# Sustainable treatment of hydrocarbon-contaminated industrial land

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## Abstract

Land contamination by petroleum hydrocarbons is a widespread and global environmental pollution issue from recovery and refining of crude oil and the ubiquitous use of hydrocarbons in industrial processes and applications. Sustainable treatment of hydrocarbon-contaminated industrial land was considered with reference to seven published works on contaminated railway land including the track ballast, crude oil wastes and contaminated refinery soils. A methodology was developed to assess the level hydrocarbon contamination of track ballast (Anderson *et al*., 2000) and in Anderson *et al*. (2002, 2003) solvent and surfactant cleaning of ballast was investigated and potential environmental impacts of the processes examined. Optimisation of *ex situ* bioremediation of diesel-contaminated soil (Cunningham & Philp, 2000) demonstrated the efficacy of the addition of microorganisms (bioaugmentation) to enhance diesel biodegradation rates at field pilot scale. This work motivated a further study that examined a novel aeration approach incorporating ventilator turbines (cowls) for soil biopiles (Li *et al*., 2004). An optimised *ex situ* bioremediation for crude oil wastes was developed in Kuyukina *et al*. (2003) which demonstrated the efficacy of bioaugmentation and the application of biosurfactants. The final study investigated the potential application of biosurfactants to *in situ* remediation (Kuyukina *et al*., 2005) in laboratory soil columns contaminated with crude oil. The collected works are informative to those seeking to remediate hydrocarbon-contaminated industrial land and the sustainability of the approaches was considered.

## Declaration

I hereby declare that I am the sole author of this critical review and demonstrate where I have made a substantial contribution to the portfolio of published work by more than one author. I further declare that the work presented has not been submitted in full or in part for the award of another degree or professional qualification. A 'Declaration of Contribution' is included alongside each published paper submitted as part of this critical review along with a summary that estimates the percentage contribution of the author. These may be found in the Appendices A to H.

_____ Date _____

## Acknowledgements

The works presented in this thesis took place over many years and would not have been possible without the support of many people. I would like to thank just a few of those here. Where appropriate, sponsors have been acknowledged in the text of the papers.

I would first like to thank Dr Jim Philp who was my teacher and mentor for many years. He sparked my interest in remediation and remains a great source of inspiration to this day. I would also like to thank Professor D. Andrew Barry who has been a constant source of knowledge and wisdom and was kind enough to act as supervisor for this thesis. Were it not for his support, this manuscript would never have been written. I am also grateful to Dr Blanca Antizar-Ladislao for acting as my internal examiner.

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**Abbreviations and Acronyms**

| | |
|---|---|
| BOD | Biochemical Oxygen Demand |
| GC | Gas Chromatography |
| GC-FID | Gas Chromatography with Flame Ionisation Detection |
| SEM | Solvent Extractable Material |
| PAH | Polycyclic aromatic hydrocarbon |
| TPH | Total Petroleum Hydrocarbons |
| UCM | Unresolved Complex Mixture |

# Part 1: Critical Review

# 1. Introduction

The seven publications forming this submission and the accompanying critique are all related to the theme of sustainable treatment of hydrocarbon-contaminated industrial land. This encompasses contaminated railway land including the track ballast, crude oil wastes and crude oil contaminated refinery soils. The author was employed as the research co-ordinator of the Contaminated Land Assessment & Remediation Research Centre (CLARRC) at The University of Edinburgh at the time the first papers discussed was published. The mission of the Scottish Funding Council scheme that founded CLARRC was to "improve the fit between higher education institutions and industry". For this reason, many of the publications that constitute this thesis were deliberately not targeted towards high impact factor academic journals but were aimed towards a wider audience an in particular practitioners from industry.

## 1.1 Background and overall context

Land contamination by petroleum hydrocarbons is a widespread and global environmental pollution issue from recovery and refining of crude oil and the ubiquitous use of hydrocarbons in industrial processes and applications. Environmental legislation and redevelopment of brownfield land are key drivers for environmental cleanup (Hartman *et al*., 2005). This is due to the potential negative impacts of petroleum hydrocarbons on human health and the environment (Heath *et al*., 1993) and a preference to reuse former industrial land over developing green field sites (Russell *et al*., 2008). The sustainability of remedial approaches has

attracted much greater attention in recent years (e.g. Baker *et al*., 2009; Sustainable Remediation Forum, 2009; Watts *et al*., 2009).

The assessment and treatment of contaminated rail ballast arising from leaks associated with diesel engines will be first considered. Disposal of contaminated ballast to landfill is costly and does not represent the most sustainable outcome as the ballast may be recovered as a recycled aggregate (CIRIA, 1999).

Significant environmental and economic benefits may be realised if a sustainable alternative to landfilling of contaminated ballast was employed. In Anderson *et al*. (2002, 2003) solvent and surfactant cleaning of ballast was investigated and potential environmental impacts of the processes examined. In Anderson *et al*. (2000), a means of quantifying organic contamination on ballast was developed as traditional laboratory extraction methods applied to soils were not readily applicable.

Migration of diesel contamination from the track leads to contamination of the adjacent ground, which may present a risk to human health and the environment. Bioremediation is potentially one of the sustainable and cost-effective treatments for hydrocarbon-contaminated land (Philp *et al*., 2009) and groundwater (Philp *et al*., 2005). An initial study on the optimisation of *ex situ* bioremediation of diesel-contaminated soil (Cunningham & Philp, 2000) demonstrated the efficacy of the addition of microorganisms (bioaugmentation) to enhance diesel biodegradation rates at field pilot scale. This work motivated further studies examining a novel aeration approach (Li *et al*., 2004) discussed in this thesis as well as other work on

bioaugmentation using immobilised microorganisms (Cunningham *et al*., 2004; Podorozhko *et al*., 2008).

Crude oils and refinery wastes are more challenging contaminants for bioremediation than refined fuels such as diesel due to the wider range of hydrocarbons including less bioavailable fractions such as high molecular weight aliphatics and asphaltenes. Further *ex situ* bioremediation research was conducted in collaboration with the Institute of Ecology and Genetics of Microorganisms (IEGM) in Perm, Russia where this topic is particularly significant as extraction and processing of crude oil has resulted in widespread contamination of soil and water.

In Kuyukina *et al*. (2003), the aim was to develop an optimised *ex situ* bioremediation approach for crude oil wastes from refinery storage pits in the Perm region. A key-limiting factor in biodegradation of such recalcitrant contaminants is the bioavailability of many of the oil fractions, and so biosurfactants were utilised to optimise the biodegradation process. These glycolipid biosurfactants were produced by the IEGM laboratory from *Rhodococcus* species (Philp *et al*., 2002) in contrast to the synthetic surfactants used previously on contaminated rail ballast. Further laboratory studies then investigated the potential application of biosurfactants for *in situ* remediation (Kuyukina *et al*., 2005) in laboratory soil columns contaminated with crude oil.

## 1.2 Structure of critical review

This critical review is divided into three sections. Chapter 1 introduces key issues surrounding the assessment and contaminated railway ballast as a source of contamination and examines means of assessing and treating ballast to enable this valuable resource to be recovered as a secondary aggregate. Attempts to optimise the speed and completeness of bioremediation of land contaminated by diesel migrating from the railway track are described in Chapter 2. In Chapter 3, the optimisation of bioremediation is examined with respect to crude oil and crude oil wastes drawing on the lessons learned from work on railway land to treat this more challenging group of contaminants.

Chapters 2 and 3 begin with a summary of the context, aims, methodologies and conclusions of the papers presented. A critique is then presented including comment on the contribution to knowledge in the field. Although, there is a natural coherence to the work presented, this is highlighted throughout the text where appropriate and forms part of the discussion in Chapter 4. The papers discussed are presented in Appendices B to H and each paper is prefaced with a declaration of the contribution made by the author.

# 2. Treatment of hydrocarbon-contaminated railway land

## 2.1 Contaminated rail ballast

Rail ballast is the aggregate that serves as the bed for rail tracks, providing stability and drainage to the track and takes its name from the historical association with shipping of aggregate as ships ballast (Claisse & Calla, 2006). Traffic loading and weathering degrades the ballast, which reduces the effectiveness and results in the accumulation of fines in the top layer (Selig and Waters 1994). In geotechnical terms the ballast is 'contaminated' by these fines and may be described as 'spent'. Aside from visually inspecting the track or drilling ballast samples in the field, ground-penetrating radar (GPR) may be employed to identify anomalies in the track bed and determine the degree of track bed deterioration (Gallagher *et al*., 1999).

Track maintenance can include removal and replacement of 'spent' ballast which may or may not be contaminated in the environmental sense largely dependent on the location of the track being maintained (Osborne & Montague, 2005). Alternatively, the fines may be washed out and the ballast 'topped up' by track mounted ballast-cleaning systems such as the £42m vehicle bought by Network Rail in 2008, one of only three such systems operating in the UK (Anonymous, 2008).

**Figure 1: Ballast contamination clearly visible adjacent to contaminated land**

The investigations considered in this section focussed on the assessment and treatment of rail ballast subject to chemical contamination and the term contaminated is used hereafter in this sense. A key motivation for the work was that the author had been undertaking bioremediation field trials on diesel contaminated land (Cunningham and Philp, 2000) adjacent to railway tracks where diesel motor units were kept overnight or for shorter periods during the day when not in service at off peak times (Figure 1). It was clear that the stationary diesel motor units acted as a substantial source of fuel and oils contaminating the track and surrounding area.

Aside from the aesthetic issue and odour produced by the hydrocarbon contamination, migration to surface or ground waters could produce a negative environmental impact (Wan, 1991). Secondly, at a different site in the UK, the author had seen a demonstration of a track-mounted rail ballast-cleaning machine (Figure 2).

**Figure 2: Track mounted ballast cleaning machine employing solvent washing**

Ballast was lifted by vacuum from the track into a receiving chamber. It was then washed using Pronatur, a proprietary blend of degreasing solvents and citrus based cleaning agents, rinsed with water and the treated material then deposited onto the track at the rear of the machine as shown in Figure 3 below.

**Figure 3: Cleaned ballast returned to the track**

Operators relied on visual estimation of the cleaning efficiency of their system. The author observed residual contamination was clearly visible on the processed ballast

and noted the presence of residual solvent from the cleaning process as is evident from a close up image (Figure 4) taken at the time.

**Figure 4: Detail of cleaned and contaminated rail ballast showing residual contamination**

This type of ballast cleaning achieved a cosmetic and environmental improvement. Although fines were also removed and 'clean' ballast returned to the track, this was a largely superficial cleaning (Figure 5) as the track mounted cleaning systems operated by Network Rail support the rails and also excavate the ballast underneath before returning a mixture of cleaned and fresh ballast to the track (Anonymous, 2008).

**Figure 5: Extent of ballast removed by vacuum lifting**

### 2.1.1 Papers presented

Anderson, P., **Cunningham, C.J.,** Barry, D.A. (2000). Gravimetric Analysis of Organic Contamination in Railway Ballast. *Land Contamination & Reclamation.* 8 (2), 71-74.

Anderson, P., **Cunningham, C.J.**, Barry, D.A. (2002). Efficiency and Potential Environmental Impacts of Different Cleaning Agents used on Contaminated Railway Ballast. *Land Contamination & Reclamation.* 10 (2), 71-77.

Anderson, P., **Cunningham, C.J.**, Hearnden, R., Barry, D.A., Philp, J.C. (2003). Optimisation and Assessment of Different Railway Ballast Cleaning Systems. *Land Contamination & Reclamation.* 11 (4), 1-7.

### 2.1.2 Aims, methodology, results and conclusions

No previous studies had compared laboratory analytical approaches to assess the extent of contamination on rail ballast. The aim of the study reported in Anderson *et al*. (2000) was to develop a simple and rapid analytical procedure that could be conducted using the minimum amount of solvent. Replicated samples of homogenised ballast were extracted in dichloromethane, hexane, methanol or ethyl acetate using a wrist action shaker.

Gravimetric determination of solvent extractable material (SEM) after centrifugation and filtration of the extracts found that ethyl acetate gave the greatest recovery. Further investigations compared wrist action shaking and ultrasonic extraction with the more laborious but exhaustive soxhlet extraction and examined the effect of sample size to solvent ratio. These determined that extraction with a wrist action shaker or ultrasonic bath with a ratio of least 100 ml of ethyl acetate to 120 g of ballast gave good accuracy and precision and could be used to determine gravimetric SEM contamination on rail ballast. As was evident from Figure 1 in the paper, the ballast was heavily contaminated with approximately 25,000 mg $kg^{-1}$ SEM.

In Anderson *et al*. (2002) the methodology developed by Anderson *et al*. (2000) was used in a study which aimed to evaluate the efficiency of solvent or surfactant cleaning approaches applied to heavily contaminated ballast. A semi-pilot scale apparatus was devised to simulate the cleaning action likely to be employed at full scale. Pronatur (Orapi Ltd, Bradford), a blend of degreasing solvents and citrus

based cleaning agents was used as this had been witnessed by the author being used in the track mounted ballast cleaning demonstration previously referred to and shown in Figure 2 above.

A base solvent terpene blend (hereafter referred to as terpene) was also used, as this was significantly more cost effective at 10% of the price of the proprietary Pronatur. At the time, the author had been introduced to BioSolve®, a proprietary blend of water-based, biodegradable surfactants promoted for use in hydrocarbon cleaning and remediation applications. This was therefore used as a contrasting approach to the solvent based cleaners and water used as a control.

The gravimetric determinations of SEM showed the ballast used was less contaminated than the previous study at approximately 11,500 mg $kg^{-1}$. Treatment using only water was able to remove 62% of the contamination to 4,360 ± 190 mg kg-1 mainly due to attrition scrubbing of the ballast. Solvents produced the highest mean reductions at 98% and 96% reducing the SEM to 250 ± 5 mg $kg^{-1}$ and 480 ± 10 mg $kg^{-1}$ for terpene and Pronatur respectively. BioSolve® also successfully treated the ballast, reducing the SEM by 91% to 990 ±110 mg $kg^{-1}$ using a 6% (v/v) solution and by 93% to 790 ± 40 mg $kg^{-1}$ using a 10% (v/v) solution. Overall there was no significant difference in the treatment efficiency of the active treatments used.

Qualitative gas chromatography (GC) using a flame ionisation detection (GC-FID) of the recovered extracts taken up in dichloromethane showed the contamination to be comprised largely of an unresolved complex mixture (UCM) as shown in Figure 2a

in the paper. This ‘hump’ is commonly encountered in simple gas chromatographic analyses of hydrocarbon mixtures due to the complexity of the sample. Advanced methodologies such as high-performance liquid chromatography (HPLC) followed by two-dimensional gas chromatography (GCxGC) with FID allow the UCM be fully resolved and individual components quantified (Mao *et al*., 2009). Interestingly, the only treatment resulting in a significant signature on the chromatogram was Pronatur and it was hypothesised that this was a result of residual solvent observed in these experiments and earlier in the field as shown in Figure 4 above.

Given the approximately equally success of the treatment approaches, the sustainability of the treatments were briefly considered. BioSolve® was only 5% of the cost of the Pronatur and 50% of the cost of the terpene. Recycling or disposal of used solvents was considered to be a less favourable environmental option than using a biodegradable surfactant and it was concluded that BioSolve® was the favoured approach.

Consideration was briefly given to the issue of small amounts of contamination and residual cleaning agents on treated ballast returned to the track (as was seen earlier in Figure 2 above). The possibility of adding microorganisms (bioaugmentation) to enhance biodegradation of residues was considered. Bioaugmentation is a key theme in later work presented, e.g. in Cunningham & Philp (2000) and Kuyukina *et al* (2003) as applied to enhancing biodegradation of hydrocarbon contaminants in soils. The issue of residual contamination on treated ballast was revisited in the next paper in the series where an assessment of potential leaching was examined.

In Anderson *et al*. (2003) the aim was to optimise the BioSolve® based treatment process and give further consideration to the sustainability of the process in terms of reusing cleaning solutions and the final disposal of effluent. The starting concentration of ballast contamination was 17,510 ± 445 mg $kg^{-1}$ SEM. It was determined that the pilot scale systems may not have been sufficiently representative of the levels of attrition likely to be encountered in a field scale treatment plant.

Astroturf®, an artificial turf made from synthetic materials, was therefore added to the experimental system to provide a greater scrubbing effect and was found to give an 8% increase in cleaning efficiency, reducing contamination by 93% to 1,245 ± 134 mg $kg^{-1}$ SEM. Relatively high standard errors on the measurements of residual ballast contamination made the influence of different treatment times and BioSolve® concentrations difficult to interpret.

However, wash times of 10-15 minutes using 1% and 3% (v/v) solutions of BioSolve® were effective in reducing contamination by around 80-90%. This was much lower than the 6% solutions used by Anderson *et al*. (2002) with significant reduction in the cost of ballast treatment and in the scale or intensity of effluent treatment required. A further reduction was achieved by recycling of cleaning solution with no loss of efficiency when treating a fresh batch of contaminated ballast.

Biochemical oxygen demand (BOD) was used as the key indicator of likely effluent strength as this would require some form of treatment prior to reuse, disposal to

sewer or to a watercourse. This was determined as being 4,890 ± 157 mg $l^{-1}$ in the worst-case-scenario of a higher 6% BioSolve® solution. As rinsing of the ballast with water after cleaning formed part of the overall treatment, BOD of the wash water was determined to be 50 ± 1 mg $l^{-1}$.

As previously considered in Anderson *et al*. (2002), the treated ballast containing residual contamination and surfactant may impact negatively on surface water through runoff or groundwater through infiltration. Stockpiled ballast may also be prone to leaching during periods of rain and leachate could find its way into surface water drainage systems. Potential leaching was assessed by taking 1 kg of rinsed, treated ballast (6% v/v BioSolve® solution) and mixing with 1 litre of tap water that yielded a very low BOD of 3.0 ± 0.8 mg $l^{-1}$. A scan of metals in the leachate using inductively coupled plasma atomic emission spectroscopy (ICP-AES) showed relatively low levels for a suite of Al, Ba, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Ni, Se, Sn and Zn.

### 2.1.3 Critique and contribution

Anderson *et al*. (2000) reported for the first time on an assessment of different extraction methodologies for the determination of organic contamination on rail ballast. The simplified methodology proposed offered considerable time savings compared with soxhlet extraction and importantly used only 25% of the solvent volume and employed a less hazardous non-chlorinated solvent.

It could be argued that the gravimetric method of determination was unsophisticated and gave minimal information of the nature of the contamination. Similarly, in Anderson *et al*. (2002), the extracts for qualitative analysis using GC-FID were not subjected to any form of clean up to remove co-extracted natural organic matter. This may again have been overly simplistic.

However, Villalobos *et al*. (2008) in a recent study on gravimetric methods for TPH determination in contaminated soils noted that interference from co-extracted substances was more likely where there was a high background natural organic matter (NOM). They cited Weisman (1998) who gave the approximate detection limit for TPH in soils as 50 mg $kg^{-1}$ and proposed that a soil with 40% organic carbon would have a detection limit one order of magnitude higher than a soil with <5% organic carbon.

Track ballast has no natural organic carbon content but hydrocarbon contamination would encourage accumulation of organic particulates such as decaying track vegetation and wind-blown soil fragments for example. These are unlikely to be >5% but it should be noted that this is a w/w measure and the bulk density of soils are much lower than aggregate used for track ballast. Residual terpene solvent was the most prominent interference as is evident on the chromatogram in Anderson *et al*. (2002), Figure 2(d) and would have contributed to the SEM levels determined leading to an underestimate of cleaning efficiency.

In August 2003, a standard procedure was introduced in the UK by Network Rail for the handling of used ballast where a checklist (Table 1) was used to determine if ballast was suitable for reuse as a recycled aggregate or for uncontained storage at local distribution centres handling ballast (Network Rail, 2003).

**Table 1: Network Rail 'Checklist C', maximum concentrations of ballast contaminants**

| Parameter | Maximum concentration (mg $kg^{-1}$ air-dried sample) |
|---|---|
| pH | 5-10.5 |
| TPH | 1,000 |
| Arsenic | 40 |
| Cadmium | 15 |
| Chromium (total) | 1,000 |
| Lead (total) | 2,000 |
| Mercury | 20 |
| Selenium | 6 |
| Cyanide (complex) | 250 |
| Cyanide (free) | 25 |
| Sulphate | 2,000 |
| Sulphide | 250 |
| Sulphur (free) | 5,000 |
| Phenol | 5 |
| Polyaromatic Hydrocarbons (PAHs) | 1,000 |

The TPH in the table represents total petroleum hydrocarbons and had a threshold of 1,000 mg $kg^{-1}$. Many of the laboratory and pilot scale treatments from our experiments were below the 1,000 mg $kg^{-1}$ for total SEM and those marginally above were likely to have passed if further clean up of the extracts were applied before analysis.

Our examination of the ballast was targeted at gross organic contamination as assessed by determination of SEM. However, with the introduction of a clean up stage prior to analysis by GC-FID and use of appropriate standards then TPH, PAHs and phenols could have been determined.

Another contaminant class of interest would be herbicides as these are commonly applied to the track to kill weeds. However, it is perhaps unlikely they would need to be applied to heavily contaminated areas as few plants are likely to be encountered at such locations (see Figure 1 in section 2.1). Interestingly, from a study that examined the degradation kinetics of the herbicides diuron and MCPA (2-methyl-4-chlorophenoxyacetic acid) on Swedish railways, Cederlund *et al*. (2007) found low microbial biomass associated with rail ballast. They also inferred that low organic matter content might constrain herbicide degradation, which supports the author's previous assertion that NOM was unlikely to be a significant interference in the gravimetric methodology described in Anderson *et al*. (2000).

