to support a reliable conclusion

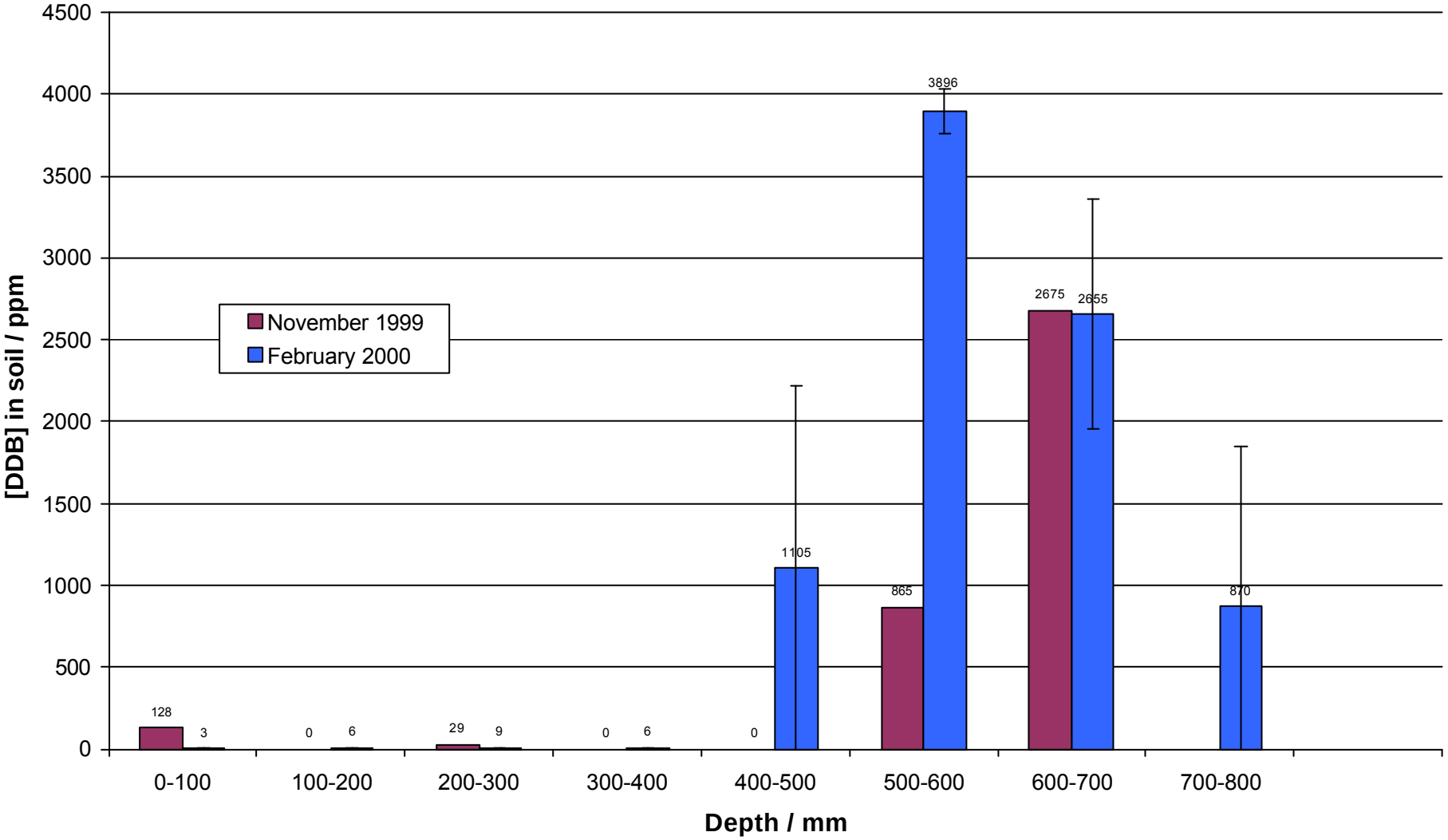

Figure 4.10 DDB distribution in the mini pit (Error bars on data from February 2000 = 1 SD, n = 2)

Chapter 5

Discussion

5.1 INTRODUCTION

The investigation had two main aims: to characterise the physical, chemical and biological conditions that occur when a soil is contaminated with cable oil; and to assess the suitability of Control Cube technology as a basis for a monitoring and control of bioremedial systems.

This chapter will begin with a discussion of the advantages and limitations of the model system and the monitoring and control measures. This will be followed by an analysis of what the recorded data suggest about the applicability of bioremediation to dodecylbenzene (DDB) cable oil in soil and ground water.

5.2 GENERAL CONSIDERATIONS

There is no reason to suppose that the hydraulic conductivity of the soil in the mini pit differed significantly from the figure of 1.54 $m.day^{-1}$ (= 1.78 x 10^{-3} $cm.s^{-1}$) measured in soil from the main pit (Lovelace, 1999) since the soil was sourced from the same site and was loaded into the mini pit in the same way. This is an order of magnitude higher than the 10^{-4} $cm.s^{-1}$ suggested by Andreoni and Baggi (1996) as a reasonable minimum and suggests that, hydrologically, this soil is a good candidate for *in situ* bioremediation. Figures for soil in actual joint bays are not available so it is not possible to say whether this is typical of conditions in the field.

Very little DDB was observed on the surface of the water in the settlement tank. This may mean that the volume of cable oil added to the mini pit was small enough that it

was all absorbed by the available volume of soil and water, or that it was particularly immobile in this soil. A thin film of non-aqueous phase liquid was seen on the surface of the water so it appears that some at least permeated through the soil block.

5.3 MONITORING SYSTEM

5.3.1 Probe choice and placement

The pH, oxidation-reduction potential (ORP) and dissolved oxygen (dO_2) electrodes used in the investigation were standard laboratory items, intended for occasional use with frequent recalibration. Recalibration of the probes was kept to a minimum in order to assess their long-term stability. They were, in general, remarkably stable over the life of the investigation.

The flow-through cell appeared to work admirably as a method of obtaining a water sample from the soil block and applying it to sensor elements without significant exposure to the atmosphere. If used in the longer term, it might have been prudent to cover the cell to exclude light and so discourage photosynthesis, but the basic design is sound.

5.3.2 Temperature

The Control Cube Water Temperature Probes used were robust and simple to use, requiring no calibration or external power. The temperatures measured at the flow - through cell and the settlement tank were extremely close, and the variation could be explained by reference solely to the relative volumes of water in each vessel.

A more meaningful value for temperature might have been obtained by burying the temperature OUT probe in the centre of the soil block. The probes were fully robust enough to allow this with no adverse effects.