Another contaminant class that could have been examined were the poly-chlorinated biphenyls (PCBs). These are a large group of compounds used as insulators and coolants in electrical transformers due to their low electrical conductivity and high boiling point. An important point raised in Shimura *et al*. (2003) was that PCBs might penetrate the surface of the ballast and be problematic to extract for analysis. The implication is that penetrated PCBs could be liberated during processing of apparently clean ballast and may present an unacceptable environmental impact

when used as a recycled aggregate. The authors also stated there was no method for extracting PCBs from ballast.

Some of the language used in the papers could also have been more carefully worded. For example, the following statement was made in Anderson *et al*. (2002):

> *"Incineration, landfilling or recycling of the waste are the options available, the choice depending on the solvent system and on the nature of the waste (hazardous, containing fines, etc.)"*

This was rather weak conclusion and a more appropriate and expansive commentary would have significantly improved the paper. For example, the European Commission's Landfill Directive (99/31/EC), as translated into UK domestic legislation, resulted in a ban on disposal of hazardous liquid waste to landfill in 2002 that was extended to all liquid wastes in 2007. An appropriate assessment would now be made using the methodology detailed in Environment Agency (2007).

Similarly, in the discussion of Anderson *et al*. (2003) it was stated that:

> *"Once separated from the waste liquor, the fines could be incorporated into a composting process or blended as part of manufactured topsoil"*

It would have been more appropriate to qualify these proposed disposal options for contaminated fines with reference to relevant quality standards and protocols. For example, the current UK guidance for composted materials is the PAS 100 document (BSI, 2005). This specifically states it is for:

> *"…materials that have been separately collected from non-biodegradables, and that have not been mixed, combined or*

*contaminated with other potentially polluting wastes, products or materials"*

Compost containing fines from the treatment of rail ballast would not meet the PAS100 specification and this could limit the circumstances under which it was used and/or the value. However, composting would potentially degrade organic contaminant and residual cleaning agents and is used as a bioremediation technique (e.g. Beaudin *et al*., 1999; Jorgensen *et al*., 2000; Antizar-Ladislao *et al*., 2006).

The use of the fines as a component of manufactured topsoil would likely be considered with reference to the appropriate UK topsoil standard, currently 'Specification for topsoil and requirements for use' (BSI, 2007).

It was also stated in the discussion of the same paper that:

*"...a constructed wetland system offers the potential as a low-cost approach for the treatment of waste liquor from washing railway ballast"*

The BOD determined for the wash water containing 6% BioSolve® was determined to be 4,890 ± 157 mg $l^{-1}$ which may have been an overly ambitious effluent for treatment by a small scale wetland at a ballast cleaning site (Environment Agency, 2003). However, this would depend on the volumes of effluent produced and the ratio of effluent to wash water discharged which would produce a total loading that may be appropriate for treatment in a constructed wetland.

### 2.1.4 Overall assessment and impact

The three papers on contaminated rail ballast contributed to knowledge in the field both in terms of analytical methodology for assessment of contamination and considerations of environmentally sustainable options for cleaning and reuse of rail ballast. The decision to publish in Land Contamination and Reclamation, a journal read by many consultants and contractors but with a lower impact factor than other potential outlets, was a deliberate choice and had the desired effect of stimulating many enquiries from industry over the years.

A conference paper (Anderson *et al*., 2002b) based on our second publication (Anderson *et al*., (2002a) won the award for best university research paper at Railway Engineering, the 5th International Conference and Exhibition held in London on the 3rd and 4th July 2002.

The knowledge gained using Biosolve® led to a collaborative project with a recovered fuel oil company in central Scotland where Biosolve® was used to treat tank bottom solids from a used oil recovery facility. The process developed made use of recovered kerosene available at the site as a solvent. Oil was extracted from the solids and the residual solids washed using Biosolve® which allowed the non-hazardous cleaned solids to be disposed of to landfill at reduced cost with a further recovery of residual kerosene/oil.

Based on these studies, field pilot trials were undertaken in 2006 with a UK consultancy employed by Network Rail. These data validated earlier laboratory scale findings on the efficacy of surfactant washing to treat contaminated ballast (unpublished data). Even six years after the last publication, an article based on the work reported appeared in a rail industry trade magazine (Mackillican, 2009) indicating the relevance of the topic to industry.

Burkhardt *et al*. (2008) recently reported on a study of diffuse pollution releases from the Swiss Federal Railways (SBB) network. Being electric, the network is not subject to high concentrations of diesel and oil contamination encountered where diesel locomotives are used. However, they reported that releases from treated wooden sleepers and lubricants from track-switches and wheel flanges were the most significant sources of hydrocarbon contamination which amounted to an estimated of 1357 tonnes per annum across the entire network.

Kiani *et al*. (2008) conducted an environmental life-cycle assessment of railway track beds. They concluded that although ballast track beds are most commonly used in the UK that concrete track beds, although initially more expensive, may have lower life cycle costs. However, a lack of robust data on the water pollution and solid waste was one of the factors that limited the conclusiveness of the comparison.

Ballast contamination from diesel locomotives and other point sources is likely to remain an important environmental issue and merits further investigation. Oil contamination of railway tracks has been studied for many years and one approach to

dealing with the issue has been to consider bioremediation to degrade the hydrocarbon contamination *in situ* (Smith *et al*., 1981). The papers presented in this section made a significant contribution to understanding of the issues surrounding the assessment and treatment of hydrocarbon-contaminated ballast and in the next section bioremediation of railway land will be considered.

## 2.2 Contaminated railway land

Contamination of land may result from many activities associated with railway operations and can include a wide range of contaminants including asbestos, fuel oils, lubricating oils, metals, PCBs, PAHs and solvents impacting soils and groundwater (DOE, 1995; Hirl, 1998). Railway sites are a typical example of industrial land where hydrocarbon-contaminated soils can be found (Kirton & Beaulieu, 2005). The context of available *ex situ* remedial approaches for such sites will first be briefly considered with particular reference to the UK situation.

Excavation and disposal to landfill has been the most attractive disposal route for contaminated soils in the UK due to relatively low costs and ease of implementation (Table 2). Remediation technologies fall broadly into the four categories of physical, thermal, chemical and biological and most may be applied *in situ* or *ex situ*. All of the treatment technologies are applicable to hydrocarbon-contaminated soils to a lesser or greater extent. An estimate of the relative proportions of disposal to landfill

or remediation technologies applied in the UK in recent years is shown in Table 2 below.

**Table 2: UK market share for contaminated land treatment 2003-2007**

| Treatment approach | 2003 | 2004 | 2005 | 2006 | 2007 |
|---|---|---|---|---|---|
| Landfill | 77% | 76% | 76% | 76% | 75% |
| Other physical | 6% | 6% | 6% | 6% | 6% |
| Containment | 6% | 6% | 6% | 6% | 6% |
| Bioremediation | 5% | 5% | 5% | 5% | 5% |
| Chemical | 4% | 4% | 4% | 4% | 4% |
| Solidification | 1% | 1% | 1% | 1% | 1% |
| Thermal | 1% | 1% | negligible | negligible | negligible |

(After MSI, 2008)

Excavation and disposal to landfill has clearly been the dominant approach despite the obvious criticism that this simply moves the problem elsewhere and is likely to be the least sustainable option. Historically, the UK remediation industry has had particular strengths in assessment of contaminated land and in validation of remedial actions rather than in remediation technologies (EIU, 2007).

Bioremediation technologies may be categorised as either *in situ* or *ex situ* strategies. The papers discussed in this and the following chapter are primarily concerned with

the latter. *Ex situ* approaches are often more rapid and are simpler to control but have the disadvantage of requiring soil to be excavated for treatment (Dott *et al*., 1995).

A common definition of bioremediation is the use of microorganisms to degrade pollutants (e.g. Atlas & Bartha, 1998). A more expansive definition from the Joint Research Council Review of Bioremediation Research in the UK published by the BBSRC, EPSRC and NERC in February 1999, defined bioremediation as being:

"*The elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes, to minimise the risk to human health and the environment"*

The replacement of 'microorganisms' with 'biological processes' reflects the inclusion of the use of plants to include phytoremediation processes.

In this section, the focus is on the use of microorganisms and the bioremediation treatments discussed are therefore designed to optimise the environment for indigenous or introduced microorganisms. For *ex situ* bioremediation of hydrocarbon-contaminated soils, this consists of providing appropriate conditions to encourage aerobic biodegradation.

These include a pH of typically between 5 and 9, soil moisture of around 50-75% of field capacity, available inorganic nutrients such as nitrogen and phosphorous as well as aeration to provide oxygen (Rosenburg & Gutnick, 1989; Leahy & Colwell, 1990). Optimal conditions for bioremediation of hydrocarbon-contaminated soils

have been extensively reviewed *e.g.* recent works by Juwarkar *et al.* (2010), Tyagi *et al.* (2010) and Rain *et al.* (2011).

The fate of petroleum hydrocarbons in soil is significantly influenced by the nature of the contaminants such as aqueous solubility, hydrophobicity, polarity and soil characteristics including density, relative mineral/organic matter content and water holding capacity (Megharaj *et al.*, 2011). Abiotic fates include soil sorption, leaching to surface water or groundwater and volatilisation to the atmosphere, all of which may result in losses of contaminant mass. Sorption may be further divided into adsorption to surfaces and absorption deeper into the soil matrix e.g. partitioning into natural organic matter.

Microbial biodegradation is not the only biological process that may result in losses of hydrocarbon contamination. Plant root uptake and translocation as well as uptake and accumulation by animals are also valid potential routes (Philp *et al.*, 2009). The fate of hydrocarbon contaminants is therefore strongly influenced by competing sorption and degradation processes.

Over time, both abiotic and biotic processes will typically result in a reduction of the contaminant mass. However, there will also be a reduction in the availability of the remaining hydrocarbon contamination for biodegradation in a process is known as 'ageing' (Semple *et al.*, 2003) where sequestration by the matrix occurs. Bioavailability impacts the remediation process in terms of the potential for microbial degradation of a contaminant during bioremediation as well as the

potential for residual contamination to impact on biological receptors from a risk assessment perspective.

It is useful to consider the distinction made by Semple *et al.* (2004) on bioavailability versus bioaccessibility. The author's definition of a bioavailable compound is *"that which is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time"* and they stress the implied immediacy of the definition. A bioaccessibile compound is defined as *"that which is available to cross an organism's cellular membrane from the environment, if the organism has access to the chemical"*.

One implication is that a contaminant may be physically unavailable to an organism, for example, by virtue of being bound for example to soil organic matter. Bioaccessibile contaminants may become bioavailable rapidly or over years or decades but the bioavailable fraction will always be less the bioaccessibile one (Semple *et al.*, 2004). Megharaj *et al.* (2011) reported that biphasic or 'hockey stick' kinetics *i.e.* an initially rapid period of degradation followed by a slower phase are commonly observed during bioremediation of soils. It would have been more complete to acknowledge the role of abiotic processes such as leaching to surface or ground water and volatilisation as also playing a role in any observed initially rapid loss of contaminants during bioremediation.

A number of laboratory methodologies for assessing bioaccessibility have been proposed such as the use of non-exhaustive chemical extractions by subcritical water

and cyclic oligosaccharides including hydroxypropyl-β-cyclodextrin (Latawiec & Reid, 2009). No one approach has been shown to be the best predictor of bioaccessibility.

However, Latawiec & Reid, (2009) proposed that techniques that rely on desorption mechanisms would be the most reliable in terms of accuracy and consistency across soil types. This is in agreement with Megharaj *et al.* (2011) who stated the ability of soils to desorb pollutants is a key determinant of the susceptibility of contaminants to biodegradation and hence the effectiveness of bioremediation. They also note that sorption remains a poorly understood process in bioremediation despite it being a critical factor.

In their recent mini-review, Vilchez-Vargas *et al.* (2010) highlighted that is has been nearly a century since bacterial isolates were first reported to be capable of using aliphatic and aromatic hydrocarbons as the sole carbon and energy sources. Biodegradability of common classes of petroleum hydrocarbons may be described as typically decreasing in the following order: n-alkanes >branched alkanes >branched alkenes >low-molecular-weight n-alkyl aromatics >monoaromatics >cyclic alkanes >polycyclic aromatic hydrocarbons (PAHs) >asphaltenes (Tyagi *et al*., 2010). Megharaj *et al.* (2011) noted that there is probably even more diversity in the microbial communities and their capabilities to metabolise contaminants than the diversity in sources and chemical complexities of the organic contaminants that are the target for bioremediation.

Bioremediation of hydrocarbon-contaminated soils may rely on the native microbial population and in most cases biostimulation is practiced, that is the provision of inorganic nutrients such as nitrogen and phosphorous. The addition of microorganisms is termed bioaugmentation and this may be done by adding exogenous strains or through enhancing the numbers of indigenous strains isolated from the same or a similar contaminated site. Many recent authors report that mixed bacterial consortia are more efficient individual bacterial strains (e.g. Tyagi *et al.*, (2010); Wang *et al.*, (2011) as consortia are more robust under field conditions and provide greater metabolic diversity.

Biodegradation of contaminants is most often the result of microbial communities as opposed to a single species and microbial diversity as determined by molecular microbiological methods is in orders of magnitude greater than culture based assays have suggested (Vilchez-Vargas *et al.*, 2010). Despite decades of research there are still no accepted standard methodologies for determination of the composition and activity of microbial communities in hydrocarbon-contaminated soils.

Until relatively recently, cultivation based methods were the only widely practiced methods to assess and monitor bioremediation. Molecular diagnostic tools such as terminal fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis (DGGE) may be used to understand the relationships between community diversity and biodegradation abilities of microbial communities. In a recent review of cultivation-independent community profiling techniques, Desai *et*

*al.* (2010) highlighted the need for bioinformatics tools to deal with the quantity of data able to be generated.

Typically, an *ex situ* bioremediation is achieved using either landfarming where relatively shallow depths of soil are spread over a wide area for treatment or by forming engineered mounds of soils as either windrows (Figure 6) or biopiles. The former are essentially landfarming of treatment piles rather than shallow lifts of soil. In practical terms, these occupy less space and may be covered to assist in maintaining optimal conditions for degradation.

**Figure 6: Windrows, covered to prevent excessive loss of moisture**

Biopiles may be actively or passively aerated and the most sophisticated approaches can include covered systems with integrated nutrient/moisture delivery and monitoring as well as capture of any volatilised contaminants (von Fahnestock *et al*., 1998). The requirement for aeration to overcome oxygen diffusion limitations in biopiles had been established in many previous studies e.g. Benazon *et al*. (1995)

who observed limited degradation of styrene below 1.5 m depth in large 780 $m^3$ biopiles (26 m x 12 m x 2.5 m).

Previous studies on railway land reported in the literature at the time had included a study where landfarming was used on hydrocarbon-contaminated soils from a shunting/repair yard in Tasmania. Line *et al*. (1996) reported on a linear reduction in TPH over 12 months of landfarming from a mean of 4,644 mg $kg^{-1}$ to around 100 mg $kg^{-1}$ when nutrients were added to stimulate the indigenous microbial population. This equated to a rate of approximately 12 mg kg $day^{-1}$.

The studies presented in this chapter were primarily motivated by a requirement to improve understanding of *ex situ* bioremediation processes in hydrocarbon-contaminated soils. This in turn was driven by the need to demonstrate that bioremediation could compete with landfill in terms of being a rapid and relatively simple approach. To achieve this, the time taken to reduce contamination to acceptable levels as well as the cost and complexity of site operations needed to be optimised.

The comparative costs of remediation technologies are typically presented as a very wide range. One of the key reasons for this being the highly site specific nature of remediation activities. These must take into account site geological, hydrogeological and contaminant heterogeneity as well as local variations for material, equipment and labour costs. The global figures in table 3 below from a recent review of bioremediation by Juwarkar *et al.* (2010) are a good example of this phenomenon.

**Table 3: A comparison of soil remediation treatment costs per tonne**

| *Remediation technique* | Min (£) | Min (£) |
|---|---|---|
| Biological | 5 | 170 |
| Chemical | 12 | 60 |
| Physical | 20 | 170 |
| Solidification/Stabilisation | 17 | 171 |
| Thermal | 30 | 750 |

The Department for Environment, Food and Rural Affairs (DEFRA) recently commissioned a study to review the current understanding and application of different contaminated land remediation techniques in the UK including current and likely future factors influencing their selection and considering issues of sustainability. The resulting report (CL:AIRE, 2011) included a survey of typical costs for different remediation approaches and these were given for smaller (<5,000 $m^3$) and larger (>5,000 $m^3$) UK sites. Costs for *ex situ* bioremediation and disposal to landfill from the report are given in table 4 below.

**Table 4: Cost data per $m^3$ for *ex situ* bioremediation and disposal to landfill**

| | **<5,000 $m^3$** | | **>5,000 $m^3$** | |
|---|---|---|---|---|
| ***Ex situ* bioremediation** | **Min (£)** | **Min (£)** | **Max (£)** | **Max (£)** |
| Total Range | 12 | 125 | 9 | 65 |
| Median | 30 | 35 | 20 | 30 |
| **Disposal to landfill** | | | | |
| Total Range | 30 | 400 | 30 | 300 |
| Median | 45 | 250 | 65 | 250 |

These data were based on a similarly small number of respondents of only 11 for *ex situ* bioremediation and 12 for disposal to landfill. There are lower costs for ex situ bioremediation compared with disposal to landfill but the range is relatively broad even for the larger sites where factors such as mobilisation/demobilisation may have less of an impact on the overall costs. As noted in the report, remediation costs tend to be given as broad ranges as site-specific factors including remedial targets vary widely.

### 2.2.1 Papers Presented

**Cunningham, C.J.**, Philp, J.C. (2000). Comparison of bioaugmentation and biostimulation in *ex situ* treatment of diesel contaminated soil. *Land Contamination & Reclamation.* 8 (4), 261-269.

Li, L., **Cunningham, C.J.**, Pas, V., Philp, J.C., Barry, D.A., Anderson, P. (2004) Field trial of a new aeration system for enhancing biodegradation in a biopile. *Waste Management*. 24 (2), 127-137.

### 2.2.2 Aims, methodology, results and conclusions

In Cunningham & Philp (2000), we reported on a field scale bioremediation study at the same location as the contaminated rail ballast previously discussed. Hydrocarbon contamination of the railway siding had occurred over decades due to leakages and spills from stationary diesel locomotives (Figure 1). Diesel is the main hydrocarbon contaminant associated with railway land (Troy & Brown, 1994). The main aim of

the study was to investigate the potential of bioaugmentation to enhance the rate of hydrocarbon degradation and therefore reduce the time taken for remediation. A secondary aim was to examine differences in performance between *ex situ* treatment approaches namely biopiles and windrows. Commercial NPK fertiliser was also compared with horse manure as the source of nutrients as this had implications for overall sustainability of the treatment.

The assessment of the baseline hydrocarbon contamination was undertaken using a similar gravimetric methodology to that used by Anderson *et al*. (2000). The soil on site was made ground containing clinker and ash, presumably from the days of steam locomotives. It was heavily contaminated by hydrocarbons, principally diesel, with almost 90,000 mg $kg^{-1}$ determined by a gravimetric oil and grease (O&G) method.

A total of nine biopiles or windrows were established as detailed in the paper (Figure 7) including a control that received no amendments but was otherwise treated as one of the static biopiles. These were diluted by approximately 50% with uncontaminated topsoil as the made ground on site also contained metals (Table 1 in the paper, p264) at relatively high levels.

**Figure 7: Layout of field trial site from Cunningham & Philp (2000)**

This brought the initial concentration of O&G in the treatment piles down to between 50,000 and 57,000 mg $kg^{-1}$. The approach to bioaugmentation was not to introduce exogenous microorganisms but to develop a mixed population of hydrocarbon degrading microorganisms from the indigenous microbial population extracted from samples taken on site. It was reasoned that a competent population could be established using a serial enrichment technique, providing diesel as the sole carbon source. The culture was then produced in sufficient volume to apply as a liquid treatment to the bioaugmented plots on site.

Monitoring of the treatments was conducted at the start of the experiment and on three subsequent sampling rounds over a period of approximately 70 days. The concentration of hydrocarbon contamination was determined by the gravimetric method and reported as O&G. The microbial populations in the treatment piles were

assessed by monitoring the numbers of hydrocarbon oxidising microorganisms using the most probable number technique (MPN) of Wrenn & Venosa (1996).

The results in the augmented piles showed over 90% reduction in O&G after only 7 days in all treatment piles. Thereafter, relatively little change was observed over the remainder of the study when a maximum of 94% reduction was achieved from a mean of 50,990 ± 3,400 mg $kg^{-1}$ down to 2,900 ± 240 mg $kg^{-1}$.

In the non-augmented systems differences between windrows and biopiles were observed. After 7 days, the concentration of O&G determined in the windrow systems was also reduced significantly by 73% from a mean of 54,500 ± 2,640 down to 14,780 ± 820 mg $kg^{-1}$. However, degradation proceeded less rapidly in the biopile systems with a mean reduction of only 13% average reduction after seven days from a starting concentration of 57,350 ± 1,490 mg $kg^{-1}$ down to 50,130 ± 1,920 mg $kg^{-1}$. There were no differences between treatments receiving either NPK or manure and therefore these data represent the averages of both for each approach.

After 36 days, the initial differences observed between non-augmented biopiles and windrows had gone. After 68 days, the non-augmented systems reached a similar endpoint to the augmented systems meaning that all approaches tested were successful in dealing with the hydrocarbon contamination.

Over the monitoring period, the control pile mirrored the behaviour of the non-augmented biopiles but with a significantly lower reduction in O&G. This was most

evident during the first seven days of the experiment where only an 8% reduction was observed. By day 36 the control pile also showed a nearly 30% reduction in O&G and by the end of the trial a considerable reduction of more than 80%. However, after 68 days the O&G in the control pile was still in excess of 16,000 mg $kg^{-1}$. At this level the site would still be considered as seriously contaminated. The rate of reduction showed evidence of slowing considerably. Nevertheless, the possibility that an acceptable end point may have been eventually reached with no intervention other than regular watering cannot be excluded.

After 13 days, all bioaugmented piles had in the order of $10^5$ colony forming units (CFU) $g^{-1}$ of soil; higher than the non-augmented systems sampled after 7 days and thereafter declined over the study period to number $10^4$ CFU $g^{-1}$ of soil. The numbers of hydrocarbon oxidising bacteria (HOX) were remarkably consistent over time in the control pile and were lower than all treatments up until 42 days into the study.

The removal rates determined were far greater than anything found in the literature. The most rapid reduction in bioaugmented treatments over 7 days equated to a hydrocarbon removal rate of 6,600 mg kg $day^{-1}$. In the previously cited example of railway land treated by landfarming over 12 months, the rate was only approximately 12 mg kg $day^{-1}$ (Line *et al*., 1996).

It is worth considering other examples from literature to establish a context for the rapid removal rate observed. Iturbe *et al*. (2004a) reported on a 100 $m^3$ biopile study (biostimulation) where TPH of petroleum and diesel contaminated soil was reduced

by 85% over 66 days from a starting concentration of around 4,600 mg $kg^{-1}$ representing a 60 mg $kg^{-1}$ $day^{-1}$. The same group later reported on a 27 $m^3$ biopile study (also biostimulation) with nearly an order of magnitude higher initial TPH in petroleum and diesel contaminated soils at 37,680 mg $kg^{-1}$. They reported an 80% reduction in TPH over 22 weeks (154 days) with a much higher removal rate of 1,372 mg kg $day^{-1}$ than found in the previous study (Iturbe *et al*., 2007). One of the highest removal rates reported came from a field study ($5m^2$ x 40cm depth plots) on diesel-contaminated soil where the maximum removal was seen using biostimulation and bioaugmentation. This reduced the initial concentration by 90% from 123,000 mg $kg^{-1}$ to 11,720 mg $kg^{-1}$ over 6 weeks equating to a removal rate of 2,780 mg $kg^{-1}$ $day^{-1}$ (Márquez-Rocha1 *et al*., 2001)

The study concluded that bioaugmentation using cultures derived from indigenous microorganisms was clearly demonstrated to be an effective technique to enhance the rate of hydrocarbon removal. Although there was no specific target level of hydrocarbons to be met, the remediation was considered to have been completed within a remarkably short period of time (7 days). Evidence was provided that static biopiles may be capable of supporting high rates of hydrocarbon degradation in augmented and non-augmented systems and that the choice of approach was a balance of time and costs/complexity and likely to be site specific.

One of the key lessons learned from the previous study was that the use of passively aerated biopiles instead of windrows might therefore offer a simpler and lower capital and operating cost bioremediation technology. This was also thought to be of

more importance during larger scale treatments than the pilot scale biopiles used previously and was the motivation of the next study discussed.

The author had observed during visits to remediation sites that very little attention was given to orientation and location of slotted plastic pipes commonly used in soil biopiles as means of providing aeration. The author proposed that a semi-passive aeration could be achieved with the same equipment used to generate airflow in a chimney using a wind-driven rotating cowl. The aim of this study was to therefore examine if such an approach could provide a more efficient means or aerating a biopile resulting in enhanced biodegradation of hydrocarbon-contaminated soil.

Assessing the efficacy of this approach benefitted greatly from collaboration with co-authors Li and Barry who provided the modelling expertise for the study. Simulations were conducted that predicted the airflows induced in biopiles with no aeration, longitudinal aeration at the base of the biopile and with a novel vertical pipe configuration. These data shown in Figures 3, 4 and 5 in Li *et al*. (2004) indicated that a more effective airflow in terms of velocity and uniformity could potentially be achieved with the latter.

To examine this in the field, two biopiles were constructed at the same railway site discussed in Cunningham & Philp (2000). These were almost four times larger (4.3 $m^3$) than those used in the previous study (1.2 $m^3$) as a larger biopile was needed to test the vertical aeration approach. Two sections of vertical pipe almost 1 metre high were placed at either end of the biopile and rotating chimney cowls (Figure 8) were

fitted to both. This was compared with the typical horizontal pipe configuration observed by the author at bioremediation field sites using two slotted pipes of the same 140 mm diameter.

**Figure 8: Biopile with vertical aeration pipes and aspirating cowl**

In this instance, 1 part multipurpose compost was mixed with 3 parts contaminated soil to alleviate potential toxicity from the metals as previously discussed and also to provide sufficient nutrients to allow a measurable biodegradation to take place. The soil also had wood chips added as bulking agent at a ratio of 14:1 wood chips to soil.

Monitoring of hydrocarbon contamination again used a simple gravimetric method and GC-FID provided further information on the nature of the hydrocarbons at the beginning and end of the study (30 days). It was thought adequate to assess the total heterotrophic population using plate counts as a gross indicator of overall aerobic microbial activity. The starting hydrocarbon concentrations were not the same in both biopiles due to heterogeneity in the distribution of hydrocarbons in the contaminated soil.

The removal rate of hydrocarbons in this study was relatively good. Both systems showed a reduction of approximately 25% but no difference in hydrocarbon removal could be inferred. Applying first order kinetics to the data to determine degradation rates gave almost identical results for both piles of 0.011 $day^{-1}$ and 0.017 $day^{-1}$ for the normal and aerated piles respectively. Assuming that a constant degradation rate co-efficient and applying zero order kinetics indicated some enhancement in the aerated pile at approximately 185 mg $kg^{-1}$ $day^{-1}$ versus 134 mg $kg^{-1}$ $day^{-1}$.

An interesting observation was that the standard errors on triplicate sample O&G results from the vertically aerated pile were typically between 1-8%. One result on day 6 was higher than the previous or starting concentration that most likely reflected heterogeneity in the pile. The horizontally aerated pile typically showed much higher standard errors of between 12 and 27% with one very low 2% error on day 3. It could be speculated that the aerated pile had a more even airflow and via abiotic losses or enhanced biodegradation that a more even distribution of contaminant was created. However, the difference was evident from day 0 and the true cause is unknown.

Microbial numbers peaked in both systems at day 15 and showed a similar pattern in both systems with slightly lower numbers observed in the aerated pile but an overall increase of one order of magnitude. It should be noted that these counts were total heterotrophic microorganisms and it is possible that the aerated pile had higher numbers of hydrocarbon degraders. In retrospect, it would have been better to include an MPN count of diesel degraders in the study.

Perhaps the most interesting results were those from the measurement of soil moisture. These data showed that the aerated pile had typically 5-10% lower moisture. The piles were watered on day 0 and again on day 6 which may have been excessive as the moisture content rose to 45-50% in both piles. Rainfall during the remaining period of the study further increased soil moisture; on day 30 the normal pile had a mean moisture level in the samples of 65%.

From this study it was concluded that the configuration of slotted pipes typically used to provide aeration in passive biopiles may be improved by adopting a different design. The simulations supported the approach and the field data stressed the importance of understanding the interrelationship between aeration and moisture.

### 2.2.3 Critique and contribution

Cunningham & Philp (2000) demonstrated that rapid bioremediation was possible in short timescales using bioaugmented static biopiles but did so using relatively unsophisticated monitoring of the removal of hydrocarbons from the various treatments. The study also did not actually compare bioaugmentation with biostimulation. In the strictest sense it examined the additional benefit of adding an indigenous enrichment culture to soils receiving additional nutrients (biostimulation).

However, it would be difficult to argue that the O&G results were not a true indicator of biodegradation being responsible for the reduction in levels of hydrocarbon

contamination observed. At the time the work was undertaken, the author had no access to more sophisticated analytical techniques such as GC-FID or GC-MS. Disadvantages of the gravimetric method included a lack of specificity, high losses of the most volatile fractions, and a high susceptibility to interferences e.g. from NOM. The advantages were that it was a relatively simple, low cost and rapid method appropriate for the determination of total hydrocarbon contamination (Villalobos *et al*., 2008).

In the O&G methodology, methanol was used alongside dichloromethane as a more polar co-solvent to extract entrained hydrocarbons from the soil samples. The use of methanol may have resulted in less efficient recoveries in the samples with low hydrocarbon levels and acetone may have been a more appropriate co-solvent (Saari *et al*., 2008). Overall, these O&G data were fairly conclusive and were therefore able to be considered as fit for purpose in terms of assessing differences between treatment approaches. However, from a contemporary viewpoint, the use of O&G would not be fit for purpose within an environmental or human health risk-based remediation.

In March 2002, the UK Department for Environment, Food and Rural Affairs (DEFRA) published the Contaminated Land Exposure Assessment (CLEA) technical guidance and software for human health risk assessment. Prior to this, many practitioners used older UK guidance with threshold and action levels (ICRCL, 1987) and also guidance from the Netherlands, which came to be known as the 'Dutch List'. ICRCL (1987) had no guideline values for petroleum hydrocarbons but

did have threshold and action levels for PAHs. The 'Dutch List' mineral oil values at the time the work was published had an intervention level, above which contamination was determined to be serious, of 5,000 mg $kg^{-1}$ and a target level of 50 mg $kg^{-1}$ VROM (2000). Under the current UK regime, the site would be assessed in terms of hydrocarbon fractions determined by GC-FID using an extraction method that separated aliphatic and aromatic carbon bandings such as that reported by Risdon *et al*. (2008).

The simple approach of mixing clean and contaminated soils was taken to alleviate potential toxicity from the high metal contents arising from ash and other fill materials deposited at the site over many years. It was reported that the 'total solvent extractable material' from the topsoil was 2,700 ± 270 mg $kg^{-1}$. For consistency it would have been more appropriate to also report this as O&G as the same gravimetric method was used on the topsoil. Soil samples were sieved before analysis removing any wood chips leaving a theoretical 5 parts topsoil to 10 parts contaminated soil i.e. 33% of the soil was topsoil. It may therefore be reasoned that the topsoil contributed approximately 2,700/3 = 900 mg $kg^{-1}$ of interference to the O&G results from NOM.

This is instructive when interpreting the final levels of O&G reached by all of the treatments after around 70 days as these were approximately 2,800 mg $kg^{-1}$. Degradation of the woodchips may have contributed significantly to the NOM but this was not quantified. It was also possible that some of the NOM was accounted for by biomass as plants began to grow on the treatment piles during the course of

the experiment. This was a result of stimulation by added nutrients and the reduction in phytotoxicity as diesel was removed (Adam & Duncan, 2002).

Reporting of the high background NOM in the topsoil was followed in the paper by the conclusion that:

> *"The overall hydrocarbon removal in the treatments were therefore higher than suggested by these data."*

Whilst this was a correct interpretation, it was incomplete. No sample was obtained from the same matrix as the contaminated soil that had not been subject to contamination by diesel. However, it may have been unrealistic to find a location on that particular site where such a sample could have been taken. Nevertheless, not taking a control sample in this manner was an oversight as it would have provided at least some estimate of the background NOM in the matrix and would have assisted in interpreting the completeness of the remediation.

Was intrinsic bioremediation observed in the control pile or did the O&G results indicate abiotic losses e.g. through volatilisation? Indeed, to what extent did abiotic losses account for O&G reductions across the treatments? It could be argued that abiotic losses were largely accounted for with the inclusion of a control where the moisture was maintained but otherwise received no additional treatment. A laboratory study on a sample of soil treated to eliminate microbial activity would perhaps have added significantly to the understanding. However, evidence from the

enumeration of hydrocarbon oxidising bacteria (HOX) also provided some insight into the possible mechanisms behind the observed O&G reductions.

It should be noted that the first non-augmented and control samples were taken after 7 days whereas the first sampling of the augmented systems occurred after 13 days. It was unfortunate that time and resource limitations necessitated sampling on different days. The significance of this was that the remarkably rapid reduction in O&G occurred before samples to enumerate the HOX were taken. It is tempting to speculate that had the samples been taken at the same time as the non-augmented piles on day seven that much higher numbers of HOX would have been observed and stronger evidence for the efficacy of bioaugmentation provided.

There were many other limitations in terms of the microbiological and chemical data available and aside from the oversights noted above; these were mainly the result of time and resource constraints. Also, although a field study, the scale was relatively small with pile volumes of approximately 1.2 $m^3$. Nevertheless, the paper made a contribution to understanding the potential of bioaugmentation to reduce the time for bioremediation of hydrocarbon-contaminated soils and suggested further investigation of the static bio-piling process that prompted the efforts of the next paper discussed.

In Li *et al*. (2004) we showed using a relatively simple analytical model that the airflow due to wind-induced pressure gradients was not uniformly distributed in static biopiles using a common configuration of horizontal land drainage pipes to

provide aeration. Airflow was found to be weakest in the centre near the base of the pile and could limit oxygen for aerobic biodegradation of hydrocarbons and restrict performance in the field. Simulations of the vertical approach suggested by the author indicated that this could be overcome by adopting this design.

Sylla *et al*. (2003) used a vertical arrangement of pipes for passive aeration during a laboratory solid waste composting trial. Natural convection was enhanced compared with horizontal pipes. However, the maximum temperature measured in both soil treatment biopiles in our study was approximately 18$^{o}$C which must be compared with the greater than 50$^{o}$C in the composting study. The use of the cowl may be considered to be an enhancement to overcome the limited airflow created due to convection during *ex situ* bioremediation.

Whilst measurement in the field of the pressure generated in the vertical pipes showed promise, the O&G results from field trial were inconclusive and it was difficult to draw firm conclusions on the influence of the approach on rates of biodegradation. The zero order kinetic model indicated a degree of enhancement in the aerated pile of 54 mg kg-1 day-1 but was drawn from very limited data. Some time after the study, the author discovered a conference paper (Cyr & Spieles, 1997) where horizontal perforated pipes with vent stacks incorporating ventilator turbines (cowls) had been trialled to enhance biopile aeration. In a very limited study on a large biopile (195 x 90 x 10 feet) of soil contaminated with diesel at 1% (w/w), the approach was concluded not have increased airflow through the pile. However, the

authors presented very little data and conceded that this conclusion was based on a qualitative evaluation of the system.

Overwatering of the piles and reasonably heavy rainfall at the time may have resulted in the moisture in the piles being higher than was optimal. Overall, better instrumentation of the pile with, for example similar pressure sensors as used by Kodres *et al*. (1999), and a more comprehensive chemical and microbial monitoring regime would have yielded more conclusive data. In both studies the soil was diluted with clean topsoil or compost to reduce the potential toxicity to hydrocarbon degrading microorganisms. Clearly, this practice impacts on the overall sustainability of the approach e.g. by increasing transport impacts from a bioremediation project and could result in an overall surplus of soil at a site. Whether the soil produced would be considered a waste could impede reuse particularly if there was no use for the soil on the site where it was produced. This issue is further elaborated upon in the discussion.

### 2.2.4 Overall assessment and impact

Even taking into account the limitations discussed previously, Cunningham & Philp (2000) was a pioneering bioremediation field study in the UK demonstrating that bioaugmentation with indigenous microorganisms could greatly enhance the treatment of hydrocarbon-contaminated industrial land. Despite being published in a journal more familiar to practitioners, this work has been cited nearly 30 times and has also provided the foundation for many future studies.

For example, in the study reported by Cunningham *et al*. (2004), immobilisation of a mixed population of hydrocarbon degraders in a polyvinyl alcohol (PVA) hydrogel was investigated to improve the mechanism of implementing bioaugmentation in the field. Immobilisation can reduce competition with indigenous microorganisms and offer protection from predation by protozoa and chemical stressors such as extremes of pH or toxic compounds (Pritchard, 1992). The approach was considered to be successful and a co-immobilised system containing PVA-entrapped microorganisms and a synthetic oil absorbent (Siahpush *et al*., 1992) showed a higher overall removal of diesel compared to conventional biostimulation and bioaugmentation treatments.

The Cunningham & Philp (2000) bioaugmentation field study was recognised in February 2001 when the authors won the Ford Motor Company Conservation and Environmental Grants (UK) programme. This supported collaboration and technology transfer with the Institute of Ecology and Genetics of Microorganisms (IEGM), Ural Branch of the Russian Academy of Sciences in Perm, Russia. Building on this, we went on to secure €120,000 funding from the International Association for the promotion of co-operation with scientists from the New Independent States of the former Soviet Union (INTAS) programme between 2002 and 2005. This international collaboration generated the three papers on bioremediation of crude oil contaminated land discussed in the following section and also furthered examination of immobilisation of hydrocarbon-oxidising bacterial cultures, examining the use of low cost natural and synthetic macro porous materials such as sawdust (Podorozhko *et al*., 2008).

Li *et al*. (2004) reported on a study into possible improvements in biopile technology and suggested significant potential for future optimisation of *ex situ* bioremediation and the paper has been cited 18 times. The biopile work also led to a field study (2003-2004) examining the potential of wind-assisted passive aeration applied to composting of paper mill sludge and green waste Figure 9 below.

**Figure 9: Semi-passive aeration system applied to a composting biopile**

*Ex situ* composting may make use of similar processes to *ex situ* biopiles using forced aeration by an electrical blower to supply air. In between periods of turning, windrows receive limited aeration through diffusion and convection, which will diminish in relation to increasing moisture contents. In a composting system the higher temperatures allow for passive aeration to occur due to convection driven by the difference in temperature between the compost pile and ambient air. When the wind assisted semi-passive aeration was applied to composting, the moisture removal observed in the Li *et al*. (2004) study that was unhelpful to bioremediation was

considered beneficial as it helped to stabilise the temperature of the media at the same time as providing oxygen for biodegradation (unpublished data).

No further work was undertaken using the wind-driven semi-passive aeration for bioremediation. However, combined with lessons learned from the composting trial, it may be a promising area for future research and the results will be useful in designing *ex situ* soil bioremediation systems. This preliminary work on composting in turn interested a colleague who went on to apply computational fluid dynamics modelling to simulate biopiles and further explored the relationships between biodegradation, aeration and moisture (Wu & Crapper, 2009a; 2009b).

Together with Cunningham & Philp (2000) these field studies attracted interest from companies and led to several collaborative projects. For example, a field trial was conducted in 2006 at a site where bunker fuel had contaminated a wide area of a former naval dockyard. Although no firm conclusions could be drawn from the study (unpublished data) the soil from the site was later used in the study published by Coulon *et al*. (2010).

## 3. Treatment of crude oil contaminated land

The previous papers discussed were concerned with land contamination by refined petroleum hydrocarbons. The papers discussed in this section addressed the more challenging issue of crude oil and crude oil wastes.

Crude oil is a complex mixture of hundreds of different hydrocarbons with wide ranging boiling points from the relatively volatile petroleum hydrocarbons (30$^{o}$C-200$^{o}$C) through heavy fuel oils (200$^{o}$C-400$^{o}$C) to asphaltenes and tars with boiling points in excess of 400$^{o}$C (Allard & Neilson, 1997). Other components include heteroaromatics containing nitrogen, sulphur and oxygen e.g. indole, thiophene and phenol.

Exploration and production of crude oil has resulted in widespread contamination of soil, surface and ground waters and a universal issue for the oil industry is dealing with wastes from the refining process (Bleckmann *et al*., 1997). In some places it has been the practice to store crude oil waste in unlined pits such as the one shown in Figure 10.

**Figure 10: Example of crude oil waste storage in an unlined lagoon in Turkmenistan**

Marine spillages of crude oil are beyond the scope of this document but are worthy of brief mention as these have often been the highest profile examples of this particular environmental challenge. Perhaps the most widely known case was that of the Exxon Valdez oil tanker which spilled more than 40 million litres of crude oil in Prince William Sound, Alaska in 1989 (Bragg *et al*., 1994). The subsequent investigations and clean up activities were one of the key drivers for production of commercial bioremediation products (Prichard, 1991; Pritchard *et al*., 1992b) continue even to this day (e.g. Boehm *et al*., 2008; Venosa *et al*., 2010).

Crude oil contamination on land has made extensive use of landfarming as a treatment approach due to ease of implementation and relatively low costs (Bleckmann *et al*., 1997). Arora *et al*. (1982) cited Grove (1980) who noted that land treatment (landfarming) has been practised by refineries since 1954. However, lengthy timescales have been reported in the literature, for example Genouw *et al*.

(1994) reported that 15 years would be required to remediate crude oil contaminated soil at a concentration of 47 g $kg^{-1}$ using landfarming.