The stainless steel probes were not observed to accumulate an appreciable amount of biofouling and, overall, a considerable amount of confidence may be placed in this data. The only exception was that the temperature data showed an anomalous increase in temperature OUT to 75 °C over a three-day period towards the end of the investigation. The pump had failed in the OFF position just prior to this event and the flow-through cell where the probe was situated was in sunlight from a skylight, nevertheless, there is no diurnal fluctuation seen in the data over this period and so it is likely that the data are erroneous. In any case, the event was transient and does not interfere with interpretation of the rest of the data.

5.3.3 Level switches

An early failure in the pump routine, when the pump remained on after the water had been removed from the extraction well, seems to have been due to the upper float switch not being actuated by the falling water level. This was remedied by adjusting the position of the switch to prevent it from fouling on the wall of the well. The switches otherwise performed without error.

5.3.4 TDR monitoring

The time domain reflectometry (TDR) data was largely self-consistent. It is possible that there was enough variation between individual probes to cause some anomalous

results (e.g. the consistently higher moisture level indicated at probe D_3 relative to D_4 (see Figure 4.4)), but results for each probe were consistent and within the expected range of values.

The frequency of data collection was high enough for the purposes of the study (initially every two or three days, less often once conditions had stabilised) but the method of obtaining the data required a high degree of operator skill and is not suitable for an on-line monitoring system. When used for a large number of probes the time taken to read all the probes would be prohibitive (Herbath, 2000) and it would be useful to automate this process.

5.3.5 pH electrode

The pH electrodes used were sealed, plastic bodied units. These were chosen for their low maintenance requirements but since the recommended life of the probes was 12 months, they would not be suitable for longer-term use than this, unless treated as consumables.

5.3.6 Dissolved O_2 probe

The dO_2 probes were far more stable than had been anticipated. The Clark electrode is often considered to require frequent recalibration but this is based on using the electrode intermittently. By keeping the membrane constantly wetted, some of the problems of instability seem to have been avoided.

The main failing of the dO_2 probe was seen, quite late in the investigation, when a hemispherical mass of jelly-like material was observed on the PTFE membrane of the probe in the settlement tank. The measured dO_2 had fallen to zero. This occurred after the addition of nutrients to the mini-pit and coincided with an increase in both temperature and sunlight incident on the tank from an overhead skylight.

It is clear that this represented growth of microorganisms on the surface of the membrane and while it was not obvious whether the organism was photosynthetic, it was accompanied by a green colouration of the water in the tank so this could not be ruled out.

5.3.7 Oxidation-reduction potential (ORP) electrode

The ORP electrodes performed reliably and did not seem to be affected much by growth of microorganisms on the probe surface. They did not require calibration and would be ideal for long-term monitoring.

5.3.8 SensorMeters

SensorMeters provided an easy-to-use solution to the collection of temperature, pH, ORP, and dO_2 data. They have the advantages of simple, usually single-point, calibration and, when used with appropriate software, they are automatically recognised so that raw analogue data can be converted to meaningful values in appropriate units.

The main disadvantage of SensorMeters was that they require a power source. They can be powered for short periods by an internal 9V battery, but for sustained use they require a DC mains adaptor. This requires access to a mains electricity socket and any interruption in the power supply results in the loss of calibration. Power fluctuations in the shed where the mini pit was situated caused two short gaps in the data.

5.3.8.1 pH/ORP SensorMeter

One of the pH SensorMeters used had a fault in the sensor identification circuit. It was not convenient to take the SensorMeter off-line to have it repaired but since the identification is separate from the sensing circuitry, it was possible to calculate pH from the raw analogue data recorded from this unit. This was acceptable in this investigation but a control installation would require that the software correctly identify the sensor.

The ORP electrodes were connected to pH SensorMeters set to their mV range. Unfortunately these units are only able to register a positive potential and so there was an artificial "plateau" in the data at a Standard Potential of about –200 mV (reflecting the Standard Potential of the Ag/AgCl reference electrode). It was possible to make spot checks using a Hanna bench pH meter but if used in a monitoring/control system, a fully bipolar sensor would be required.

5.3.8.2 Dissolved Oxygen SensorMeter

No specific problems were noted with the use of the Dissolved Oxygen SensorMeter. It was convenient to calibrate in air since this indicated when the probe was uncovered

due to a pumping failure but it could equally have been calibrated to read 100% in air- or oxygen-saturated water.

5.3.9 Control Cube and Control Disc

5.3.9.1 Control Cube

The Control Cube is a flexible control/datalogging interface, and can easily be configured as a stand-alone datalogger. The model used had eight sensor and eight control channels. Since a total of ten sensors were used (two each of pH, dO_2, ORP and temperature and two float switches), and only a single control output (the mains controller for the peristaltic pump), it was convenient to separate the functions of pumping and data collection by using two Control Cubes.

This separation of functions would not be appropriate in an unattended installation but was acceptable in this case since the experimental mini pit was checked every two to three weeks.

Control Cube is capable of storing up to 10 data files, each containing eight channels of 500 data points, plus a time channel. Each data file therefore represents a little less than three weeks at the one-hour regular recording interval used in this investigation. Once a data file is filled, it is automatically saved and a new file started. Thus almost 30 weeks of data could potentially be stored without intervention. It is unlikely that an installation would be left for so long between inspections but the limit of 10 files means it is important that old files are deleted occasionally. A gap in the data occurred from 23 September 1999 to 01 October 1999 because the time between downloads was longer

than a single data file. This would not have been a problem if older files had been deleted, but the most recently completed file was the tenth, the Control Cube memory was full and a new file could not be started.

It should be possible to extend the recording interval by combining the timing functions of Control Cube with a counter, also available in the application. However, this would have to be considered a temporary measure, and a commercial remediation system would need to be more flexible and easier to program for these longer periods.

Another limitation of Control Cube is the requirement for 13 A, 240 V AC power. Paradoxically, underground electrical transmission cables are often situated well away from a mains electricity source.

5.3.9.2 Control Disc

The Control Disc routines (Appendix 1 & 2) used in this study were rather simple, but they revealed limitations in Control Disc when applied to this kind of work. This is not a criticism – the software was never produced with this application in mind – but it does mean that the software would not be suitable for an application such as on-line monitoring and control of a bioremediation installation, where significant integration and manipulation of data streams is required.

One limitation imposed by the software is that any counter is limited to a maximum of 999. In this investigation, this represented about 40 days of pumping. It is possible to decrement a counter, or produce a routine that will use more than one counter and

"carry" results from one to another to greatly increase this capacity. Careful programming and testing can circumvent this.

At least one of the two failures of the pumping system could have been avoided by careful testing of the routine:

The first version of the routine halted the pump if both float switches were in the down position. When the upper switch fouled on an obstruction in the extraction well, the upper switch remained up and even though the lower switch indicated that the water had been pumped out, the pump remained switched on. This could have been avoided if the control routine took its cue to halt from the lower switch alone. The final version of the routine (Appendix 1) did this. It would not have been possible to account for both switches becoming stuck, but the event indicates the importance of careful testing of routines.

It is unclear what caused the second failure. It occurred immediately following the repeated addition of small quantities of water back into the extraction well in order to measure the volume pumped per cycle. This may have caused the upper switch to be raised again before the water level had dropped far enough for the lower switch to fall. This was a very unusual condition and, as the term of the investigation was almost completed, the cause could not be investigated in detail.

5.4 EFFECT OF CHANGING CONDITIONS

One of the disadvantages of an *in situ* operation, such as was simulated by the mini pit, compared to a bioreactor with perfect mixing, is that in addition to the normal Monod kinetics of population dynamics, bacterial degradation is affected by the slow rate of mixing of nutrients, water and oil in the soil matrix. This leads to a delay before a change in logged parameters is observed in response to the addition of contaminants, inocula and nutrients.

This intrinsic delay might render the system unsuitable for control by a negative feedback algorithm. It is likely that a fuller understanding of the system would allow the development of a predictive model, which would in turn raise the possibility of using a feed forward control system.

5.5 MONITORED DATA

The data monitored using the Control Cube shows some interesting trends, once the changes due to the known perturbations are taken into account. The low ORP and reduction in dO_2 in the groundwater are hallmarks of microbial activity and have been observed in BTEX contaminated soil (Weidemeier *et al.*, 1996).

5.5.1 pH

The pH of water will reflect the materials dissolved in it. The pH of the water being pumped from the soil was consistently lower at approximately 6.0 than that in the settlement tank (6.5 – 7.0). This was consistent with a soil pH of 5.7 based on a 5:1 soil:water extraction measured by Andrews (1999) and was rather lower than the

suggested levels for optimum bioremediation of 6.5 – 8.5 (Ritter & Scarborough, 1995) or 7.8 (Dibble & Bartha, 1979).

The rise in pH on exposure to the atmosphere may mean that microorganisms in the settlement tanks under aerobic conditions removed the dissolved substances. By definition, the products of anaerobic respiration are not fully oxidised and so may provide aerobic organisms with a food source.

Alternatively, it might be that the dissolved substances were volatile and simply escaped into the atmosphere from the water surface. For instance, carbonates play an important role in the composition of ground water: It is likely that the low pH is largely due to the presence of 164.8 $mg.kg^{-1}$ bicarbonate, H_2CO_3 in the soil (Andrews, 1999). Bicarbonate in solution exists in dynamic equilibrium with carbon dioxide. For convenience, the two are considered together as $H_2CO_3^*$.

Other significant equilibria which both affect, and are influenced by, the pH of the resulting solution are: $H_2O \Leftrightarrow H^+ + OH^-$; $CO_{2\,(g)} + H_2O \Leftrightarrow H_2CO_3^*$; $H_2CO_3^* \Leftrightarrow H^+ + HCO_3^-$ and $HCO_3 \Leftrightarrow H^+ + CO_3^{2-}$ (Appelo & Postma, 1993). The relative proportion of each species in solution at different pH levels is shown in Figure 5.1.

Once the water is in contact with the atmosphere, much of the $H_2CO_3^*$ will be lost as CO_2 diffuses into the atmosphere and the equilibrium between CO_2 and H_2CO_3 is shifted, with an accompanying change in pH.

In a field situation, it would probably be necessary to manipulate the soil pH by addition of a buffer to bring it into a range that favours organisms that degrade the contaminant. This underlines the point that indigenous organism have a competitive advantage over introduced organisms and argues for biostimulation and against augmentation with allochthanous microorganisms as a remedial strategy.

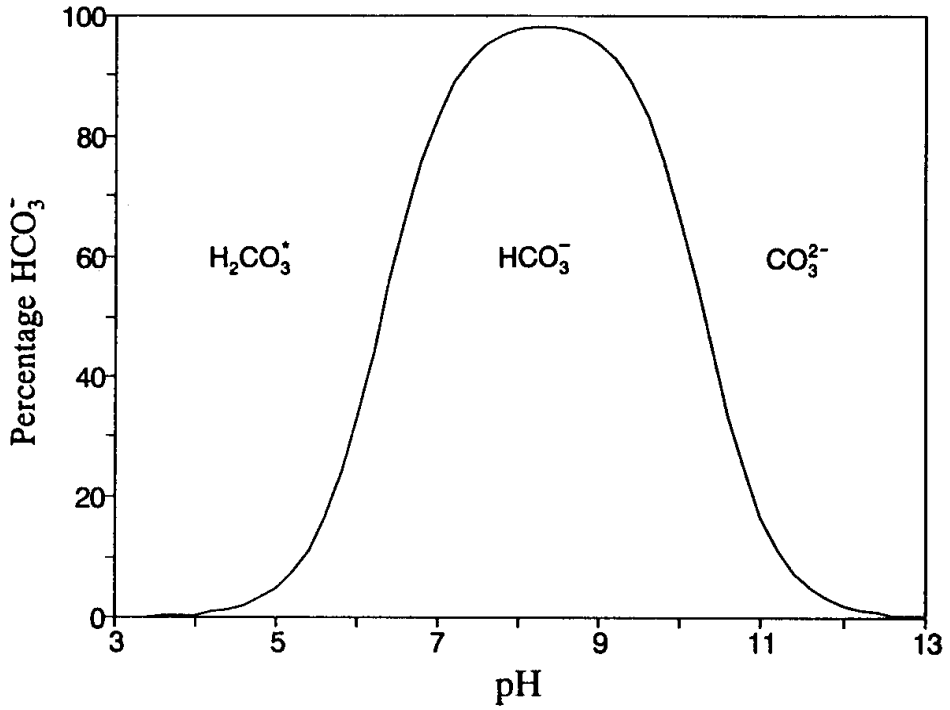

Figure 5.1 Percentage of HCO_3^- of S CO_2, plotted as a function of pH with a fractionation factor (Appelo & Postma, 1993) ($H_2CO_3^*$ = sum of H_2CO_3 and $CO_{2\,(aq)}$)

5.5.2 Oxidation-reduction potential (ORP)

Respiration involves the transfer of electrons from food - highly reduced electron donor molecules such as hydrocarbons - through a chain of coupled redox reactions, to a terminal electron acceptor (TEA), with the resultant transfer of energy from the food to a storage compound such as adenosine triphosphate (ATP).