The field and laboratory experiments discussed in this section were conducted in collaboration with the Institute of Ecology and Genetics of Microorganisms (IEGM) in Perm, Russia. Within IEGM, the Laboratory of Alkanotrophic Microorganisms (LAM) has worked for many years with alkanotrophic nocardioform bacteria such as *Rhodococcus*. Environmental applications of the genus have been widely explored e.g. indication of the presence and the bioremediation of petroleum hydrocarbons. Microorganisms of the genus *Rhodococcus* can utilise a wide range of hydrocarbon substrates (e.g. gaseous and liquid n-alkanes, mono- and heterocyclic aromatic compounds), grow under microaerophilic conditions, at low temperature (<10$^{o}$C) and can tolerate heavy metals (Ivshina *et al*., 1995; Bell *et al*., 1998).

In Anderson *et al*. (2002) the proprietary blend of synthetic surfactants Biosolve® was used to assist cleaning of contaminated rail ballast. Synthetic surfactants are generally considered to exhibit toxicity and may be resistant to biodegradation (Mulligan *et al*., 2001). In the remediation applications discussed in this section, natural biosurfactants were employed. Production of biosurfactants has a physiological role as they allow microorganisms to grown on insoluble substrates. Members of the genus *Rhodococcus* produce glycolipid biosurfactants containing trehalose as the carbohydrate (Lang & Philp, 1998). Biosurfactant complexes from *R. ruber* have been reported to exhibit significantly less toxicity than synthetic surfactants and also from rhamnolipids from *Pseudomonas aeruginosa* (Ivshina *et*

*al*., 1998). However, not all synthetic surfactants present toxicity or biodegradability limitations and some such as alkyl polyglucosides have been reported to have low toxicity and excellent biodegradability (Park *et al*., 2007).

The application of biosurfactants to bioremediation of hydrocarbons has been widely reported in the literature (e.g. Banat, 1994; Ron & Rosenberg, 2002). Biosurfactants from *Rhodococcus* were recently reviewed by Kuyukina & Ivshina (2010a) and more generally the trehalose lipid biosurfactants by Franzetti *et al*. (2010). The majority of components of crude oil have low solubility in water and tend to bind to soil particles reducing availability to microorganisms for degradation. This has been well described as a major limitation to the bioremediation of hydrocarbon contamination (Huesemann, 1997; Lai *et al*., 2009).

(Bio)surfactants can overcome this by mobilising hydrocarbons due to reduced oil/water interfacial tension and secondly by enhancing solubilisation of the contaminants into hydrophobic cores of surfactant micelles that form. Therefore, surfactants enhance bioremediation by increasing the surface area of hydrophobic substrates available for biodegradation and increase the bioavailability of these substrates (Ron & Rosenberg, 2002). Surfactant efficiency may be described by the critical micelle concentration (CMC) defined as the point where surface (liquid/air) or interfacial (liquid/liquid and solid/liquid) tension reduction reaches a maximum and micelles are formed (Kuyukina & Ivshina, 2010a).

Surfactant enhanced soil flushing may be applied as an *in situ* technique to enhance contaminant transport. Mobilised contaminants may be biodegraded in the vadose zone *in situ* or, if permitted to migrate to groundwater, removed in a 'pump and treat' system (Scheibenbogen *et al*., 1994). *Ex situ* surfactant enhanced soil washing has been described, for example, Urum *et al*. (2005) reported that a commercial rhamnolipid biosurfactant preparation (Jeneil Biosurfactants Company, USA) was effective in removing crude oil from soil assisted by floatation achieved by air sparging.

## 3.1 Papers presented

Kuyukina, M.S., Ivshina, I.B., Ritchova, M.I., **Cunningham, C.J**., Philp, J.C., Christofi, N. (2003). Bioremediation of crude oil contaminated soil using slurry-phase biological treatment and landfarming techniques. *Soil and Sediment Contamination*. 12 (1), 85-99.

Kuyukina, M.S., Ivshina, I.B., Makarov, S.O., Litvinenko, L.V., **Cunningham, C.J.**, Philp, J.C. (2005). Effect of biosurfactants on crude oil desorption and mobilisation in a soil system. *Environment International.* 31(2), 155-161.

## 3.2 Aims, methodology, results and conclusions

In Kuyukina *et al*. (2003), the aim was to develop an optimised *ex situ* bioremediation approach for crude oil wastes from storage pits in the Kokuyskoye oil field in the West Urals of Russia. Samples of a heterogeneous mixture of soil and oily wastes contained in one of the two 900 $m^3$ pits were assessed gravimetrically for total hydrocarbon content. The mean was determined to be 200 g $kg^{-1}$ expressed as

Total Recoverable Petroleum Hydrocarbons (TRPH). This was comparable to the gravimetric approach used by Cunningham & Philp (2000) but in this instance the solvent was chloroform according to the favoured method of the IEGM laboratory as described in Capelli *et al*. (2001).

A rapid reduction of almost 90 g $kg^{-1}$ total hydrocarbons in a short timescale was reported by Cunningham & Philp (2000) using bioaugmentation. However, the hydrocarbon contamination was mainly from diesel and the crude oil wastes were expected to require a more intensive approach.

An oleophilic bio-fertiliser had been developed with colleagues at IEGM and this was used for bioaugmentation and biostimulation. The formulation has been described in detail by Ivshina *et al*. (2001). Briefly, two strains of *Rhodococcus* from the Regional Specialised Alkanotrophic Microorganism Collection at IEGM were combined with NPK mineral salts in the ratio of 70:5:1 and a *Rhodococcus* biosurfactant complex at a concentration of 10 g $l^{-1}$.

Land farming was originally intended to be compared with the use of a slurry-phase bioreactor where conditions could be better optimised to enhance degradation rates. However, it was later decided to combine the techniques in a treatment train approach where oily wastes were pre-treated in the slurry-phase bioreactor before the solids were separated and applied to land farming cells. The same bio-fertiliser was used to inoculate the slurry-phase bioreactor. Total heterotrophic microorganisms

and hydrocarbon oxidising bacteria (HOX) were monitored weekly during the experiment.

For the land farming cells, dilution with 3 parts of clean soil to 1 part oily waste was used to bring the initial levels of contamination down to 46 g $kg^{-1}$ TRPH in two of the cells (C1 and C2) and a higher dilution with 10 parts clean soil (C3) brought the initial concentration down to 16 g $kg^{-1}$ TRPH. This was done in anticipation of there being a potential inhibitory effect from higher concentrations of oily wastes. Bio-fertiliser was added at the rate of 2 kg $m^2$ and 1 kg $m^2$ in cells C2 and C3 respectively, and this was done weekly for the first four weeks followed by one further application after seven weeks.

A significant decrease in TRPH was observed in all of the land farming cells after one week. The losses in the cells (C1, C2) with the higher starting concentration of 46 g $kg^{-1}$ TRPH were approximately 15 g $kg^{-1}$ (33%) and 22 g $kg^{-1}$ (48%) respectively. By contrast the cell (C3) with an initial concentration of 16 g $kg^{-1}$ showed a 27% decrease to 10 g $kg^{-1}$. After 10 weeks the TRPH in the control cell (C1) had reduced by 66% to 15.5 g $kg^{-1}$ TRPH compared with the bioremediation treatment cell (C2), which showed a reduction of 87% down to 6 g $kg^{-1}$ TRPH. In treatment cell (C3) with the higher dilution rate of 10:1 clean soil to oily waste, TRPH reduction levelled off after 6 weeks to 1 g $kg^{-1}$ (94% reduction) and remained at this level after 10 weeks.

Thin-layer chromatography with a flame ionisation detector (TLC-FID) was used to provide fractional analysis of hydrocarbons during crude oil bioremediation studies (e.g. Oh *et al*., 2000). In this study sampling was limited to time zero and two events after 5 and 10 weeks due to budgetary constraints. These TLC-FID data were presented in Table 2 in the paper but have been represented as relative percentage changes graphically in Figure 11 for ease of interpretation.

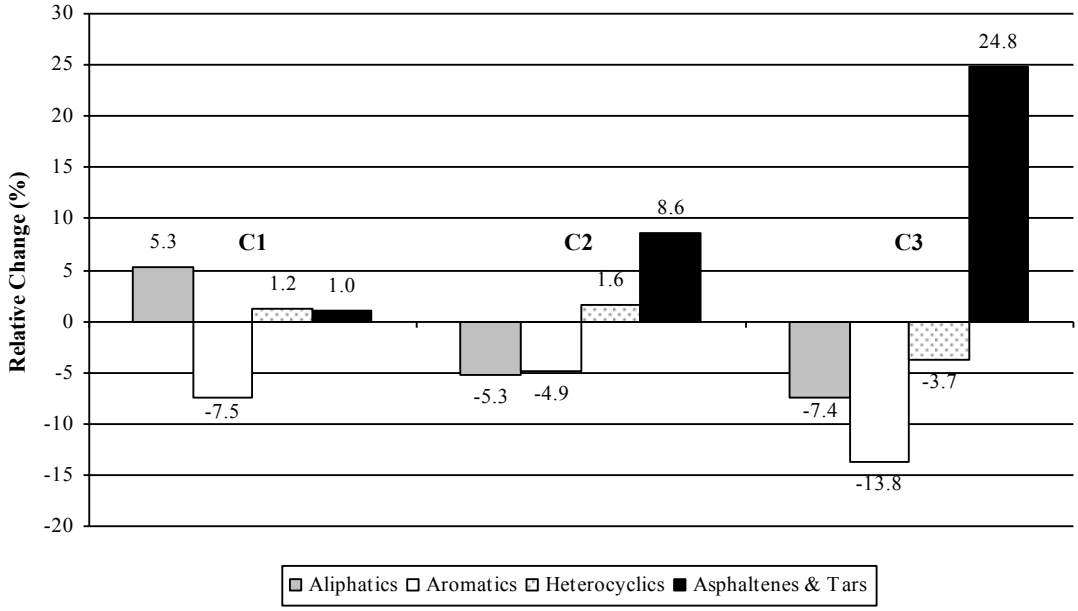


**Figure 11: Relative percentage change in fractional composition of residual oil in land farming cells over 10 weeks**

For example, in treatment cell C3, after 10 weeks the asphaltenes/tar fraction in the remaining hydrocarbon fraction represented 28.7% of the total compared with only 3.9% of the total at the beginning of the experiment.

The heterotrophic counts indicated the general health of the microbial population in each system. These data and all other microbial counts show the mean of three

replicates ± one standard deviation and were also presented in tabular form in the paper so these have been represented graphically in Figures 12 and 13 for ease of interpretation.

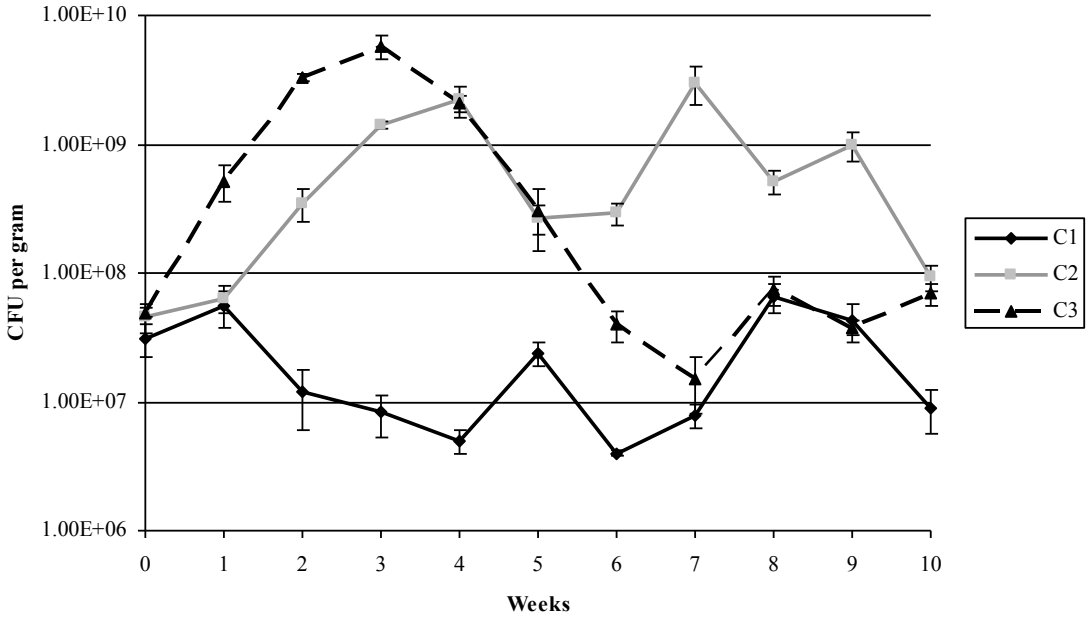


**Figure 12: Numbers of total heterotrophic microorganisms in land farming cells**

One notable trend was a decline in the total heterotrophs in C3, which received half the application rate of bio-fertiliser to cell C2 from a maximum of 5.8 x $10^9$ CFU $g^{-1}$ at week 3 to 1.5 x $10^7$ CFU $g^{-1}$ after 7 weeks of treatment. Numbers broadly remained stable after this time for the remainder of the experiment. The HOX data for treatment cells (C2 and C3) receiving the bio-fertiliser showed clearly higher numbers of HOX than the control cell (C1).

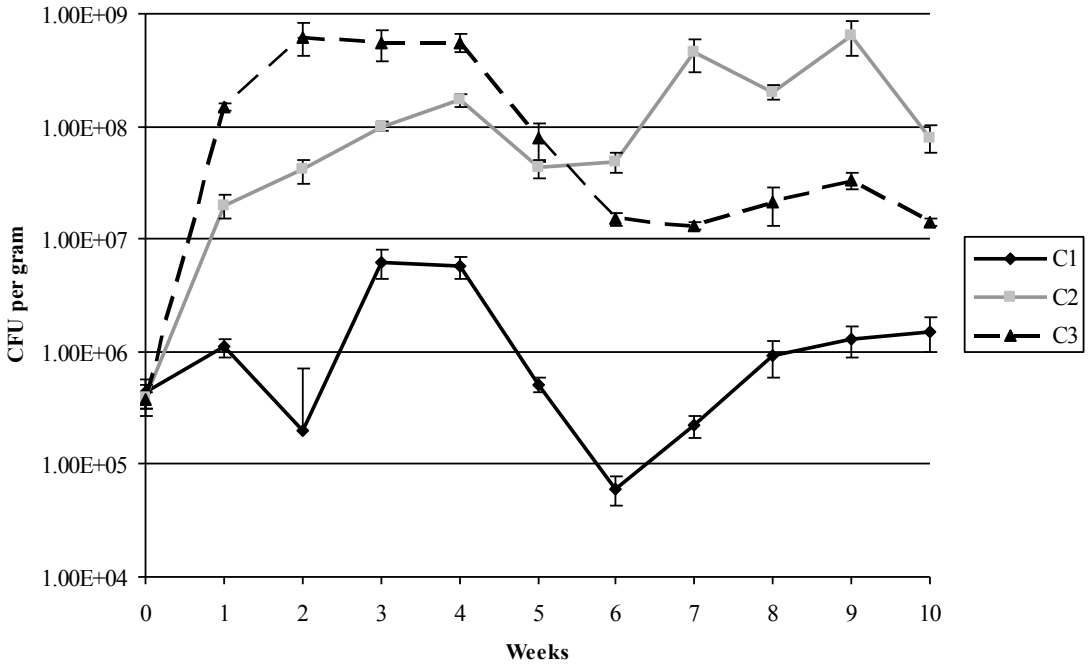


**Figure 13: Numbers of hydrocarbon oxidising bacteria (HOX) in land farming cells**

The experiments were conducted during the summer in the Perm region where the mean summer air temperature is around 22$^{o}$C (Kuyukina *et al*., 2005). The reduction in numbers observed at week 6 may have been related to changes in ambient temperature although no recording took place. The same pattern was also observed in the total heterotrophic counts although C2 did not appear to exhibit this behaviour.

After 8 weeks of treatment in the slurry bioreactor, the aqueous phase TPRH was reported to have reduced by 99% from 4.9 g $l^{-1}$ down to 0.6 g $l^{-1}$. This did not represent the total mass of hydrocarbon as there was significant residual hydrocarbon remaining attached to surfaces of the bioreactor and to the solid fraction of the oily waste. Biosurfactant in the reactor would have been thought to alleviate this

'hydrocarbon creep' as has been observed in lab studies where oil tended not to stick to glass vessels when surfactant was used (Churchill *et al*., 1995).

Contaminant removal in slurry systems has been reported to depend primarily on degradation activity of the microbial population (Robles-González *et al*., 2008). Introduction of the bio-fertiliser to the slurry-bioreactor had previously been observed to increase the total heterotrophic microbial population from 7.9 x $10^5$ CFU $ml^{-1}$ to 1.5 x $10^7$ CFU $ml^{-1}$ which represented a nearly 20 fold increase. The HOX population were most greatly increased from a baseline of 5.1 x $10^4$ CFU $ml^{-1}$ by more than 1800 times to 9.2 x $10^7$ CFU $ml^{-1}$ and persisted at between $10^7$ and $10^8$ CFU $ml^{-1}$ thereafter.

The remaining solid fraction from the slurry-phase bioreactor was mixed with the same topsoil as the other landfarming cells at a ratio of 1:1(S1) and a more dilute ratio of 1:4 (S2). This resulted in an initial concentration of 24 g $kg^{-1}$ and 9 g $kg^{-1}$ respectively. No further bio-fertiliser was added and cells were otherwise tilled and watered as for C1, C2 and C3.

Over 5 weeks of treatment the TRPH reduced by 86% to 3.4 g $kg^{-1}$ in S1 and by 89% to around 1 g $kg^{-1}$ in S2. Fractional analysis of hydrocarbons by TLC-FID was also carried out and these data showing the relative compositional change over 5 weeks are shown in Figure 14. It should be noted that degradation of other hydrocarbon fractions results in oxygenated intermediates, which can accumulate and contribute to the asphaltene fraction (Dibble & Bartha, 1979).

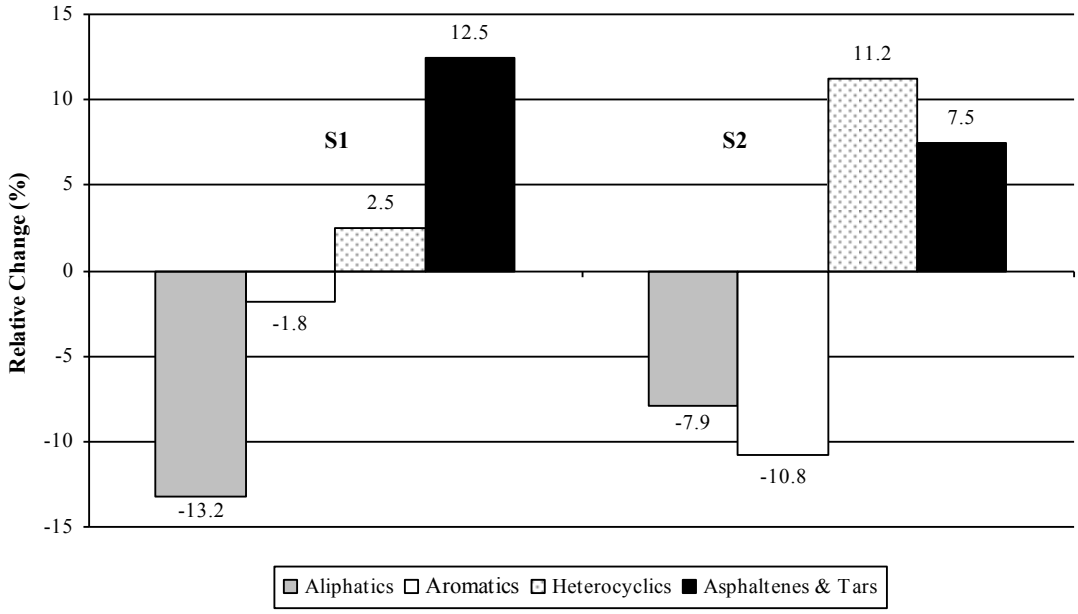


**Figure 14: Relative percentage change in fractional composition of residual hydrocarbons in land farming cells over 5 weeks following pre-treatment in the slurry bioreactor**

The treatment train cells S1 and S2 which received the solid fraction from the slurry-bioreactor were also sampled weekly and the total heterotrophic microbial population (Figure 15) and HOX (Figure 16) enumerated. Error bars are ± one standard deviation and these data all represent the mean of three replicates.