A large number of possible terminal electron acceptors (TEAs) exist but the most common in soil are oxygen ($O_2 \Rightarrow CO_2$), nitrate ($NO_3^- \Rightarrow N_2$), iron ($Fe^{3+} \Rightarrow Fe^{2+}$), sulphate ($SO_4^{2-} \Rightarrow H_2S$) and carbon dioxide ($CO_2 \Rightarrow CH_4$). If the TEA is oxygen, the process is termed aerobic respiration. If another TEA is used, the process is described as anaerobic.

In general, the microbial community will use available TEAs in the following order:

$$O_2 > NO_3^- > Fe^{2+} > SO_4^{2-} > CO_2$$

So, aerobic organisms will use oxygen where available, anaerobes will be able to use denitrification and other anaerobic processes. Obligate anaerobes will predominate where the only available TEAs are, for instance, iron and sulphate. By combining knowledge of what TEAs are available, and the prevailing redox (ORP) conditions it is possible to infer what respiratory processes predominate.

The soil analysis carried out at Silsoe (see Table 3.1) indicated that nitrate, sulphate and bicarbonate were present and available for use as a TEA by soil microorganisms. It was clear from the extremely low oxygen level in the extracted water that the oxygen dissolved in the injected water was quickly depleted and alternative TEAs would have to be utilised.

The low ORP observed in the ground water (approximately –600 mV) is indicative of the presence of obligate anaerobes and hence extremely low oxygen levels. Figure 5.2 shows the ORP ranges typical of a variety of redox reactions. The ORP in the ground water is consistent with reduction of iron(III) to iron(II), reduction of sulphate to sulphide, and methane fermentation.

No data is available on the iron content of the soil so it is not possible to comment on whether this was a significant electron acceptor. Methane, the product of CO_2 reduction is odourless and so is not easily detected. This investigation did not include measurement of methane.

The "rotten egg" smell of hydrogen sulphide, noted when the settlement tank became anaerobic after the pumping system failed in the ON position, suggests that microbial activity in the ground water included sulphate-reducing organisms. A similar odour was reported by Herbath (2000) on dismantling anaerobic tanks used to investigate the removal of DDB from soil under a variety of nutritional and an/aerobic regimes.

It is likely that aerobic organisms were able to thrive in close proximity to the injection well, which was supplied with air-saturated water from the settlement tank. Soil samples were not taken from this part of the mini pit, in order to ensure that the well was not damaged.

The combination of low ORP, known availability of sulphate in the soil and the observation of the distinctive odour demonstrates 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, but it was not necessarily associated with cable oil degradation.

5.5.3 Dissolved oxygen

That the water being pumped out of the soil contained no measurable oxygen suggests that there was some aerobic process occurring. This may have been biological but not necessarily so. If conditions in the soil were already strongly reduced, the oxygen would be quickly consumed by chemical oxidation of the reduced products of anaerobic respiration.

Since there were only two points of dO_2 monitoring, there was no way of knowing how quickly the oxygen is used up but it is probable that any available oxygen was depleted relatively close to the injection well.

If DDB-degrading organisms are aerobic as reported by Holt & Bernstein (1992) and Nichols (1996), then oxygen availability will be a limiting factor, and any attempt at field scale bioremediation would have to address this limitation. This could be achieved in one of three ways: direct injection of air or oxygen into the contaminated soil; sparging of the ground water with air or oxygen to saturate it prior to reinjection or; addition of an oxygen-evolving agent such as hydrogen peroxide to the ground water.

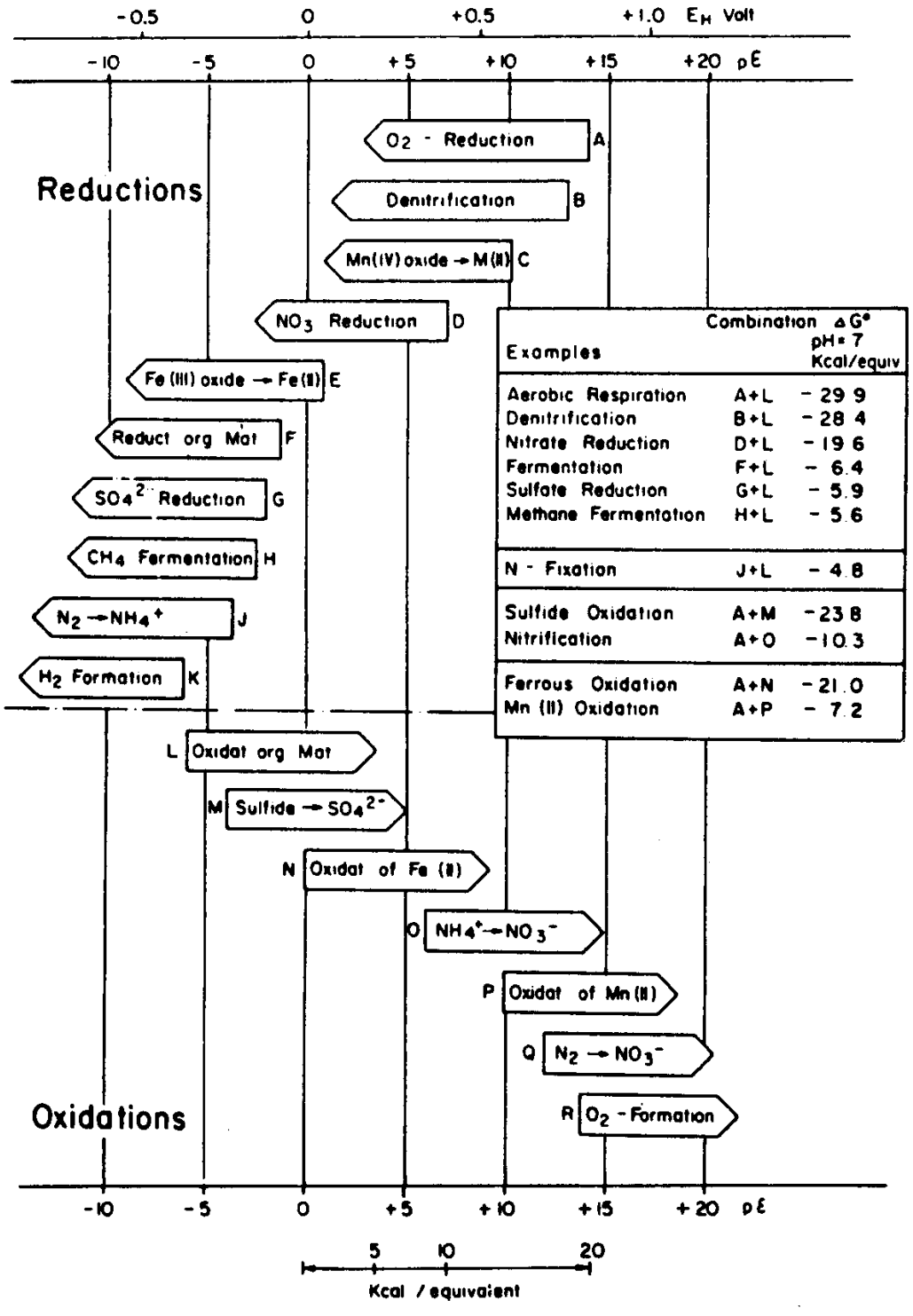

Figure 5.2 Sequence of important redox processes at pH 7 in natural systems (Appelo & Postma, 1993)

5.6 MICROBIOLOGY

Since only two sets of microbiological counts were obtained, it is not possible to comment in detail on the development of the microbiological community. However, it is possible to make some general observations.