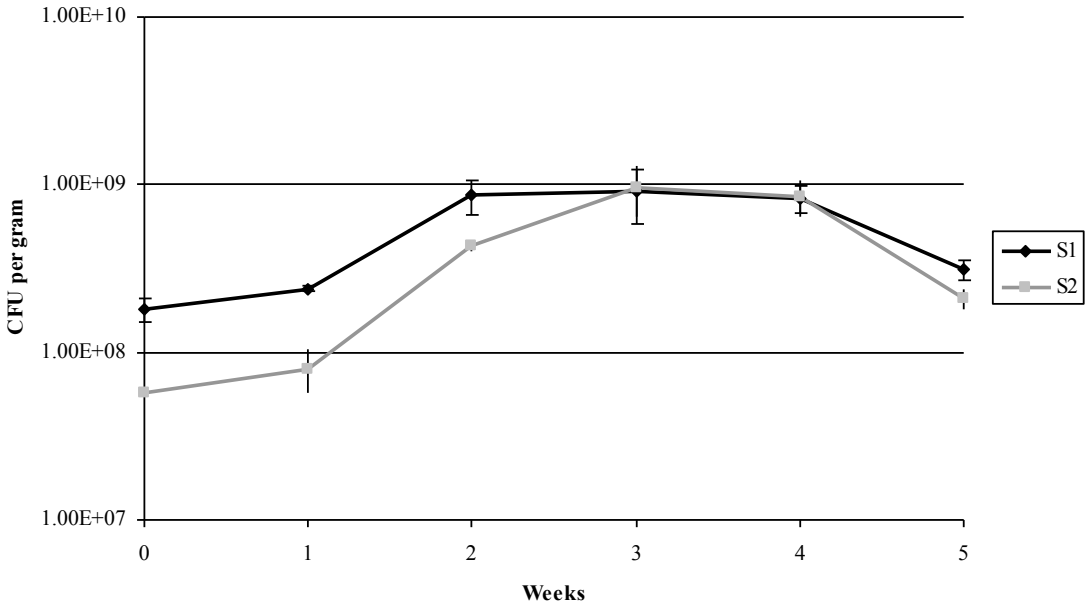


**Figure 15: Numbers of total heterotrophic microorganisms in land farming cells receiving solids from the slurry bioreactor**

Both of the treatment cells showed stimulation of the heterotrophic microbial population compared to the control treatment cell (C1) where numbers were between $10^6$ and $10^7$ CFU $g^{-1}$. Treatment S2 had a much higher dilution being made up from 80% topsoil to 20% partially treated solids from the slurry bioreactor. Higher levels of inoculation from the slurry reactor solids in S1 with only 50% dilution with topsoil probably explained the higher numbers in S1 for the first three weeks of treatment.

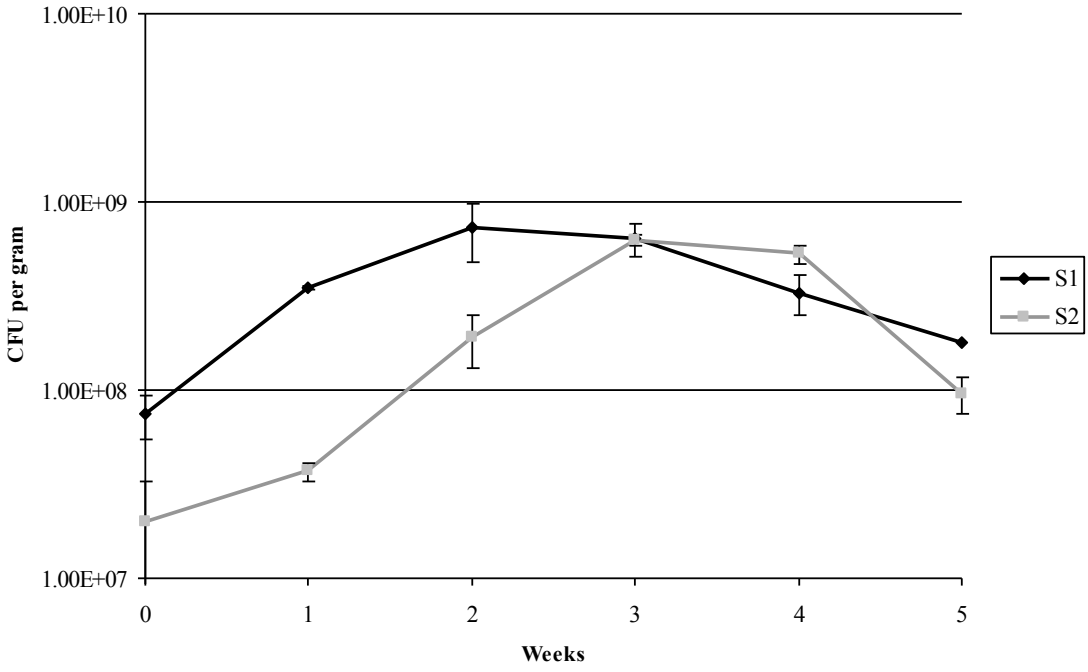


**Figure 16: Numbers of hydrocarbon oxidising bacteria (HOX) in land farming cells receiving solids from the slurry bioreactor**

Similar to the data for heterotrophs in Figure X above, treatment cell S2 had significantly lower numbers of HOX for the first three weeks of treatment. After three weeks the numbers showed a similar pattern and a decline in population. Note from Figure 4 in the paper that the majority of oil reduction was seen during the first three weeks and numbers may well have been related to declining substrate availability. The numbers of HOX were in the order of $10^7$ and $10^8$ CFU $g^{-1}$ throughout.

After 8 weeks, half of the surface of the landfarming cells C1, C2 and C3 was seeded with an equal mixture of *Trifolium pratense* (Red Clover), *Bromus exaristatus* (Brome) and *Phleum pratense* (Timothy). A clean topsoil control plot (K) of similar dimensions was similarly seeded. The Table 3 referred to in the paper was in fact not

present in the final publication and these data have been reproduced graphically in Figures 17 and 18.

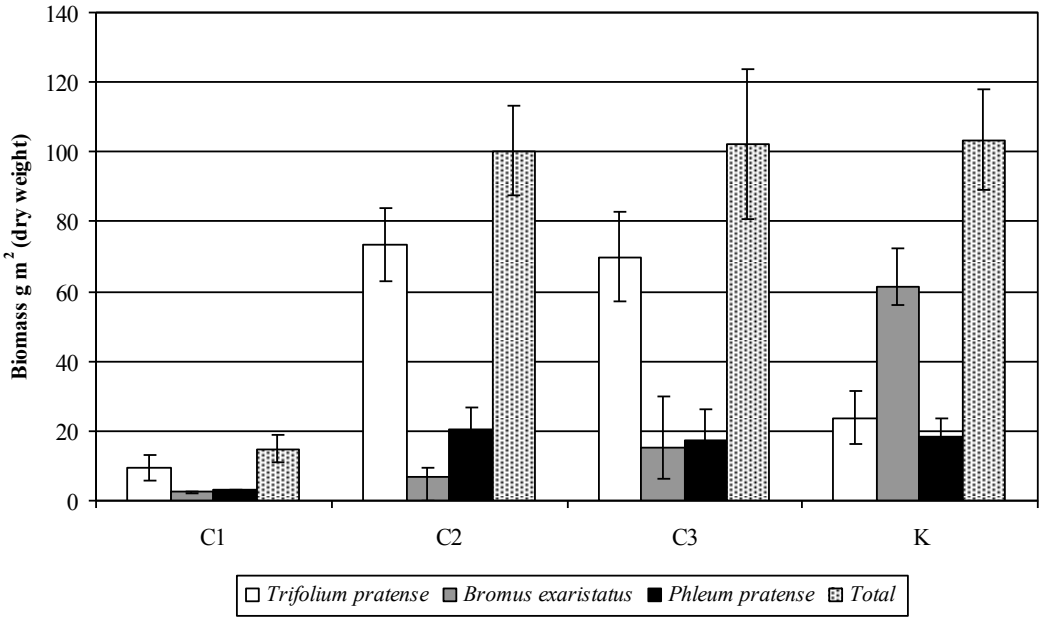


**Figure 17: Plant biomass (dry weight) in treated land farming cells**

Figure 17 above shows the mean plant biomass as grams per square meter of area ± one standard deviation for each of the plant species. The error bars on the mean total plant biomass are the square root of the sum of the squares of the individual standard errors for each species. After one month, the plant biomass data showed a clear inhibition to the growth of all species in the untreated contaminated cell (C1) where the total biomass was determined to be approximately 15 g $m^2$ with 18 g $kg^{-1}$ TRPH present at the time of sowing. This compared with 104 g $m^2$ in the clean topsoil. A similar total biomass was found in both treatment cells C2 (100 g $m^2$) and C3 (102 g $m^2$) where the TRPH at the time of seeding were 6 g $kg^{-1}$ and 1 g $kg^{-1}$ respectively.

The dominant species in the control soil was the perennial grass *B. exaristatus* representing nearly 60% of the total biomass. In all of the contaminated cells, *T. pratense* dominated with 64%, 73% and 68% in C1, C2 and C3 respectively. The third species *P. pratense* was approximately evenly represented in all systems including the control at between 17% and 20% of the total biomass.

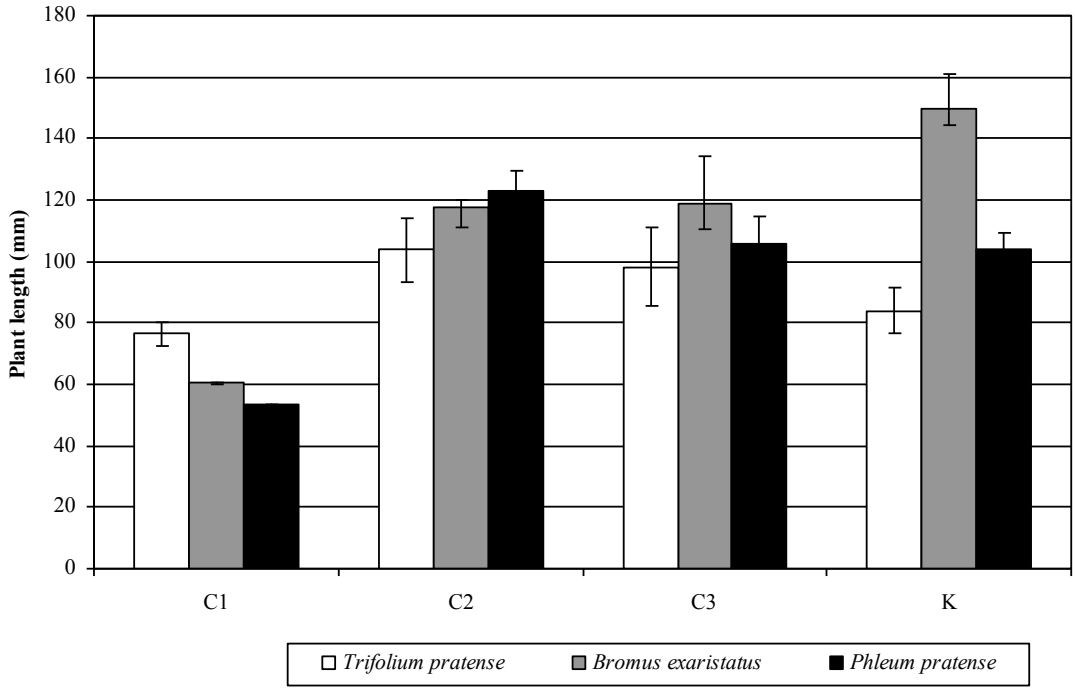


**Figure 18: Plant length (mm) in treated land farming cells**

The plant length data was the total of root and shoot length and these data showed far less relative variation than the biomass data. However, these data also showed the clear inhibition of all species in the untreated contaminated cell (C1). The mean length of all species was 63 mm, which was only 44% of the clean topsoil (K)

samples at 113 mm. The mean lengths found in treatment cells C2 and C3 were 115 mm and 108 mm respectively and were not significantly different from the control.

It was also concluded that *T. pratense* (Red Clover) was the most effective species in recovering soil fertility due to its ability to fix nitrogen. This may have explained the domination in the contaminated systems as the plant was not competing with microorganisms for soil nitrogen. Overall it was concluded from the study that the soil slurry-bioreactor had been successfully proven to be an effective means of pre-treating oily wastes prior to bioremediation using land-farming cells.

In contrast to the previous paper discussed, in Kuyukina *et al*. (2005) we reported on the potential use of biosurfactants to enhance the *in situ* remediation of crude oil contamination via mobilisation of hydrocarbons into the subsurface for biodegradation. The aim was to compare the efficiency of *Rhodococcus ruber* (strain IEGM 231) biosurfactant against the synthetic nonionic surfactant Tween 60 in mobilising crude oil from a local oilfield through laboratory soil columns. A model soil was prepared consisting of 50% sand, 30% clay and 20% peat and this was deliberately chosen to be relatively representative of soils in the Perm region which have high clay content. In the laboratory, triplicate columns of 57 cm length and 3 cm diameter were contaminated by an overall 10% (w/w) of crude oil from a local oil well.

The biosurfactant yield was more than 60% greater for *R. ruber* grown on *n*-hexadecane (9.9 g $l^{-1}$) than when *n*-dodecane was used as the sole carbon source (6.5

g $l^{-1}$). The emulsification ability was determined for both biosurfactants using 5 ml of *n*-hexadecane with an equal volume of distilled water and 0.2 ml of biosurfactant. Solutions were vigorously mixed using a vortex mixer and left to stand. In the paper, values were reported after 30 minutes, 1 hour and 24 hours. The latter is the most commonly used value to report the emulsifying ability and an emulsification index (E24) was calculated by dividing the measured height of the emulsion layer by the total height of the mixture and multiplying by 100. The E24 value was 1.8 times higher for the biosurfactant grown on *n*-hexadecane at around 45% compared with 25% for the one grown on *n*-dodecane.

Initial column studies examined the effect of temperature on the penetration of water and crude oil (Figure 1 in the paper). These data were described as non linear due to fluid accumulation around the walls of the columns during the first 30 minutes. In Figure 2 in the paper, it was shown that a biosurfactant solution was more mobile and completely penetrated the soil column more rapidly than the Tween 60 solution at all temperatures studied (15$^{o}$C, 22$^{o}$C and 28$^{o}$C). The most relevant temperature was taken to be 22$^{o}$C as this represented the mean summer temperature in the Perm region. At 22$^{o}$C case the biosurfactant solution penetrated completely in 75% of the time (4.5 hours) of that taken by the Tween 60 solution (6 hours).

Collaboration with Perm State University (Russia) led to the development of a simple model to simulate penetration of water and crude oil. The model simulations were shown (Figure 4 in the paper) from 30 minutes into the experiments due to the lack of linearity of the data seen during the early stages. This was caused by the

preferential accumulation of fluid on the sides of the columns. For the data collected after 30 minutes the simulation values were found to be a good fit with the experimental data.

The main results of Kuyukina *et al*. (2005) were the column data on the effectiveness of *Rhodococcus* biosurfactants versus Tween 60 to mobilise crude oil at different temperatures as shown in Figure 5 in the paper. Both were applied at twice the CMC as the main aim was to achieve maximum solubilisation of oil for subsequent degradation in the sub surface. Oil removal was determined to be temperature dependent in all the treatments with higher efficiencies observed in all treatments at 28$^{o}$C. The greatest removal was very successful with almost 82% of the oil removed at 28$^{o}$C by the biosurfactant produced using *n*-hexadecane as the carbon source. Comparative removal efficiencies were 59%, 46% and 34% for the *n*-dodecane produced biosurfactant, Tween 60 and distilled water respectively.

Removal efficiencies were less when the temperature was reduced by only 6$^{o}$C to 22$^{o}$C. At this temperature the greatest removal of 65% was by the biosurfactant produced using *n*-hexadecane as the carbon source. Comparative removal efficiencies at 22$^{o}$C were 53%, 28% and 10% for the *n*-dodecane produced biosurfactant, Tween 60 and distilled water respectively. At 15 $^{o}$C, the removal efficiencies were 43%, 27% and 5% for the *n*-dodecane produced biosurfactant, Tween 60 and distilled water respectively.

Biosurfactant grown on *n*-hexadecane was not able to be used at 15 $^{o}$C as it would not flow at this temperature which was thought to be due to the presence of residual *n*-hexadecane as the temperature was below its melting point. It was therefore concluded that the biosurfactant produced using *n*-hexadecane as the carbon source was the most effective at the temperature (22$^{o}$C) thought to be the most relevant to the Perm region. The composition of the crude oil and the fractions washed from the soil columns as determined by TLC-FID was shown in Figure 6 of the paper and the relative percentage changes from these data have been represented in Figure 19 below.

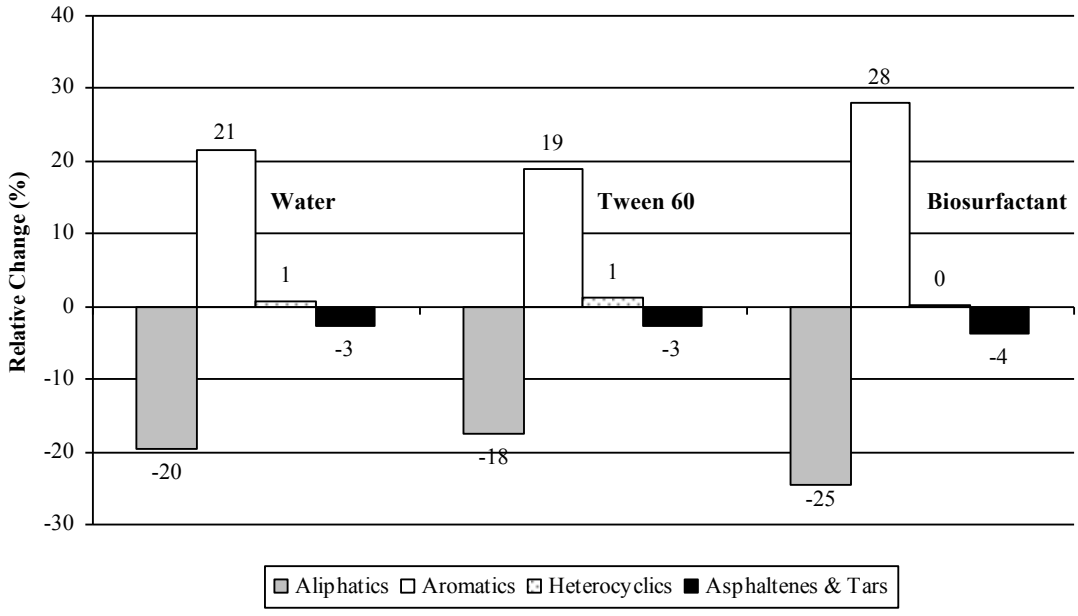


**Figure 19: Relative percentage change in fractional composition of residual hydrocarbons from soil column washing**

The most notable difference between treatments was that the biosurfactant resulted in a relatively high percentage of aromatics in the recovered oil. The aromatic fraction increased by 28% to 39% representing an increase of 3.6 times from a baseline of

11% in the original crude oil. The distilled water and Tween 60 also had higher approximately 20% more at 32% and 30% respectively. It was noted that the asphaltene fraction recovered by the biosurfactant was less than half that of that observed for the water and Tween 60 columns at only 1%.

It was concluded that the *Rhodococcus* biosurfactants mobilised those components of crude oil from soil that would be relatively more amenable to biodegradation than those resulting from the application of Tween 60. This coupled with more effective overall removal rates provided evidence that these surfactants have potential applications for *in situ* remediation.

### 3.3 Critique and contribution

In Kuyukina *et al*. (2003) it was demonstrated that *ex situ* bioremediation was an effective strategy for the rapid treatment of crude oil wastes. The control cell C1 where oily wastes were diluted 1:3 with clean topsoil then tilled and watered weekly without any further amendments provided a baseline for a minimal intervention of a 66% reduction to 16 g $kg^{-1}$ TRPH. What was not commented on in the paper was that after 10 weeks of treatment this represented a substantial reduction using only dilution with topsoil the rate of reduction and suggest that removal of TRPH may have reached a similar endpoint to the most successful treatment regime of around 1 g $kg^{-1}$ TRPH after around 23 weeks. This would be considered a positive outcome and indeed several studies have reported removal rates resulting from active interventions that were less than was observed.

However, one of the aims of the study not stated in the paper was to complete bioremediation during the relatively short warm period of around 17 weeks, typical of the West Urals region of Russia where the oily wastes were generated (Ivshina *et al*., 2001). The comparable treatment cell (C2), which started at the same level of TRPH, showed a reduction of 87% down to 6 g $kg^{-1}$ TRPH. Again, crude extrapolation of the data may suggest that a similar end point could have been achieved after around 16 weeks.

In the bioremediation treatment cell with the higher dilution rate of 10:1 clean soil to oily waste the TRPH reduction levelled off after only 6 weeks to 1 g $kg^{-1}$ TRPH and remained at this level after 10 weeks. At the time of publication this represented an acceptable end point as no risk-based criteria were applied in the region. The soil was deemed fit for purpose for 'general economic purposes', which may be equated to what in the UK was termed commercial/industrial land use.

The slurry-phase bioreactor was not used as a standalone treatment compared with the landfarming cells but as a pre-treatment. It was a fairly crude system with no trapping of volatile organic compounds or separation of free phase hydrocarbons. With the benefit of hindsight, the slurry bioreactor should have been more carefully designed to allow greater process control such as mixing intensity, which is known to be a critical factor in performance (Robles-González *et al*., 2008).

Degradation of the aromatic fraction in the slurry-reactor was commented upon stating that:

> *"A notable characteristic of the bioreactor was a high degradation rate of aromatic hydrocarbons not readily degradable under normal soil conditions"*

What was observed was a 9% reduction in the relative percentage of the aromatic fraction over 8 weeks. The statement could be criticised as being not entirely accurate as the control cell (C1) with no amendments produced a relative reduction in the aromatic fraction of 7.5% over 10 weeks. In the final paragraph on page 90 it had been erroneously stated that fractions in C1 had not significantly changed. The point that was attempted to be made was that the asphaltene and tar fraction had not changed significantly (1%). The change aromatic composition in C1 was actually greater than the cell with similar initial TRPH (C2) where the result was 4.9%. The greatest shift in composition was seen in C3 with the aromatic fraction being proportionally 13.8% less of the total TPRH after 10 weeks.

The deliberate contamination of clean topsoil used to dilute the oily wastes and treated solids from the slurry-bioreactor would have been questionable in the UK context from a legislative perspective. Taking the two 900 $m^3$ pits at the trial site as an example and assuming that they were only 75% full yields 1350 $m^3$ of oily waste to be treated. Using the lower 1:3 level of dilution in treatment C2 would require 4050 $m^3$ of clean topsoil and the 1:10 dilution (C3) would require 13500 $m^3$ of clean topsoil. Another consideration would be the land area required to undertake landfarming at 20 cm depth, which would be approximately 27000 $m^2$ (2.7 ha) and 73000 $m^2$ (7.3 ha) for the 1:3 and 1:10 dilutions respectively.

It was stated in the paper that the addition of the bio-fertiliser:

> *"……resulted in a 100- to 1000-fold increase in the number of hydrocarbon oxidizing bacteria in cells C2 and C3 compated [sic] with a 1-fold increase in the control cell (C1)"*

This was not an entirely accurate description of the data as the increase in HOX ranged from a just over 50 fold increase after 1 week to a greater than 1600 fold increase at week 9 in treatment cell C2. The increase in C3 ranged from 35 fold after 7 weeks to a greater than 1600 fold increase after week 2. The 1 fold increase in the control (C1) treatment cell was also not accurately described as a mean 4 fold increase was observed over the period of the trial.

The microbial counts and the hydrocarbon oxidising bacteria (HOX) were presented as evidence for the efficacy of bioaugmentation. One of the limitations of the study was that no molecular techniques were employed to provide direct evidence that the HOX counts represented persistence of the two strains of *Rhodococcus* introduced in the bio-fertiliser complex. One approach would have involved the use of a quantitative competitive polymerase chain reaction (PCR) method as described by Schwartz *et al*. (2000). Nevertheless, removal of TPRH was positively correlated with higher numbers of HOX. It has been proposed that an increase in HOX is sufficient to provide evidence of survival of an introduced consortium (Mittal & Singh, 2010).

The significant decrease in TRPH observed in all of the land farming cells after one week was stated to be:

*"....due mostly to physiochemical processes, for example, volatilization and photooxidation of petroleum hydrocarbons"*

However there was no elaboration on this and in the absence of fractional analysis of the hydrocarbons after one week, there was no direct evidence presented. No comparison was drawn with the early HOX numbers and initial hydrocarbon reduction. Treatment cells C1 and C2 with the higher initial TRPH concentration (46 g $kg^{-1}$) had HOX counts of 1.1 x $10^6$ CFU $g^{-1}$ (3 fold increase) and 2.0 x $10^7$ CFU $g^{-1}$ (51 fold increase) at week 1 corresponding with a 33% and 48% TPRH reduction respectively. A higher reduction of TRPH was therefore associated with higher numbers of HOX.

In treatment cell C3 which had a much lower initial TRPH concentration of 16 g $kg^{-1}$, this was reduced by 27% to 10 g $kg^{-1}$ with corresponding HOX numbers at week 1 of 1.5 x $10^8$ CFU $g^{-1}$ (>400 fold increase). One possible explanation is that despite the application of both biosurfactant and biosurfactant producing strains, partitioning of the hydrocarbons in the clean topsoil limited their removal. It would also be the case that there was proportionally less hydrocarbon contamination at the top layer of the cell than in the more concentrated systems. It may be argued therefore that abiotic losses would be expected to be greater in C1 and C2.

The assertion that the highest rates of biodegradation were achieved following preliminary stimulation in the slurry bioreactor can be justified but was an incomplete conclusion. The conclusion was only valid if the argument is accepted

that the rapid initial reduction observed in the control (C1) and treatment (C2) was mostly due to physiochemical processes rather than biodegradation.

In treatment cell S1 (1:1 dilution of pre-treated solids with topsoil), the reduction in TRPH was from 24 g $kg^{-1}$ to 7 g $kg^{-1}$ (70%) in 3 weeks representing removal of around 0.8 g $kg^{-1}$ $day^{-1}$. Excluding the first week of treatment, the next most rapid rate of removal was nearly 40% lower and was observed in cell C2 (1:3 dilution of oily sludge with topsoil) from 24 g $kg^{-1}$ to 10 g $kg^{-1}$ between weeks 1 and 4 representing 0.5 g $kg^{-1}$ $day^{-1}$. By comparison, the initial reduction during the first week in C2 was 3.1 g $kg^{-1}$ $day^{-1}$.

One of the most comparable studies was that reported by De-qing *et al*. (2007). In this large field study, 960 $m^3$ of oily sludge was treated by landfarming and bioaugmentation was compared with biostimulation. The study used a patented preparation 'Rhoder' (Russian Fedaration No. 2090697) which consisted of *Rhodococcus ruber* and *Rhodococcus erythropolis* (Ouyang *et al*., 2005). Note that these were the same species used in our study. De-qing *et al*. (2007) also used the same methodology to determine total hydrocarbons as Kuyukina *et al*., (2003) so a useful comparison may be drawn. A summary of the total oil content results are given in Table 5.

**Table 5: Reduction in total hydrocarbons from De-qing *et al.* (2007)**

| Treatment | Total hydrocarbon content (g $kg^{-1}$) | | Reduction |
|---|---|---|---|
| | Initial | After 160 days | |
| A | 101 | 48 | 53% |
| B | 101 | 61 | 40% |
| C | 101 | 81 | 20% |
| D | 130 | 110 | 15% |

Treatment B was an area of 15 x 80 m that was 0.7 m deep and received approximately 1kg $tonne^{-1}$ soil of the 'Rhoder' preparation and a combination of urea and potassium dihydrogen phosphate to bring the C:N:P ratio to 100:10:1. Treatment A was a 15 x 6 m sub-set of this area covered by a greenhouse. Treatment C received the same nutrients but no bioaugmentation and treatment D was the untreated control.

**Table 6: Reduction in total hydrocarbons from Kuyukina *et al*. (2003)**

| Treatment | Total hydrocarbon content (g $kg^{-1}$) | | Reduction |
|---|---|---|---|
| | Initial | After 70 days | |
| C1 | 46 | 16 | 66% |
| C2 | 46 | 6 | 87% |
| C3 | 16 | 1 | 94% |

Although the starting concentrations were at least 50% lower in our study, a more rapid degradation of the crude oil was evident. The 'Rhoder' culture was applied only once at the start of the study and although the *Rhodococcus* strains are known

biosurfactant producers, no additional biosurfactant was added. It should also be noted that our study was conducted during the summer months in Perm where the mean summer air temperature is around 22$^{o}$C (Kuyukina *et al*., 2005). The mean air temperature near the start of the De-qing *et al*. (2007) study was 5.9$^{o}$C and near the end had only risen to 6.4$^{o}$C. The greatest reduction was found in the soils covered by a greenhouse where the temperatures were reported to be approximately 10$^{o}$C above ambient. Another significant point to note from these data in Tables 3 and 4 is that in our study, the reduction in the control cell (C1) was only 28% less than the most successful treatment (C3) and in De-qing *et al*. (2007) it was 38% less.

Our study would have been significantly improved by the inclusion of corresponding treatments that received nutrient addition and the biosurfactant complex without bioaugmentation as carried out by De-qing *et al*. (2007). Aside from the influence of temperature, in their study the bioaugmentation could be seen to double the removal of hydrocarbons from 20% using biostimulation alone to 40% where the 'Rhoder' preparation was applied.

A point not elaborated upon in our paper was the use of the residual water from the slurry-bioreactor to maintain moisture at around 20% in the S1 and S2 treatment cells. At the time the solids were removed, this was reported to have a residual TPRH of 0.6 g $l^{-1}$. Therefore, these cells received a small additional input of hydrocarbon but also of bio-fertiliser. Another point that received little consideration was the question of toxicity from heavy metals and these were not determined in the oily waste or the topsoil as it was assumed that dilution with topsoil would be sufficient

to alleviate any potential inhibition of hydrocarbon degrading microorganisms (Bleckmann *et al*., 1994).

Although seeding with the mix of perennial grasses was described as phytoremediation, it was more useful in the study as a field based plant ecotoxicity assay. It was not unreasonable to suggest that seeding with plants would result in a beneficial effect on the soil health and could have resulted in further oil degradation. For example, Ouyang *et al*., (2005) transplanted lawns of *Festuca arundinacae* (Tall Fescue) onto oil contaminated soils that had undergone 56 days of bioremediation and observed an additional 5-6% decrease in hydrocarbon content.

Seeding with *Trifolium pratense (*Red Clover) has been reported to result in a reduction in petroleum contamination in soils (e.g. McCutcheon & Schnoor, 2003). *T. pratense* was not accurately described in Kuyukina *et al*., (2003) as it is not a member of the grass family Poaceae but belongs to the family Fabaceae (Legumes). In one greenhouse study where agricultural soil was contaminated with 15 g $kg^{-1}$ used motor oil, seeding with *T. pratense* without fertiliser addition produced the greatest reduction in hydrocarbon contamination (as oil and grease) of 42% after 50 days (Dominguez-Rosado & Pichtel, 2004).

In Kuyukina *et al*. (2005), enhancing *in situ* remediation of crude oil contaminated land was considered. This shorter laboratory study again made use of *Rhodococcus* biosurfactants. This paper added to the knowledge of the potential efficacy of biosurfactants in the removal of crude oil from soils and has been cited more than 30

times. The stated aim was to consider application in terms of *in situ* remediation i.e. flushing of contamination through the subsurface for subsequent microbial degradation.

Kuyukina *et al*. (2005), was generally better presented with fewer errors and needed less re-interpretation than the previous paper discussed in this section. The maximum biosurfactant yield was reported to be greatest at 9.9 g $l^{-1}$ when *R. ruber* was grown on *n*-hexadecane. However, no comparison made with other studies as to whether this represented a comparatively high yield or otherwise. For example, Zheng *et al*. (2009) recently reported a significantly greater yield of 13.3 g $l^{-1}$ for a *R. ruber* strain isolated from oil production water in Daqing Oilfield, China. However, *n*-hexadecane was applied at 5% (v/v) compared to 3% (v/v) in our study although cultivation was for 44 hours versus 72 hours.

Results of the emulsion index determination were also presented without comparison to other values reported in the literature. Colombo Fleck *et al*. (2000) reported that a biosurfactant producing strain of *R. ruber* strain gave very high emulsification of diesel with an E24 value of 58%. They compared this with a similar study by Pruthi & Cameotra (1995) where biosurfactant from *Pseudomonas aeruginosa* produced an E24 value of only 30%. In our study, the E24 value was 1.8 times higher for the biosurfactant grown on *n*-hexadecane at around 45% compared with around 25% when the *R. ruber* strain was grown on *n*-dodecane. A similar result was found by Zheng *et al*. (2009) who reported that biosurfactant from a strain of *R. ruber*

produced the highest emulsification when grown on *n*-hexadecane but a higher value for the E24 of around 60%.

The application of biosurfactants at twice the CMC was chosen as the main mechanism we tried to employ was solubilisation of the hydrocarbons in the crude oil. However, Urum *et al*. (2004) in a similar crude oil washing experiment concluded that removal was attributed to mobilisation due to reduced interfacial tension. In their experiments, a 15% aqueous solution of commercial rhamnolipid (Jeneil Biosurfactants Company) did not exhibit micelle solubilisation.

It was commented that the rate of penetration of crude oil through the soil columns at 28$^{o}$C was double that observed at 15$^{o}$C (Figure 1 in Kuyukina *et al*. (2005)). However, this was not accurate as one hundred percent penetration was reached after 4.5 hours and 6 hours respectively making the former 1.3 times more rapid rather than twice as stated. Figure 2 in Kuyukina *et al*. (2005) showed the effect of temperature on Tween 60 and *Rhodococcus* biosurfactant penetration through oil contaminated soil columns. Biosurfactant penetrated more rapidly as there was less sorption to the soil matrix than Tween 60.

Biosurfactants were demonstrated to be much more effective at mobilising crude oil from the soil columns than Tween 60 at all temperatures studied (15$^{o}$C, 22$^{o}$C and 28$^{o}$C) with one notable exception. The biosurfactant produced using *n*-hexadecane as carbon source was described in the paper as being:

*"...not effective in cold conditions as it froze at temperatures below 16°C"*

Although the meaning was conveyed by the use of the term 'froze' this was of course not an accurate means to describe what was observed. The behaviour was attributed to the presence of residual *n*-hexadecane (which has a melting point of 16-18°C) in the biosurfactant solution.

In a similar study, Scheibenbogen *et al*. (1994) applied a model aliphatic and aromatic hydrocarbon mixture and found that *P. aeruginosa* rhamnolipid biosurfactant removed 59% of total hydrocarbons from columns. However, they added 0.1% (w/v) sodium pyrophosphate to enhance micelle formation and only achieved a maximum removal of 36% without supplementation. In our paper, the ability of biosurfactants to mobilise and remove crude oil was stated as being between 1.9 and 2.3 times greater than Tween 60. In fact the lowest difference was seen at 28°C where the *n*-dodecane produced biosurfactant was only 1.3 times more effective removing a mean of 59% versus 46% for the Tween 60 treatment.

The compositional TLC-FID data showed that the fractions in the recovered oil using Tween 60 and distilled water were the most similar. In the paper it was perhaps misleading to only state that the Tween 60 and biosurfactant washed oil fractions were similar. In all treatments the proportion of aliphatics had decreased significantly from 83% to between 58% and 65%. A difference observed using the biosurfactant was that the relative increase in the aromatic fraction was greater at 39% compared to 32% using water and 30% using Tween 60. This coupled with the low recovery of

the asphaltene fraction, which represented only 1% of the total hydrocarbons, suggested that biosurfactant washing liberated oil with favourable characteristics for subsequent biodegradation in the subsurface.

Whilst the results were described as positive and enhanced mobilisation of crude oil was viewed as a success, there was no mention of potential negative impacts to the environment. One concern of deliberately mobilising hydrocarbons in the subsurface is the potential for horizontal or vertical migration of contaminants beyond the desired treatment or recovery zone (Mulligan *et al*., 2001). Another aspect not considered was the potential for enhanced mobilisation of metals into the subsurface. Microbial rhamnolipid surfactants have also been reported to have applicability in the removal of heavy metals from soil (Mulligan 2005). The potential of *Rhodococcus* biosurfactants appears not have received attention from researchers (Kuyukina & Ivshina 2010b). Aquifer heterogeneity, diverging and converging groundwater flows and seasonal fluctuations in the water table add to the complexity of predicting contaminant transport in the subsurface.

Overall it was concluded that the *Rhodococcus* biosurfactants have potential application in the *in situ* remediation of oil contaminated sub soils and ground waters. However, there was no elaboration on the method of application. The use of biosurfactants to remove hydrocarbons from soil could also have been described as being applicable to *ex situ* washing as has been reported in the literature e.g. Deshpande *et al*., (1999).

### 3.3 Overall assessment and impact

The field study undertaken at the Kokuyskoye oil field reported in Kuyukina *et al*. 2003 was undoubtedly limited by budgetary constraints and would have benefitted from more careful experimental design. The resulting paper also contained many errors and the data could have been presented significantly more clearly. The majority of the previous section has been taken up with this paper as there were many points where an improvement in both presentation and interpretation of the results could have been made. Nevertheless, the study was a successful demonstration of a rapid bioremediation process for crude oil wastes and achieved some of the most rapid results reported in the literature. Despite all of the limitations, the paper contributed to the knowledge in the field and has been cited 18 times.

Perhaps the biggest limitation of Kuyukina *et al*. (2005) was that all of the column experiments on crude oil mobilisation were carried out almost immediately after contamination of the model soil by crude oil. Pacheco *et al*. (2010) recently reported that biosurfactant from a strain of *R. erythropolis* showed a significant reduction in crude oil removal efficiency in aged contaminated sandy sediments which went from nearly 100% down to only 18% after two months. Nevertheless, the paper contributed to the knowledge in the field and has been cited 31 times. The modelling work was continued with an improved qualitative model developed which was reported on in Kuyukina *et al*. (2007).

One of the impacts of the papers discussed in this section was that they led to the author being awarded a NATO Collaborative Linkage Grant with colleagues at the IEGM. There was recognition of a need to make more compound or class specific assessments of hydrocarbon contaminants in site investigations as well as during and after bioremediation projects. However, as was previously noted, access to laboratory analytical equipment such as GC-FID or GC-MS was often limited in Russia due to financial constraints. The project examined the use of spectrophotometric methods as a means of assessing total PAH concentrations in soil building on the work of Touraud *et al*. (1998) and Cloarec *et al*. (2002) and the results published in Ivshina *et al*. (2007).

As a result of papers discussed in this section, the author was the only researcher in Europe invited by the Chevron Energy Technology Company to submit a response to a call for proposals in 2010. The call text revealed that the company viewed their largest environmental liability as being weathered crude oil and PAH impacted vadose zone soils. They identified remediation of heavy hydrocarbons/PAHs as the most significant research driver (Schaun M Smith, personal communication, 15th January, 2010). A proposal was prepared and colleagues from IEGM in Russia were partners along with the University of Aberdeen. The overall hypothesis in the proposal was that a combination of intensive (slurry reactor) and passive *ex situ* bioremediation would cost effectively treat weathered hydrocarbons, meeting risk based cleanup goals and producing 'fit for purpose' materials for reuse at contaminated sites.

Although the proposal was shortlisted and then highly ranked it was not successful. However, there was significance to being invited. In McMillen *et al*. (2004), employees of Chevron Texaco published a review of lessons learned from bioremediation of exploration and production wastes. The first lesson was that "special bug products are not needed". They proposed that most soils contain a sufficient population of hydrocarbon degrading microorganisms and that the cost of bioaugmentation, and oleophilic fertilisers rarely justify the increased cost over 'standard' fertilisers such as urea for bioremediation of crude oil contamination. The invitation to submit a proposal was based on the papers discussed in this thesis that advocated bioaugmentation and the use of oleophilic fertilisers. Specifically, the work on bioremediation of crude oil and crude oil wastes had attracted the attention of a major oil company.

## 4. Discussion and conclusions

All of the studies presented were concerned with industrial land contamination by petroleum hydrocarbons as this is well established as a widespread and global environmental pollution issue. The assessment and treatment of contaminated rail ballast was first considered. Disposal of contaminated ballast to landfill is costly and significant environmental and economic benefits may be realised if a sustainable alternative to landfilling of contaminated ballast was employed.

In Anderson *et al*. (2000) a methodology was developed for the determination of total hydrocarbon contamination on ballast. This was the first time that an assessment

of different extraction methodologies for hydrocarbon-contaminated rail ballast had been published. The simplified methodology proposed was significantly quicker than soxhlet extraction and importantly used 25% less solvent. A further benefit was that it avoided the use of the chlorinated solvent dichloromethane which had often been the default solvent of choice at the time. Further applications were proposed for the method including assessment of contamination on other aggregate materials, for example shingle beaches impacted by oil spills. However, a gravimetric method of determination is relatively unsophisticated and provides minimal information of the nature of the contamination.

In two further studies reported in Anderson *et al*. (2002, 2003), we investigated solvent and surfactant cleaning of ballast and examined the potential environmental impacts of the processes. Despite the limitations and omissions previously discussed these provided useful insights into ballast cleaning options and initiated several further studies (unpublished data). The papers continue to attract interest from industry with a summary article having been published recently (Mackillican, 2009).

Consideration of the fate of the wastes generated by the ballast cleaning processes described was poorly considered and this issue was worthy of further discussion here. In Anderson *et al*. (2002) it was determined that the pilot scale systems, which would have more accurately been described as bench scale, underestimated the effect of attrition likely to be encountered in a field scale treatment plant. In Anderson *et al*. (2003) this was simulated by incorporating Astroturf® to provide a greater scrubbing effect and the concentration of BioSolve® was reduced significantly. This would

reduce the BOD of the effluent produced, at 6% this was determined to be nearly 5000 mg $l^{-1}$.

Co-location of a ballast cleaning operation at a site where *ex situ* bioremediation of soils was taking place would offer the possibility of amending contaminated soil with residual surfactant and wastewater in an integrated treatment facility. The residual BioSolve® surfactant from ballast cleaning may have a beneficial effect on an *ex situ* bioremediation. Several studies have reported on the use of Biosolve® to enhance bioremediation of hydrocarbon-contaminated soils (e.g. Becker, 2002; Sanscartier *et al*., 2009). However, even with recycling of the residual BioSolve® back into the process, the volumes of effluent generated may still be impractical at sites other than large bioremediation facilities. In addition, the treated soils would need to meet relevant environmental or human health criteria for their intended end use and the vexed question of whether the soil produced would be considered a waste could impede reuse.

In the UK, the Environment Agency has been leading developments in this area which, are almost equally dependent on the EU Waste Framework Directive (2006/12/EC) and on European case law. A recent development has been the introduction of a recovery permit which alongside site investigation and remedial performance data may offer the clearest route to treated soils to cease being considered as waste (Environment Agency, 2010). Interestingly, railway ballast is considered in the same document as a material potentially suitable for recovery to land as a fill material.

The comment accompanying the entry for ballast stated that it must be "free from significant oil contamination" in order to cease to be waste. There was no elaboration as to the definition of what significant means in this context. A voluntary code of practice has been introduced in the UK for the remediation industry (CL:AIRE, 2008). The Environment Agency supports that by following the code of practice, developers can make the decision that materials arising on site need not be considered waste if they are to be reused on the same site.