5.6.1 Cable oil-degrading microorganisms

Microorganisms capable of using cable oil as a sole carbon source (cable oil-degrading microorganisms, CDMs) were found to grow under aerobic conditions. This supports the findings of Nichols (1996). No data were generated to indicate which isomers were utilised or what breakdown products resulted.

The aerobic 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, an important group of microorganisms that play a major role in the mineralisation of organic materials in soil. The group includes the genus *Nocardia*, which has been demonstrated to degrade LABs (Bhatia & Singh, 1996)

Parallel work by Herbath (2000) demonstrated anaerobic organisms capable of survival on DDB and mineral salts alone, and reported a significant reduction in DDB levels within six months in soil under anaerobic conditions at 8 °C, suggesting that natural attenuation of DDB in anaerobic ground water is a significant factor.

There is evidence that short-chain alkylbenzenes are degraded anaerobically. Toluene (methylbenzene) is degraded under methane–producing conditions by organisms

isolated from landfill sites (Wang & Barlaz, 1998). A review article by Heider *et al.* (1999) describes catabolism of toluene to benzoyl coenzyme A 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. Recent work by Anderson & Lovley (2000) has shown that benzene can be degraded under sulphate-reducing conditions. There have not been any reports of higher LABs being degraded anaerobically prior to Herbath (2000).

Herbath's (2000) findings suggest that in some cases, natural attenuation with monitoring may be a plausible method of dealing with cable oil contamination. This has the great benefit of low cost since it does not involve pumping of ground water, injection of materials etc.

If natural attenuation in anaerobic conditions proves to be too slow, there remains the option of a more proactive approach, manipulating conditions to favour the faster metabolism of aerobic microorganisms.

5.6.2 Distribution of microorganisms

As might be expected, there seems to be enhanced numbers of microorganisms in the capillary zone and below, where there is more available water and an increased carbon source in the form of DDB. It is not possible to fully differentiate the two effects.

The greatly reduced microbial counts found in November compared with August were possibly due to the depressed temperature at this time. However, the numbers found were appreciably lower than those found by Herbath (2000) in the main pit and in aerobic and anaerobic tanks.

The addition of aerobically cultured bacteria from the main pit seemed not to have impacted greatly on the microflora of the mini pit. The numbers were much lower than those found in the main pit and in any case, conditions in the regions of soil where there is a significant concentration of carbon source are anaerobic and so not conducive to the growth of the introduced organisms. If this is the case in soil from the same source, it strongly suggests that bioaugmentation is not likely to be effective in the field. It is not clear whether the low numbers are due to an inability of the culture to thrive in the mini pit, or if the effect is due to the added cells not migrating through the soil block effectively. It is worth noting that a colony-forming unit (CFU) can represent one or more bacterial cells or spores. This is significant since Actinomycete spores may be able to persist in conditions where active cells could not. 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 bioremediation by these microorganisms is taking place.

5.6.3 Effect of nutrient addition

The lack of microbiological data after the addition of nutrients prevents firm conclusions from being drawn. However, the biofouling that occurred in the settlement tank clearly indicates that nutrients were a potential limiting factor prior to their addition.

Although orthophosphate was used in the nutrient mix, the clogging of soil pores by insoluble products described by Aggarwal *et al. (*1991) did not seem to adversely affect the natural rate of flow. The concentrations added were well above the maximum recommended. It is likely that this was not a problem because the high concentration was added only once and will have been diluted fairly quickly in its passage through the soil block. The recirculated water will have had a much lower concentration of orthophosphate. Continuous addition of high concentrations of orthophosphate may result in a reduced hydraulic conductivity and any addition of nutrients in the field should be tailored to the chemical composition of the target soil.

Since the flow rate through the soil did not change, it would also appear that bacterial numbers remained low enough that soil pores were not fouled. This probably reflects limited bacterial growth due to the low oxygen levels in the soil – supporting the theory that degradation of DDB, the major carbon source in the mini pit, is oxygen limited.

5.7 FLUOROMETRY

The fluorometry data, while based on limited sampling, confirms that the DDB is concentrated at or near the water table. This is what would be expected given the physical characteristics of the cable oil (see Table 2.1). This localisation of the oil at the water/air interface is consistent with there being free oil floating on the water surface and smeared/adsorbed onto soil particles, with a small proportion in solution. The fact that this condition persisted five and seven months post-contamination argues against appreciable biodegradation, and supports the case that in the absence of other limiting factors (oxygen in this case), the rate of dissolution of the oil will be limiting.

Chapter 6

Conclusions

6.1 BIOREMEDIATION

The main points that can be drawn from the microbiological, sensor and fluorometry data are:

- Cable oil in soil is found mainly at the top of the saturated zone
- Cable oil-degrading microorganisms (CDMs) occur naturally in soil
- CDMs which were isolated are aerobic, probably Actinomycetes
- Conditions in the saturated zone are largely anaerobic
- Conditions are not optimal for *in situ* bioremediation by the organisms identified in this study
- Bioremediation could be accelerated by biostimulation

Herbath (2000) has suggested that anaerobic biologically mediated degradation of cable oil can take place. If rates were high enough, this would be the preferred method of dealing with cable oil on grounds of cost and ease of application, requiring only that cable oil levels be monitored to demonstrate that they are decreasing.

Organisms able to degrade cable oil aerobically (CDMs) are likely to do so at a faster rate than anaerobic ones. Since conditions in ground water are predominantly anaerobic, the use of such organisms would require a more active approach. It would be necessary to supply oxygen as injected air/oxygen or, e.g. H_2O_2. Aerobic CDMs have been demonstrated in anaerobic regions of the soil, possibly as inactive spores that are able to grow once oxygen is available.

To prevent clogging of soil pore spaces by too vigorous growth in an aerobic bioremediation installation, it would be necessary to tailor the types and amounts of nutrients and terminal electron acceptors to the prevailing conditions. The intrinsic delay in response of such a complex system would require predictive modelling and feed-forward control if rates were to be maximised

To implement this, a greater knowledge of the chemistry and microbiology of cable oil degradation would be required.

6.2 DATA LOGGING AND CONTROL SYSTEM

The Control Cube and associated sensors are of great use for monitoring parameters of interest during bioremediation. The ease of use makes it possible to obtain far more information than would be the case if readings were taken manually. The maximum of eight channels of simultaneous data may be a limitation, although it might be possible to produce software capable of integrating data from two or more Control Cubes simultaneously.

Control Cube is unsuitable in its current form as a basis of a control system for this application because it is not capable of integrating information from sensors in a predictive manner. However, some features of Abington's data logging and control systems would be desirable in any future installation:

- Automatic sensor recognition
- Robust sensors

- Simple calibration

Additional features would have to include the ability to integrate data into a predictive model of biodegradation to allow feed-forward control. A simple feedback mechanism would be unsuitable because the delays inherent in the system would lead to oscillations in nutrient levels, cell numbers etc. This would hamper, rather than encourage optimum rates of degradation and could lead to eutrophication of downstream waters.

A monitoring/datalogging/control system would also have to be capable of operation independently of mains electricity. This might be through the use of battery-powered equipment, an on-site generator or, more elegantly, by inductively coupling the equipment to the cable itself to obtain power.

Chapter 7

Suggestions for further work

7.1 MICROBIOLOGY

- Carry out species level identification of cable oil-degrading microorganisms
- Investigate potential for degradation of cable oil under aerobic and anaerobic conditions utilising a range of different soil types and terminal electron acceptors
- Investigate transport of cable oil degrading bacteria in contaminated soils
- Further study the role of nutrients in microbial degradation
- Investigate role of nutrients on fungal activity (e.g. white rot fungus, *Phanerochaete chrysosporium*) and effects on DDB degradation

7.2 BIOCHEMISTRY

- Use compound-specific isotope analysis (CSIA) to trace the fate and transport of cable oil in the subsurface.
- Investigate the isomeric composition of cable oil before, during and after degradation.
- Investigate toxicity changes during degradation

7.3 MODELLING

- Model fate of cable oil in a variety of soil types
- Development of a feed-forward control algorithm to optimise the use of additives and maximise the rate of *in situ* bioremediation

7.4 CONTROL SYSTEM

Future work might also include the use of the Control Cube or other control equipment to control treatment of recirculated ground water (e.g. air sparging, dosing with fertilisers, pH conditioning) based on a predictive ("feed-forward") mathematical model.

7.5 PLANNED RESEARCH

National Grid Company plc have already committed to funding future research in this field. The author will be registering for a PhD at Edinburgh University in October 2000. It is intended to use this opportunity to address the need for further data on the microbiology and biochemistry of cable oil degradation, and it would be expected to lead to the construction of a predictive model.

References

AELION, C.M. (1996). Impact of aquifer sediment grain size on petroleum hydrocarbon distribution and biodegradation. *Journal of Contaminant Hydrology* **22**, 109-121.

AGGARWAL, P.K., MEANS, J.L. & HINCHEE, R.E. (1991). Formulation of nutrient solutions for *in situ* bioremediation. *In Situ Bioreclamation*, 51-66. Edited by R.H Hinchee & R.F. Olfenbuttel. Butterworth-Heinemann

ANDERSON, R.T. & LOVLEY, D.R. (2000). Anaerobic bioremediation of benzene under sulfate-reducing conditions in a petroleum-contaminated aquifer. *Environmental Science and Technology*. B34B, 2261-2266.

ANDREONI, V. & BAGGI, G. (1996). Bioremediation of contaminated sites: problems and perspectives. *Annali di microbiologia ed enzilogia* **46**, 125-136.

ANDREWS, R.P. (1999). *Study Number SSHQ/117/99: Chemical and physical analysis of two soil samples*. Soil Survey and Land Research Centre, Cranfield University. Report prepared for Yolande Herbath.

APPELO, C. A. J. & POSTMA, D. (1993). Redox processes. In: *Geochemistry, Groundwater and Pollution*. A. A. Balkema, Rotterdam. 239-294.

ATLAS, R.M. (1993). *Handbook of Microbiological Media*. CRC Press, Boca Raton.

BAKER, K.H. & HERSON, D.S. (1994). *Bioremediation*. McGraw-Hill.

BALBA, M.T., AL-AWADHI, N. & AL-DAHER, R. (1998). Bioremediation of oil-contaminated soil: microbiological methods for feasibility assessment and field evaluation. *Journal of Microbiological Methods* **32**, 155-164.

BAYONA, J.M., ALBAIGES, J., SOLANAS, A.M. & GRIFOLL, M. (1986). Selective aerobic degradation of linear alkylbenzenes by pure microbial cultures. *Chemosphere* **15**, 595-598.

BHATIA, M. & SINGH, H.D. (1996). Biodegradation of commercial linear alkyl benzenes by *Nocardia amarae*. *Journal of Biosciences* **21**, 487-496.

BREGNARD, T.P.-A., HOHENER, P., HANER, A. & ZEYER, J. (1996). Degradation of weathered diesel fuel by microorganisms from a contaminated aquifer in aerobic and anaerobic microcosms. *Environmental Toxicology and Chemistry* **15**, 299-307.

CHAINEAU, C.-H., MOREL, J.-L. & OUDOT, J. (1995). Microbial degradation in soil microcosms of fuel oil hydrocarbons from drilling cuttings. *Environmental Science & Technology* **29**, 1615-1621.

CHAPELLE, F.H., BRADLEY, P.M., LOVLEY, D.R. & VROBLESKY, D.A. (1996). Measuring rates of biodegradation in a contaminated aquifer using field and laboratory methods. *Ground Water* **34**, 691-698.

CHAWLA, S. & LENHART, S.M. (2000). Application of optimal control theory to bioremediation. *Journal of Computational and Applied Mathematics.* **114**, 81-102.

CHESTON, A.E. (1997). Bioremediation of joint bays. *M.Sc. Thesis.* Cranfield Institute of BioScience and Technology.

CRELLIN, J. (1999). *Personal Communication*, 19 August 1999. Abington Partners, Bath.

DAS, K. & KEENER, H.M. (1997). Moisture effect on compaction and permeability in composts. *Journal of Environmental Engineering* **123**, 275-281.

DAVE, H., RAMAKRISHNA, C., BHATT, B.D. & DESAI, J.D. (1994). Biodegradation of slop oil from a petrochemical industry and bioreclamation of slop oil contaminated soil. *World Journal of Microbiology & Biotechnology* **10**, 653-656.