Hydrocarbon contamination of railway land is common and a major source is from the migration of diesel contamination from the track. Bioremediation is one of the approaches that may offer the most sustainable and cost-effective treatment for hydrocarbon-contaminated soils. In Cunningham & Philp (2000) the focus of the study was to assess the efficacy of bioaugmentation for *ex situ* bioremediation of diesel-contaminated soil. At the time the paper was published, bioaugmentation had been much debated in the literature for many years (e.g. Morgan & Watkinson, 1989; Atlas, 1991; Pritchard, 1992; Vogel, 1996). Some authors, e.g. Koronelli (1996) gave a specific reason to justify bioaugmentation, in his case that in Russia, inoculation with active hydrocarbon degrading bacteria had been found to be important due to the cold climate.

One of the justifications for bioaugmentation given in the literature was that in some cases oil pollution incidents were of such magnitude as to cause sterilisation of the soil (Nwachukwu, 2001). The author went on to say that that this explains why some

oil-impacted land remains compacted and unproductive for years. This author suspected that incidences of this nature were extremely rare and would only occur when site specific conditions conspired to saturate the ground in an ecosystem that was already non productive. However, the topic of the study reported by Nwachukwu (2001) was inoculation of sterilised agricultural soils and it is difficult to imagine anything other than a rare and catastrophic oil spill resulting in effective sterilisation of agricultural land.

At the time of writing a similar debate may be found in the literature (e.g. Silva *et al.*, 2010) and the contaminated land industry has seen a number of proprietary cultures offered for bioremediation of hydrocarbon contamination (Mohammed *et al*., 2007). In one recent example, Tyagi *et al*. (2010) stated that:

*"There is a mixed debate on which of the two techniques, bioaugmentation or biostimulation, is a better strategy for bioremediation"*

Unfortunately, the authors do little to progress the debate as in their concluding remarks they simply note that bioaugmentation and biostimulation can be used as complementary techniques. In most cases it is likely that biostimulation will be required as inorganic nutrients will limit biodegradation so the question will be to augment or not. Fantroussi & Agathos (2005) proposed that bioaugmentation was best suited to confined systems like slurry bioreactors as conditions may be more readily optimised to suit the augmented population. However, it is worth noting that they considered the introduction of an exogenous population and not the re-

application of greater numbers of hydrocarbon degraders isolated from the site in question.

It is not entirely clear what made the bioaugmented treatment reported in Cunningham & Philp (2000) so rapid. The bioaugmentation culture was derived from a composite soil sample taken from different locations on the site. An extraction in 0.85% (w/v) saline supplemented with 0.2% (w/v) tetra-sodium pyrophosphate was carried out to descorb microorganisms from the soil that was further enhanced by sonication for 1 minute. Thereafter, duplicate aliquots inoculated the first of three enrichment steps in a mineral medium, each lasting one week with artificially weathered diesel as the sole carbon source, with a final 10 day incubation of the batch culture in a larger vessel. The indigenous microbial population from the site were clearly competent degraders of the hydrocarbon contamination as evidenced by the success of biostimulation in non-augmented treatments.

In many studies, the enrichment procedure began with the direct seeding of a mineral medium with 1-5 g of contaminated soil and the target contaminant provided as the sole additional carbon source (e.g. Capelli *et al*., 2001; Bento *et al*., 2005; Genovese *et al*., 2008**;** Wolicka *et al*., 2009). Authors also reported shorter enrichments, e.g. and some plated cultures with aliquots of shake flask media, select the most prolific colonies and begin another enrichment cycle in a shake flask culture (e.g. Wolicka *et al*., 2009). Whereas Bento *et al*. (2005) centrifuged a suspension of waste and mineral media and used the supernatant as the source of microorganisms for their study; in this study we discarded the supernatant and used the pellet.

Perhaps these differences and an overall more exhaustive approach to developing the culture for inoculation accounted for the success of bioaugmentation observed in Cunningham & Philp (2000). Devinny & Chang (2000) made the distinction between seed and mass inoculation. In the former the aim is to provide competence that may be lacking in the indigenous microbial population and in the latter to shift the community structure in favour of target contaminant degraders. The approach taken in all of the studies presented was the latter and this was considered more appropriate for petroleum hydrocarbon industrial land. Interestingly, in one of the most rapid field bioremediation studies on diesel that reported a removal rate of 2,780 mg $kg^{-1}$ $day^{-1}$, the bioaugmentation of a mass culture was achieved by a novel approach. The liquid culture was first used to inoculate a smaller volume (200 kg) of soil and this was then added to the larger 2 $m^3$ field system (Márquez-Rocha1 *et al*., 2001).

An alternative was the approach taken by Li *et al*. (2000) who built on previous work by Corseuil & Weber (1994) and developed a continuous bioaugmentation system where a column of activated carbon was inoculated with indigenous microorganisms grown on 1% sterilised paraffin. A nutrient medium and paraffin were continuously added to the column and the effluent kept for bioaugmentation of the contaminated soil. One of the advantages proposed for this approach was that 'mature' microbes selectively sloughed from the activated carbon would be less adherent than what they termed 'freshly grown' microorganisms and would move more readily through soil pores. The authors didn't describe how the initial recovery of the hydrocarbon degrading microorganisms was carried out but in their laboratory study, only 1.2%

degradation took place without bioaugmentation which reduced the TPH by 42% from an initial concentration of 200 g $kg^{-1}$ after 32 days.

From the work described by Li *et al*. (2004) it was concluded that the configuration of slotted pipes typically used to provide aeration in passive biopiles may be improved by alterative configurations. Enhanced passive biopile approaches may find most application for on site remediation projects where time and space are likely to be more critical and perhaps less likely to be deployed at a central treatment facility where economies of scale would favour more intensive windrow approaches. Nevertheless, many remediation contractors will continue to apply a non-engineered solution randomly deploying slotted pipes in treatment piles without an understanding of the potential to further optimise the treatment process.

Further work on this is merited and the modelling inspired by our study by Wu & Crapper (2009a, 2009b) made some progress towards this. A hydraulics-based approach simulated a biopile taking into account the external wind and temperature, degradation processes within the pile and most importantly, the location of aeration pipes and the venting pressure, and considering the distribution of treatment over various regions within the pile. Results indicated that the model produces reasonable results, with biodegradation related to the temperature within the pile and the temperature in turn related to wind speed and aeration details. This gave an insight into the practical design of biopiles. However, translation into a change in field practice may be slow to be realised.

In Cunningham & Philp (2000) a mixed microbial population isolated from the site in question was employed for bioremediation of diesel contamination. By contrast, in Kuyukina *et al*. (2003), two well-characterised strains of *Rhodococcus* were combined with NPK mineral salts in the ratio of 70:5:1 and a *Rhodococcus* biosurfactant complex. Gogoi *et al*. (2003) stated that an appropriate mixed culture was required for effective bioremediation of crude oil wastes. However, in their pilot-scale landfarming study, the addition of a mixed degrading population resulted in only a small increase in biodegradation efficiency and a 75% removal of hydrocarbons from an initial concentration of around 40 g $kg^{-1}$ was achieved after 1 year. Others have reported more rapid degradation of crude oil from bioaugmentation with a single degrading strain along with biostimulation compared with biostimulation alone (e.g. Nwachukwu, 2001).

One of the most interesting and overlooked results from the study reported in Kuyukina *et al*. (2003) was the relatively high reduction in oil content observed in the control treatment. Dilution with 3 parts of clean soil to 1 part oily waste was used to bring the initial levels of contamination down to 46g $kg^{-1}$ TRPH in the control (C1) and treatment (C2) cells. After only one week the TRPH had reduced by 33% to 31 g $kg^{-1}$ and by 48% to 24 g $kg^{-1}$ respectively. After 10 weeks the TRPH in C1 had reduced by a further 33% to 15.5 g $kg^{-1}$ and in C2 by a further 39% to 6 g $kg^{-1}$.

The control cell was comprised of 75% topsoil and 25% oily sludge and the topsoil had been sourced from a nearby agricultural field where cereal crops were grown.

This would have introduced NPK from fertilisers applied during cultivation. Another possible explanation for the high removal rate in the control cell was the relatively high natural population of hydrocarbon degraders, which had a mean HOX count over the study period of $1.7 \pm 0.7 \times 10^6$ CFU $g^{-1}$. Mishra *et al*. (2001) stated that indigenous HOX counts of between $10^3$ and 10 $CFU^4$ $g^{-1}$ were inadequate for bioremediation of oily sludge contaminated soil

It could be argued that dilution with topsoil alone was a highly successful technique and after 10 weeks of treatment was only 21% less than the most comparable treatment. To put this into perspective, in De-qing *et al*. (2007) the difference between the most successful treatment and the control after 160 days was 38% (Table 3) but this comparison was made with a treatment under glass in a greenhouse. Unfortunately, further sampling of the control pile was not made, as it would have been advantageous to follow the reduction in the control pile to discover when a plateau would have been reached. However, as has already been commented on, one of the aims of the study not stated in the paper was to complete bioremediation during the relatively short summer of around 17 weeks typical of the West Urals region of Russia. It may be speculated that the control could not have met this timescale.

All of the microbiological data discussed in this thesis relied on culturable microorganisms. This was mainly due to budgetary constraints and to a lesser extent by limited access to facilities. Since the publication of Cunningham & Philp (2000) study, there have been numerous advances in culture-independent molecular tools

and techniques. An understanding of microbial community structure and functionality could have aided interpretation in some of the papers previously discussed. Such tools and techniques were recently reviewed (*e.g.* Stenuit *et al*., 2008; Desai *et al.*, 2010) and will not be covered in detail here. Culture independent techniques have been reported to add value to bioremediation studies although the utility of the data may be questioned. In one example, Claassens *et al*. (2006) found that sites with greater removal of soil hydrocarbon contamination also possessed diverse phospholipid fatty acid (PLFA) profiles.

In a recent study, molecular fingerprinting was described as a complementary tool to assess the effect of different interventions such as choice of inorganic nitrogen amendment (de L. Rizzo *et al*., 2010). However, as concluded in Bundy *et al*. (2002), these issues are site specific and hydrocarbon contamination of different soils are likely to result in different community profiles. Some authors still propose that an increase in hydrocarbon degrading microorganisms is sufficient to provide evidence of survival of an introduced consortium (Mittal & Singh, 2010). Respirometry may also be a useful indicator in the laboratory during treatability studies (Aspray, *et al*., 2007). and in the field (Møller *et al*., 1996). The survival of an inoculum has been called the 'Achilles' heel' of bioaugmentation (Singer *et al.*, 2005) so research is needed to provided a robust method of assessing survival.

In Kuyukina *et al*. (2005) *Rhodococcus* biosurfactants were shown to be more effective than the synthetic surfactant Tween 80 in removal of crude oil in column studies. As previously discussed there was no elaboration on the proposed method of

application in the field. Flushing of the vadose zone with biosurfactant solution could be achieved by flooding or spraying the surface of a contaminated area or via trenches or infiltration galleries (Iturbe *et al*., 2004b). It was perhaps an oversight that no comment was made on the conditions required for subsequent biodegradation from mobilised hydrocarbons. Given the previous combined application of bioaugmentation and biosurfactants in the *ex situ* treatment of crude oil contamination it would have been reasonable to assert that the same constraints applied to *in situ* biodegradation in terms of inorganic nutrients and availability of oxygen as the terminal electron acceptor.

It was concluded that the *Rhodococcus* biosurfactants mobilised those components of crude oil from soil that would be relatively more amenable to biodegradation than those resulting from the application of Tween 60. The aliphatic fraction would be expected to be more resistant to removal than the aromatics and this was observed in the study. It was also noted that the asphaltene fraction recovered by the biosurfactant was less than half that of that observed for the water and Tween 60 columns at only 1%. Perhaps the biggest limitation of Kuyukina *et al*. (2005) was that all of the column experiments on crude oil mobilisation were carried out almost immediately after contamination of the model soil by crude oil. No weathering of the system was allowed to take place and this should have been either included in the study or taken into account in the conclusion with an indication that the results were best related to fresh spills of crude oil.

As previously noted in this document but not discussed in Kuyukina *et al*. (2005) was the potential for horizontal or vertical migration of contaminants beyond the desired treatment or recovery zone. Another aspect not considered was the potential for enhanced mobilisation of metals into the subsurface. Microbial rhamnolipid surfactants have also been reported to have applicability in the removal of heavy metals from soil (Mulligan 2005). The potential of *Rhodococcus* biosurfactants appears not have received attention from researchers (Kuyukina & Ivshina 2010b). Iturbe *et al*. (2004b) reported that vanadium was effectively removed at an efficiency of nearly 95% by a synthetic surfactant Canarcel TW80 and this may have been of relevance to our study although the concentration of vanadium in crude oil is highly variable depending on the oil source (Bell *et al*., 2004).

A key limitation to the use of biosurfactants is their cost of production. This is clearly evidenced by their less than 2% share of the global industrial surfactant market (Kuyukina & Ivshina, 2010a) and commercialisation at a large scale has yet to occur. Calvo *et al*., (2009) recently suggested that future research effort must be focused on development of novel recombinant hyper-producing strains for high-level production of biosurfactants.

Although superficially the use of biosurfactants may appear to be inherent more sustainable than synthetic ones, consideration must be given to the substrates and method of production. From this perspective one of the potential disadvantages of *Rhodococcus* biosurfactants is the requirement for a petrochemical substrate typically an *n*-alkane. However, several studies have reported on alternative substrates.

Ciapina *et al*. (2006) found that a strain of *R. erythropolis* was able to produce biosurfactant with a relatively low yield (1.7 g l-1) but with a high emulsification index (E24) of 67% using glycerol as the sole carbon source. An added advantage was that growth on glycerol released more biosurfactant into the medium whereas growth on *n*-hexadecane resulted in more cell wall associated production. This has implications for reduced downstream processing and was the reason why sonication was used to maximise surfactant yield in the papers discussed in the previous section.

Sadouk *et al*. (2008) investigated the ability of a *R. erythropolis* strain to produce biosurfactant when grown on 3% (v/v) used sunflower oil from a factory in Algeria where it was used to fry potato crisps. Another potential is the use of renewable substrates such as rapeseed oil and Ruggeri *et al*. (2009) found a small number of 18 environmental isolates including one *Rhodoccocus* strain were able to grow this as the sole carbon source although biosurfactant yields and E24 values were low. An alternative approach may be to use natural plant products such as guar gum and locust bean gum. Torres *et al*. (2007) compared these with the synthetic surfactant sodium dodecyl sulphate (SDS) for removal of crude oil in soil columns and found the natural products removed around 48% of TPH compared with 36% for SDS at the same application rate.

An issue worthy of consideration is that many physical, chemical, thermal and biological treatments for hydrocarbon-contaminated land are referred to as being ‘innovative’. There is no doubt that technical developments are required to reduce risks and increase the effectiveness, efficiency and sustainability of remediation.

However, overuse of the term innovative can only serve to reduce the much-needed confidence in remediation technologies that offer a cost effective alternative to excavation and disposal to landfill.

Historically, this approach has been the most widely applied technique when tackling contaminated sites due to its short timescale, simplicity and comparatively low costs compared with other treatment options. As much as 75% of contaminated land was treated in this way in the UK in 2007 and bioremediation accounted for only 6% of the UK market (MSI, 2008). Despite being practiced for more than half a century, bioremediation has been variously considered by authors to be proven or innovative. Quotes from nearly two decades ago suggested acceptance of the technology:

*"Land treatment is an environmentally attractive alternative for the disposal of petroleum refinery wastes"* Arora *et al*. (1982)

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*"Bioremediation is cost effective, available and demonstrated"* Ryan *et al*. (1991)

In a paper by Spira *et al*. (2006), the European approach to increasing application of innovative soil and groundwater remediation technologies was reviewed. The authors went some way to recognising that the term 'innovative' was over applied. They provided examples of permeable reactive barriers as well as *in situ* thermal, chemical and biological techniques. The following quotation (the quotation marks around innovative are from the authors) illustrates the point:

*"The above-illustrated four "innovative" technologies reveal that there is already some experience from applications available"*

Innovative may be seen from the perspective of those faced with funding the cost of remediation as meaning not sufficiently proven. This is of course entirely subjective but understandable given that almost all practitioners would concede that sites must be considered on their own merits and specific implementation of most remedial techniques are site specific.

That is not to say that there are no potentially disruptive or truly innovative alternative remediation technologies that could be applied to hydrocarbon-contaminated land. Colleagues in the School of Engineering at The University of Edinburgh have developed, and at the time of writing were in the process of commercialising, a smouldering combustion technique that could be applied *in situ* or *ex situ*. This is ideally suited for hydrocarbon-contaminated land and potentially more sustainable than other techniques as there is no requirement for a continuous input of energy and the process is self-sustaining and self-terminating. Results from bench scale trials showed that the technique could the concentration of TPH from 38000 mg $kg^{-1}$ to <0.1 mg $kg^{-1}$ (Switzer *et al*., 2009).

Consideration of sustainability as part of the remedial decision making process will undoubtedly favour less transport, energy and emission intensive options. Remediation may in the past have benefitted from a perception that it was inherently a ‘good’ thing as a site was being cleaned and brought into reuse or the environment was in some way being improved.

There are many different approaches that may be employed to consider the sustainability of a remediation project including Life cycle assessment (LCA) and Net environmental benefit analysis (NEBA). The latter specifically includes valuing 'ecosystem services'. For example, being able to swim in a water body has a value that may be increased or diminished depending on whether remediation will improve or reduce water quality or perhaps even impact the ability to access this resource.

However, recent thinking on applying sustainability principles to remediation has challenged this opinion. Baker *et al*. (2009) recently compared the route to acceptance of sustainability in remediation with the development of risk assessment and asserted that with time the value of sustainable remediation will be appreciated by practitioners. One of the key developments in sustainable remediation was the formation of the US Sustainable Remediation Forum (SURF) in 2006. SURF was formed by a group of remediation practitioners and has defined sustainable remediation as being:

*"A remedy or combination of remedies whose net benefit on human health and the environment is maximized through the judicious use of limited resources"*

A comprehensive 'white paper' was published (Sustainable Remediation Forum, 2009) that aimed to bring together current thinking and experiences from SURF members. Key stakeholders were identified as being site owners, regulators, remediation industry and the public. One of the key issues identified early in the document was that each stakeholder has a different perspective and that the 'sustainability' of a particular remedial activity needs to be considered as being project (or site) specific. Net environmental benefit has been added to the drivers

stakeholders must evaluate alongside the efficacy, cost and regulatory acceptance of remediation technologies or approaches being proposed.

Barriers to the more widespread implementation of sustainable remediation were stated as including a lack of agreed-upon metrics, regulatory consensus and financial incentives. However, perhaps the key barrier was the lack of a well-defined framework and as a consequence of the creation of the US Sustainable Remediation Forum (SURF), a similar group called SURF UK was formed in in 2007 with the mission:

*"to develop a framework in order to embed balanced decision making in the selection of the remediation strategy to address land contamination as an integral part of sustainable development"*

The framework (CL:AIRE, 2010) developed dates that it is designed to complement the existing best practice guidance in the UK '*Model Procedures for the Management of Land Contamination'* (Environment Agency, 2004). However, searching the model procedures document yields one use of the word sustainability among the more than 200 pages of guidance. Nevertheless, SURF UK was keen to highlight alignment with the model procedures. For example, the following quotation was taken from the section on remedial options appraisal:

*"Sustainability of the strategy (i.e., how well it meets other environmental objectives, for example on the use of energy and other material resources, and avoids or minimises adverse environmental impacts in off-site locations, such as a landfill, or on other environmental compartments, such as air and water)"*

The new guidance provided in the SURF-UK framework has built a comprehensive and systematic means of including environmental, economic and social indicators into remedial options appraisal. They highlight an important distinction between the SURF-UK framework and a United States Environmental Protection Agency initiative 'green remediation'.

The latter has a much narrower focus around the use of renewable energy and maximising the environmental benefits of remediation. The SURF-UK framework is designed to consider the broader sustainable development objectives of a project i.e. including wider considerations of land use in a development.

An extensive review of relevant environmental indicators was carried out by SuRF-UK (Bardos *et al.*, 2009) and the most recent versions of the indicators developed and the issues that indicators might need to consider are presented in Tables 7-9 below.