DIBBLE, J.T. & BARTHA, R. (1979). Effect of environmental parameters on biodegradation of oil sludge. *Applied Environmental Microbiology* **37**, 729-739

DUTCH INTERVENTION VALUES. (1994). *Circular on intervention values for soil remediation: DBO/07494013*, Ministry of Housing, Spatial Planning and Environment Directorate-General For Environmental Protection, Department of Soil Protection (IPC 625), PO Box 30945, 2500 GX The Hague. 9 May 1994.

EGANHOUSE, R.P. (1986). Long-chain alkylbenzenes: their analytical chemistry, environmental occurrence and fate. *International Journal of Environmental Analytical Chemistry* **26**, 241-263.

CROWCROFT, P. (2000). Policy Number EA S/2703/1/1/Final 1 3 May 2000. In *Part IIA EPA (1990) (England) Process Documentation*. Environment Agency: Bristol.

GLEDHILL, W.E., SAEGER, V.W. & TREHY, M.L. (1991). An aquatic environmental safety assessment of linear alkylbenzene. *Environmental Toxicology and Chemistry* **10**, 169-178.

HAIGH, S.D. (1995). Fate and effects of synthetic lubricants in soil: biodegradation and effects on crops in field studies. *The Science of the Total Environment* **168**, 71-83.

HARRISON, A.B. & BETTS, W.B. (1996). Assay of bacterial and fungal activity in diesel contaminated soil using a 14C-glucose utilization method. *Letters in Applied Microbiology* **23**, 43-46.

HAYWORTH, J.S. & BURRIS, D.R. (1997). Nonionic surfactant-enhanced solubilization and recovery of organic contaminants from within cationic surfactant-enhanced sorbent zones. 1. Experiments. *Environmental Science & Technology* **31**, 1277-1283.

HEIDER, J., SPORMANN, A.M., BELLER, H.R. & WIDDEL, F. (1999). Anaerobic bacterial metabolism of hydrocarbons. *Federation of European Microbiological Societies Microbiology Reviews.* **22**. 459-473

HERBATH, Y. (2000). The application of *in-situ* techniques for the bioremediation of soil and groundwater contaminated with cable oil. *Ph.D. thesis (unpublished – expected date of submission 30 September 2000)*. Cranfield Institute of BioScience and Technology.

HMSO. (1990). *Environment Protection Act.* HMSO: London.

HMSO. (1995). *Environment Act.* HMSO: London.

HMSO. (2000). *Contaminated Land (England) Regulations.* HMSO: London.

HOLT, M.S. & BERNSTEIN, S.L. (1992). Linear alkylbenzenes in sewage sludges and sludge amended soils. *Water Research,* **26**, 613-624.

HUESMANN, M.H. (1995). Predictive model for estimating the extent of petroleum hydrocarbon biodegradation in contaminated soils. *Environmental Science & Technology* **29**, 7-18.

JOHNSTON, J.J., BORDEN, R.C. & BARLAZ, M.A. (1996). Anaerobic biodegradation of alkylbenzenes and trichloroethylene in aquifer sediment down gradient of a sanitary landfill. *Journal of Contaminant Hydrology* **23**, 263-283

KISER, K. (1995). Fighting contamination with bioremediation. *Scrap processing and recycling* **52**, 44-46, 48,50,52.

KOUSSIA, E. (1999). *In vitro* study of biodegradation of cable oil and the effect of nutrient supplement on biodegradation rate. *M.Sc. Thesis.* Cranfield Institute of Bioscience and Technology.

LACKEY. L.W., PHELPS. T.J., KORDE, V., NOLD, S., RINGELBERG, D., BIENKOWSKI, P.R. & WHITE, D.C. (1994). Feasibility testing for on-site bioremediation of organic wastes by native microbial consortia. *International Biodeterioration & Biodegradation* **33**, 41-59.

LEAHY, J.G. & COLWELL, R.R. (1990). Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews* **54**, 305-315.

LI, K.Y., ANNAMALAI, S.N. & HOPPER, J.R. (1993). Rate controlling model for bioremediation of oil contaminated soil. *Environmental Progress* **12**, 257-261.

LI, K.Y., ZHANG, Y. & XU, T. (1995). Bioremediation of oil-contaminated soil – a rate model. *Waste Management* **15**, 335-338

LOVELACE, G. (1999). *Personal Communication*, 27 September 1999. Soil Survey and Land Research Centre, Cranfield University.

MANGAS, E, VAQUERO, M.T., COMELLAS, L. & BROTO-PUIG, F. (1998). Analysis and fate of aliphatic hydrocarbons, linear alkylbenzenes, polychlorinated biphenyls and polycyclic aromatic hydrocarbons in sewage sludge-amended soils. *Chemosphere* **36**, 61-72.

MARGESIN, R. & SCHINNER, F. (1999). Biological decontamination of oil spills in cold environments. *Journal of Chemical Technology and Biotechnology* **74**, 381-289.

MAYR, C., WINDING, A. & HENDRIKSEN, N.B. (1999). Community level physiological profile of soil bacteria unaffected by extraction method. *Journal of Microbiological Methods.* **36**, 29-33.

McCARTY, P.L. & SEMPRINI, L. (1993). Engineering and hydrogeological problems associated with *in situ* treatment. *Hydrological Sciences* **38**, 261-272.

MINSKER, B.S. & SHOEMAKER, C.A. (1998). Dynamic optimal control of in-situ bioremediation of ground water. *Journal of water resources planning and management* **124**, 149-161.

MOUNTAIN, S., HULLMAN, A. & ANDREOTTI, G. (1998). Oil in the soil. *Civil Engineering* **68**, 52-55.

NICHOLS, J.A. (1996). The environmental impact of cable oils. *M.Sc. Thesis*. Cranfield Institute of BioScience and Technology.

O'LEARY, K.E., BARKER, J.F. & GILLHAM, R.W. (1995). Remediation of dissolved BTEX through surface application: a prototype field investigation. *Groundwater Monitoring and Remediation* **Fall 1995**. 99-109.

OUYANG, Y., MANSELL, R.S. & RHUE, R.D. (1995). Emulsion-mediated transport of nonaqueous-phase liquid in porous media: a review. *Critical Reviews in Environmental Science and Technology* **25**, 260-290.

PATEL, D. (2000). Personal communication, National Grid Company plc, Leatherhead. 24 May 2000.

PHELPS, T.J., SIEGRIST, R.L., KORTE, N.E., PICKERING, D.A., STRONG-GUNDERSON, J.M., PALUMBO, A.V., WALKER, J.F., MORRISSEY, C.M. & MACKOWSKI, R. (1994). Bioremediation of petroleum hydrocarbons in soil column lysimeters from Kwajalein Island. *Applied Biochemistry and Biotechnology* **45/46**, 835-845.

PRINCE, R.C. (1991). Bioremediation of oil spills, with particular reference to the spill from the Exxon Valdez. In: *Microbial Control of Pollution*. Fry, J.C., Gadd, G..M., Herbert, R.A., Jones, C.W. & Watson-Craik, I.A. (eds). Cambridge University Press, Cambridge. 19-34.

RAYMOND, R.L. (1974). Reclamation of hydrocarbon contaminated groundwaters. *US Patent #3,846,290*. 5th November 1974.

RITTER, W.F. & SCARBOROUGH, R.W. (1995). A review of bioremediation of contaminated soils and groundwater. *Journal of Environmental Science & Health* **A30(2)**. 333-357.

ROUSE, J.D., SABATINI, D.A., SUFLITA, J.M & HARWELL, J.H. (1994). Influence of surfactants on microbial degradation of organic compounds. *Critical Reviews in Environmental Science and Technology* **24**, 325-370.

ROWLAND, S.J., (1996). Chemical composition of cable oil. *PEP Research and Consultancy Limited.* Report prepared for the National Grid Company plc.

SAWYER, D.T., SOBKOWIAK, A. & ROBERTS JR, J.L. (1995). *Electrochemistry for Chemists, 2nd Ed*. John Wiley & Sons, Inc, New York

SCHWAB, A.P., SU, J., WETZEL, S., PEKAREK, S. & BANKS, M.K. (1999). Extraction of petroleum hydrocarbons from soil by shaking. *Environmental Science and Technology*. **33**, 1940-1945.

SHERBLOM, P.M., GSCHWEND, P.M. & EGANHOUSE, R.P. (1992). Aqueous solubilities, vapor pressures and 1-octanol-water partition coefficients for C_9-C_{14} linear alkylbenzenes. *Journal of Chemical Engineering Data* **37** 394-399.

SHEVAH, Y. & WALDMAN, M. (1995). In-situ and on-site treatment of groundwater. *Pure & Applied Chemistry* **67**, 1549-1561.

SHOUCHE, M.S., PETERSEN, J.N. & SKEEN, R.S. (1993). Use of a mathematical model for the prediction of optimum feeding strategies for *in situ* bioremediation. *Applied Biochemistry and Biotechnology*. **39/40**, 763-779.

SINGLETON, I. (1994). Microbial metabolism of xenobiotics: fundamental and applied research. *Journal of Chemical Technology and Biotechnology* **59**, 9-23.

SMITH, M.R. & RATLEDGE, C. (1989). Catabolism of alkylbenzenes by *Pseudomonas* sp. NCIB 10643. *Applied Microbiology & Biotechnology* **32**, 68-75.

SMITH, M.R. (1990). The biodegradation of aromatic hydrocarbons by bacteria. *Biodegradation* **1**, 191-206.

SWEED, H.G., BEDIENT, P.B. & HUTCHINS, S.R. (1996). Surface application system for in situ ground-water bioremediation: site characterization and modeling. *Ground Water* **34**, 211-222.

TAKADA, H. & ISHIWATARI, R. (1990). Biodegradation experiments of linear alkylbenzenes (LABs): isomeric composition of C_{12} LABs as an indicator of the degree of LAB degradation in the aquatic environment. *Environmental Science and Technology* **24**, 86-91

TAKADA, H. & ISHIWATARI, R. (1991). Linear Alkylbenzenes (LABs) in urban riverine and coastal sediments and their usefulness as a molecular indicator of domestic wastes. *Water Science & Technology* **23**, 437-446.

TEBBUTT, R. (1998). Natural attenuation of cable oils. *M.Sc. Thesis.* Cranfield Institute of BioScience and Technology.

TURSMAN, J.F. & CORK, D.J. (1992). Subsurface contaminant bioremediation engineering. *Critical Reviews in Environmental Control* **22**, 1-26

VESPER, S.J., MURDOCH, L.C. HAYES, S. & DAVIS-HOOVER, W.J. (1994). Solid oxygen source for bioremediation in subsurface soils. *Journal of Hazardous Materials* **36**, 265-274.

WANG, Y.-S. & BARLAZ, M.A. (1998). Anaerobic biodegradability of alkylbenzenes and phenol by landfill derived microorganisms. *Federation Of European Microbiological Societies Microbiology Ecology* **25**, 405-418.

WEBER JR, W.J. & CORSEUIL, H.X. (1994). Inoculation of contaminated subsurface soils with enriched indigenous microbes to enhance bioremediation rates. *Water Resources* **28**, 1407-1414.

WEIDEMEIER, T.H, SWANSON, M.A., WILSON, J.T., KAMPBELL, D.H., MILLER, R.N. & HANSEN, J.E. (1996). Approximation of biodegradation rate constants for monoaromatic hydrocarbons (BTEX) in ground water. *Ground Water Monitoring and Remediation* **Summer 1996**, 186-194.

WEISSEMANN, S. & KUNZE, C. (1994). Microbial activity in heating oil contaminated soil under field and controlled conditions. *Angew. Bot.* **68**, 137-142.

WEISSENFELS, W. D., KLEWER, H.-J. & LANGHOFF, J. (1992). Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity. *Applied Microbiology & Biotechnology* **36**, 689-696.

YANG, X., ERICKSON, L.E. & FAN, L.T. (1995). A study of the dissolution rate-limited bioremediation of soils contaminated by residual hydrocarbons. *Journal of Hazardous Materials* **41**, 299-313.

Appendices

Appendix 1
Control Disc routine for mini pit pump controller

```
Start PUMP
     Action "Counter 1" = 0
     Repeat Forever
          If {"Connect-your-own digital 1" is Equal to OFF}
And {"Connect-your-own digital 2" is Equal to OFF} Then
Action Increase "Counter 1" End If
          If {"Connect-your-own digital 1" is Equal to OFF}
And {"Connect-your-own digital 2" is Equal to OFF} Then
Action Set "Mains switch A" = ON End If
          If {"Connect-your-own digital 1" is Equal to ON}
And {"Connect-your-own digital 2" is Equal to ON} Then
Action Set "Mains switch A" = OFF End If
     Again
End
```

Where:

“Connect-your-own digital input 1” = Upper float switch

“Connect-your-own digital input 2” = Lower float switch

“Mains switch A” = Mains electricity supply to peristaltic pump

Appendix 2
Control Disc routine for mini pit monitoring

```
Start MINIPIT LOGGER

     Repeat Forever

          Action Log Data

          Action Wait 3600

     Again

End
```