**Table 7: UK Sustainable remediation environmental indicators (March 2011)**

| Category | Issues that indicators might need to consider |
|---|---|
| Impacts on air | Emissions that may affect climate change or air quality, such as greenhouse gases (e.g. $CO_2$, $CH_4$, $N_2O$), $NO_X$, $SO_X$, particulates (especially PM5 and PM10), $O_3$, VOC's, ozone-depleting substances, etc. *(Note: Does not include any odorous effects, bioaerosols, allergens or dust, as these are included in 'Social 3: Impacts on neighbourhoods or regions')* |
| Impacts on soil and ground conditions | Changes in physical, chemical, biological soil condition that affects the functions or services provided by soils. May include soil quality (chemistry), water filtration and purification processes, soil structure and/or organic matter content or quality; erosion and soil stability, geotechnical properties, compaction and other damage to soil structure affecting stability, drainage, or provision of another ecosystem good or service. Impacts on geological Sites of Special Scientific Interest (SSSIs) and geo-parks. |
| Impacts on water | Release of contaminants (including nutrients), dissolved organic carbon or silt/particulates, affecting suitability of water for potable or other uses, water body status (under the Water Framework Directive) and other legislative water quality objectives, biological function (aquatic ecosystems) and chemical function, mobilisation of dissolved substances. Effects of water abstraction included, such as lowering river levels or water tables or potential acidification. *(Note: Does not include any water abstraction use or disposal issues, as this is covered in 'Environmental 5: Use of natural resources and generation of wastes'.)* |
| Impacts on ecology | Includes: Direct consequences for flora, fauna and food chains, especially protected species, biodiversity and impacts on Sites of Special Scientific Interest. Introduction of alien species. Significant changes in ecological community structure or function. Impacts of light, noise and vibration on ecology. Use of decontamination equipment that affect fauna (e.g. affecting bird or bat flight, or animal migration, etc.). *(Note: Does not include effects on soil and aquatic ecosystems, which are covered in 'Environmental 2: Impacts on soil and ground conditions' and 'Environmental 3: Impacts on water', whilst impacts of light, noise and vibration on humans are covered in 'Social 3: Impacts on neighbourhoods and regions'.)* |
| Use of natural resources and regeneration of wastes | Consequences for land and water resources, use of primary resources and substitution of primary resources within the project or external to it, including raw and recycled aggregates. Use of energy/fuels taking into account their type/origin and the possibility of generating renewable energy by the project. Handling of materials on-site, off-site and waste disposal resources. Water abstraction, use and disposal. |
| Intrusiveness | Impacts on flooding or increased risk of flooding; alteration of landforms that affect environment, (e.g. a "natural" view). *(Note: Does not include effects on built environment and protection of archaeological resources, which are covered in 'Social 3: Impacts on neighbourhoods or regions', whilst affects on ecology are covered in 'Environmental 4: Impacts on ecology'.)* |

After SuRF-UK (2011)

**Table 8: UK Sustainable remediation social indicators (March 2011)**

| Category | Issues that indicators might need to consider |
|---|---|
| Human health and safety | Risk management performance of the project in terms of delivery of mitigation of unacceptable human health risks. Risk management performance in the short term, including: risks to site workers, site neighbours and the public from remediation works and their ancillary operations (includes hazardous process emissions such as bioaerosols, allergens, PM10 as well as impacts from operating machinery and traffic movements, excavations, etc). |
| Ethical and equity considerations | How are social justice and/or equality addressed? Is the spirit of the 'polluter pays principle' upheld with regard to the distribution of impacts and benefits? Are the effects of works disproportionate to, or more beneficial towards, particular groups?<br><br>What is the duration of remedial works and are there issues of intergenerational equity (e.g. avoidable transfer of contamination impacts to future generations)? Are the businesses involved operating ethically (e.g. open procurement processes)?<br><br>Does the treatment approach raise any ethical concerns for stakeholders (e.g. use of genetically modified organisms)? |
| Impacts on neighbourhoods or regions | Impacts to local community, including dust, light, noise, odour and vibrations during works and associated with traffic, including both working-day and night-time / weekend operations. Effect of antisocial use of site, and its impact of other regeneration activities. Impacts on the built environment, architectural conservation, conservation of archaeological resources. Effect of the project on local culture and vitality.<br>*(Note: Does not include effects or perceptions of a "natural" view, which is covered in 'Environment 6: Intrusiveness'.)* |
| Community involvement and satisfaction | Impacts of works on public access to services (all sectors – commercial, residential, educational, leisure, amenity). Inclusivity and engagement in decision making-process. Transparency and involvement of local community, directly or through representative bodies. |
| Compliance with policy objectives and strategies | Compliance of the works with policies, regulatory standards and good practice as set out nationally, by local authority, at the request of community and/or in line with industry working practices and expectations. |
| Uncertainty and evidence | How has sustainability assessment been carried out and what has it considered? Quality of investigations, assessments (including sustainability) and plans, and their ability to cope with variation. Accuracy of record taking and storage. Requirements for validation/verification. |

After SuRF-UK (2011)

**Table 9: UK Sustainable remediation economic indicators (March 2011)**

| Category | Issues that indicators might need to consider |
|---|---|
| Direct economic costs and benefits | Direct financial costs and benefits of remediation for organisation, consequences of capital and operation costs, and sensitivity to alteration (e.g. uplift in site value to facilitate future development, minimisation of risk or threat of legal action). |
| Indirect economic costs and benefits | Long term or indirect impacts and benefits, such as financing debt, allocation of financial resources internally, changes in site/local land/property values, and fines and punitive damages (e.g. following legal action, so includes solicitor and technical costs during defence).<br><br>Consequences of an area's economic performance. Tax implications. Financial consequences of impact on corporate reputation. |
| Employment and employment capital | Job creation, employment levels (short and long term), skill levels before and after, opportunities for education and training, innovation and new skills. |
| Gearing | Creating opportunities for inward investment, use of funding schemes, ability to affect other projects in the area / by client (e.g. Cluster) to enhance economic value. |
| Life span and project risks | Duration of the risk management (remediation) benefit, e.g. fixed in time for a containment system); factors that might impact the chances of success of the remediation works and issues that may affect works, including community, contractual, environmental, procurement and technological risks. |
| Project flexibility | Ability of project to respond to changing circumstances, including discovery of additional contamination, different soil materials, or timescales. Robustness of solution to climate change effects.<br><br>Robustness of solution to altering economic circumstances. Requirements for ongoing institutional controls. Ability to respond to changing regulation or its implementation. |

After SuRF-UK (2011)

The studies discussed in this thesis almost all gave consideration to sustainability albeit in a non-systematic and qualitative manner. The ballast cleaning studies described in Anderson *et al*. (2002, 2003) discussed environmental impacts of the process and the benefits of recycling rail ballast. Cunningham & Philp (2000) and Li

*et al*. (2004) were concerned primarily with enhancing the speed of bioremediation to compete with landfilling of hydrocarbon-contaminated soils. Remediation of crude oil wastes from refinery storage pits reported in Kuyukina *et al*. (2003) presented bioremediation as an 'acceptable' alternative to incineration and this study and Kuyukina *et al*. (2005) made use of biosurfactants in place of synthetic ones.

With the benefit of hindsight all of the studies discussed in this thesis could have been significantly improved in design and execution even taking resource and time constraints into account. The cost of conducting replicated and data intensive field trials remains a key challenge that precludes almost all commercial full-scale bioremediation projects from being reported in the literature.

Indeed, the USEPA noted in their review of commercial bioaugmentation agents for marine oil spills that the extreme resource intensiveness of field studies was a barrier to more widespread field studies on bioremediation agents (USEPA, 2004). It is evident that Kuyukina *et al*. (2003) would have been significantly improved by an increase in the number of treatments to include at least one system that received nutrient addition and the biosurfactant complex without bioaugmentation.

Diplock *et al*. (2009) recently proposed that that largest research challenge for bioremediation was translating laboratory treatability data into field scale predictions. More recently, Ramos *et al.* (2011) commented that exploitation of knowledge gained from both laboratory and field studies had not been fully realised. This echoes the position of Head (1998) from more than a decade before who said

that one of the greatest challenges was providing evidence that a chosen treatment will be effective in the field.

Diplock *et al*. (2009) also highlighted the need for commercial projects to collect more intensive data to enhance our understanding. Others have recognised the need to improve sharing of data between projects. Watanabe (2001) proposed the establishment of a database that collected the results of microbial community data assessments of contaminated sites and those where bioremediation had been applied to build understanding. This same thinking could easily be proposed for physical, chemical and thermal techniques.

Several EC funded initiatives have brought together information on remedial technologies with the aim of fostering the transfer of lessons learned between countries and to increase awareness of the range of technologies available. These include the European Co-ordination Action for Demonstration of Efficient Soil and Groundwater Remediation (EURODEMO) and PROMOTE which focussed on the verification of site investigation and remediation technologies for soil and groundwater.

Inevitably, there were many interesting aspects of the papers discussed and the current status of research in the remediation field. One of these was the potential role of genetically modified organisms (GMOs) for treatment of hydrocarbon-contaminated land. However, the author agrees with Stroud et al. (2007) who stated that:

*"field application of genetically modified organisms is improbable given the current environmental regulations and increasing unpopularity with the general public"*

The opinion of this author is that the situation in the UK is unlikely to change for some time. From personal experience, even the importation of a natural strain from the US bioaugmentation to enhance reductive dechlorination of Trichloroethylene was problematic.

Notwithstanding the limitations of this thesis, the body of work presented represents a contribution to knowledge in several aspects of treatment of hydrocarbon-contaminated industrial land including diesel contaminated railway land and track ballast, crude oil wastes and crude oil contaminated soils. The studies have separately and collectively enhanced understanding in the field.

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## Part 2: Collected papers

1. Anderson, P., **Cunningham, C.J.,** Barry, D.A. (2000). Gravimetric Analysis of Organic Contamination in Railway Ballast. *Land Contamination & Reclamation.* 8 (2), 71-74.

2. Anderson, P., **Cunningham, C.J.**, Barry, D.A. (2002). Efficiency and Potential Environmental Impacts of Different Cleaning Agents used on Contaminated Railway Ballast. *Land Contamination & Reclamation.* 10 (2), 71-77.

3. Anderson, P., **Cunningham, C.J.**, Hearnden, R., Barry, D.A., Philp, J.C. (2003). Optimisation and Assessment of Different Railway Ballast Cleaning Systems. *Land Contamination & Reclamation.* 11 (4), 1-7.

4. **Cunningham, C.J.**, Philp, J.C. (2000). Comparison of bioaugmentation and biostimulation in *ex situ* treatment of diesel contaminated soil. *Land Contamination & Reclamation.* 8 (4), 261-269.

5. Li, L., **Cunningham, C.J.**, Pas, V., Philp, J.C., Barry, D.A., Anderson, P. (2004) Field trial of a new aeration system for enhancing biodegradation in a biopile. *Waste Management*. 24 (2), 127-137.

6. Kuyukina, M.S., Ivshina, I.B., Ritchova, M.I., **Cunningham, C.J.**, Philp, J.C., Christofi, N. (2003). Bioremediation of crude oil contaminated soil using slurry-phase biological treatment and landfarming techniques. *Soil and Sediment Contamination*. 12 (1), 85-99.

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## Appendix A: Summary of contribution to papers

In addition to the declaration of contribution provided in the following appendices, the table below estimates the percentage contribution to each of the papers as numbered on the previous page.

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
|---|---|---|---|---|---|---|---|
| Idea/Concept | 90 | 90 | 90 | 50 | 80 | 30 | 40 |
| Aims | 80 | 80 | 80 | 60 | 70 | 40 | 25 |
| Sample collection | 90 | 50 | 50 | 90 | 90 | 25 | 0 |
| Analysis | 40 | 40 | 40 | 100 | 50 | 30 | 0 |
| Interpretation | 75 | 75 | 75 | 70 | 50 | 30 | 25 |
| Writing | 75 | 75 | 75 | 80 | 50 | 30 | 25 |

## Appendix B: Anderson *et al.* (2000)

Anderson, P., **Cunningham, C.J.** Barry, D.A. (2000). Gravimetric Analysis of Organic Contamination in Railway Ballast. *Land Contamination & Reclamation.* 8 (2), 71-74.

### *B.1 Declaration of contribution*

The author had been undertaking bioremediation field trials on diesel-contaminated land (Cunningham and Philp, 2000) adjacent to railway tracks that were the source of the contamination. Some attempts were made to stimulate degradation of the ballast *in situ* by applying nutrients and fertiliser and the need arose to make an assessment of treatment effects. This prompted the author to work with Anderson developing the methodology and the author was heavily involved in undertaking the laboratory work along with Anderson. The author was also closely involved in the interpretation of results and at all stages of writing the paper.

*Land Contamination & Reclamation*, 8 (2), 2000
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# Gravimetric analysis of organic contamination in railway ballast

Peter Anderson, Colin J. Cunningham, and D. A. Barry

Abstract
*Railway ballast provides both the foundation and drainage for railway track. It can become heavily contaminated with diesel fuel due to leakage and spillage. Typical analytical methods for soils may not be applicable to the assessment of ballast. The efficiency of different commonly available solvents as ballast extractants was investigated. Ethyl acetate performed best, yielding 3870, 6065 and 8990 mg kg$^{-1}$ more contaminant than dichloromethane, hexane and methanol, respectively. Mechanical shaking and sonication were compared for different sample weights, solvent volume ratios and extraction times. Using ethyl acetate, efficient practical assessment of contaminated ballast is achieved using a ratio of at least 100 ml of solvent to 120 g of ballast.*

Key words: ballast, contamination, diesel fuel, railway gravimetric, solvent extraction

## INTRODUCTION

Railway ballast consists of crushed hard rocks and stones, typically between 28–50 mm. It is placed as a top layer of the substructure in which the sleepers are embedded. Ballast provides both the foundation and drainage material for railway track and represents a considerable (£30m) annual cost to the UK rail industry (Selig and Waters 1994; Collinson 1998). Fuel, principally diesel, represents the largest organic contaminant of both ballast and railway land generally. Leakage from stabled diesel motor unit (DMU) sets represents a major source of contamination. Other organic contaminants may include creosotes and petroleum products used for the preservation of railway ties, polychlorinated biphenyls (PCBs), herbicides, pesticides, deicing fluid and toilet waste.

Increasingly, cleaning and recycling of ballast have, in the last decade, become a regular practice as part of regular track maintenance. Sampling and assessment of ballast condition is an on-going task. Typically, contaminated ballast is removed for treatment at a washing plant or lifted using a large vacuum excavator as part of a mobile washing system operating on the railway line (Tiefel *et al.* 1994). Use of solvent in the cleaning process creates the risk of residual material becoming more mobile when cleaned ballast is returned to the track. When ballast becomes too rounded to serve as track foundation, it is used in other engineering applications, e.g. as fill. However, such 'spent' ballast remains a potential contaminant if improperly cleaned. Thus, an appreciation of ballast cleaning efficacy is necessary both for the routine ballast assessment, and also to reduce the contamination risk resulting from ballast replacement or disposal.

As a means of assessing the efficacy of ballast cleaning processes, typical gravimetric analytical protocols may not be suitable, as they are not easily adapted to quantify the level of ballast contamination (US EPA Methods 3540C and 3550B 1996). Such techniques are most suited to relatively fine well-characterised, homogenous material (e.g. 1–2 mm). In a previous study on the occurrence and levels of polycyclic aromatic hydrocarbons (PAHs) in ballast and railway right-of-way ditches, ballast was sampled and extracted immediately on site with 0.5 l ethyl acetate in a 1 l beaker using a swirling action for two minutes (Wan 1991). Extracts were rotary evaporated and fractionated to provide a final extract for analysis by gas chromatography mass spectrometry (GC-MS).

However, to the authors' knowledge, no studies have been reported in the literature comparing extraction methods for assessing organic contamination in railway ballast. This work was undertaken to address

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some preliminary factors by first examining the effectiveness of four different readily available solvents, viz. dichloromethane, hexane, methanol and ethyl acetate. Standard extraction methods – mechanical shaking and sonication – were compared over different extraction times for a range of sample size: solvent volume ratios, all benchmarked against Soxhlet extraction. This involves passing solvent continuously through the sample, held in a porous thimble, by distilling the solvent to a condenser centred over the thimble. A syphon system removes the extract back into the refluxing solvent and the net effect is continuous extraction by fresh solvent (Dean 1998). The results presented in this paper represent the initial phase of a wider study on contaminant transport from railway ballast and the potential environmental impacts associated with reuse of ballast cleaned by organic solvents. Further applications include the assessment of contamination on other larger sized materials, for example shingle beaches impacted by oil spills.

## EXPERIMENTAL

Ballast samples (granite of size 20–40 mm with an elongation index of 40–60%) were collected from several locations at an operational railway siding where contamination by diesel was visually evident. These were homogenised in the laboratory and refrigerated at 4$^{o}$C. Methanol, dichloromethane, ethyl acetate and hexane used in extractions were all of pesticide grade (Rathburn Chemicals, Walkerburn, Peebleshire, UK).

### Extraction procedures

Extractions were performed using a wrist-action shaker (Stuart Scientific, Bibby Sterilin Ltd., Staffordshire, UK) at 500 oscillations per minute and an ultrasonic bath (Decon model FS200B, Hove, UK) pre-set to 120 W with a swept frequency range of between 35-45 kHz. For both methods, 100 ml of solvent was added to 500 ml stoppered conical flasks containing the ballast samples. After extraction, decanted solvent was centrifuged for two minutes at 6000 rpm (Eppendorf, model 5416, Hamburg, Germany), filtered through Whatman No. 542 filter paper under vacuum and transferred into pre-weighed 250 ml round-bottomed flasks (four-point balance, Mettler-Toledo, model AT 261 Delta range, Bedford, UK). Extracts were evaporated to near dryness at a temperature of 35$^{o}$C using a rotary evaporator (Rotavapor – R, Buchi, Flawil, Switzerland) and finally displaced under nitrogen. For Soxhlet extraction, 60-70 g ballast was inserted into cellulose thimbles (33 × 100 mm, Whatman, Maidstone, UK), 400 ml solvent was added to pre-weighed round bottomed flasks and refluxed for 24 hours. Flasks were re-weighed and the contamination determined gravimetrically.

For the evaluation of different solvents, 100 ml of dichloromethane, hexane, methanol and ethyl acetate were used to extract 30 g of ballast using wrist action shaker for a time of ten minutes. Extractions were carried out in triplicate and the third replicate was re-extracted twice more in 100 ml of fresh solvent to give additional recovery data. To investigate the effect of sample size and extraction time, triplicate extractions were carried out using both the wrist action shaker and ultrasonic bath for sample weights of 30, 60, 90 and 120 g for extraction times of two, six and ten minutes. A third replicate was re-extracted twice more with fresh solvent over the same duration.

## RESULTS AND DISCUSSION

The total amounts and proportions (expressed as percentages) extracted for the four solvents are shown in Table 1. The total amounts recovered after three successive extractions showed the superior performance of ethyl acetate, in that it extracted 3870, 6065, and 8990 mg kg$^{-1}$ more contaminant than dichloromethane, hexane and methanol, respectively. Hexane is not an effective solvent for extraction of high molecular weight petroleum products (Total Petroleum Hydrocarbon Criteria Working Group 1998), which may be prevalent in contaminated railway ballast. The low

**Table 1. Comparison of extraction solvents to remove contamination from railway ballast***

| | Amount extracted (mg kg$^{-1}$) [RSD (%)] | | | | Percentage extracted (%) | | |
|---|---|---|---|---|---|---|---|
| Solvent | First | Second | Third | Total | First | Second | Third |
| Dichloromethane | 22710 [18.4] | 2500 | 730 | 25930 | 87.5 | 9.6 | 2.8 |
| Ethyl acetate | 27130 [20.7] | 2050 | 705 | 29800 | 90.8 | 6.8 | 2.4 |
| Hexane | 20100 [19.1] | 2840 | 795 | 23735 | 84.3 | 12.0 | 3.3 |
| Methanol | 11220 [21.5] | 6360 | 3240 | 20810 | 53.8 | 30.6 | 15.6 |

* Extractions were performed on 30-g samples using wrist action shaker for ten minutes. Triplicate extractions were carried out for the first cycle. The third replicate was re-extracted a second and third time to provide additional recovery data.

recovery obtained using methanol is likely related to the non-polar fraction present in diesel and also in other potential contaminants such as lubricating and gear case oils. Ethyl acetate was found to be more effective than dichloromethane and has the added advantage of being non-chlorinated and less hazardous (Health and Safety Executive 1998). All subsequent extractions were therefore carried out using ethyl acetate.

A 30 g sample of ballast typically comprised between four and six stones only, so it was not surprising to find the relative standard deviation (RSD) values for triplicate extraction approaching or in excess of 20%. The optimal conditions for sonication and mechanical shaking are dependent on sample size, volume of solvent and extraction time. To improve the reproducibility of the results, larger samples of ballast (60, 90 and 120 g) were extracted as described using 100 ml of ethyl acetate. These data are shown in Figures 1 and 2.

Gentle swirling for 10–15 seconds removed between 65% for 30 g and 49.2% for 90 g after the first extraction with a concomitant increase in the amount extracted in second and third cycles. This indicated that the contaminants coating the ballast were easily removed, given that only minimal shaking was required to extract approximately 50% of the total contaminant load. The concentration of organic contamination removed for the 30 and 60 g sample sizes (measured in mg $kg^{-1}$ ballast), was not affected by either the time (i.e. two, six or ten minutes) or by the method of extraction. This is supported by the fact that 90% of the total extractable material present was removed after the first extraction with 6–9% and 1–3% removed after the second and third extraction cycles. RSD values decreased with increasing sample mass. The RSD value, calculated using triple extractions, for the 60 g sample size was 15%, compared with the result of approximately 20% for the 30 g sample size.

By increasing the sample mass to 90 and 120 g, the percentage extracted after the first cycle for sonication decreased considerably. For the extraction of the 90 g sample sizes, the percentage removed after two minutes sonication was 76.2% and, for ten minutes, 84.2%. For the 120 g sample size, the percentage extracted over ten minutes dropped to 79.8% while for two minutes this was as low as 68.5%. Correspondingly, there was an increase in the proportions removed after the second and third extraction cycles. For the extraction of 90 g sample size, the proportions removed after two minutes sonication were 19.8 and 4.0% and, for ten

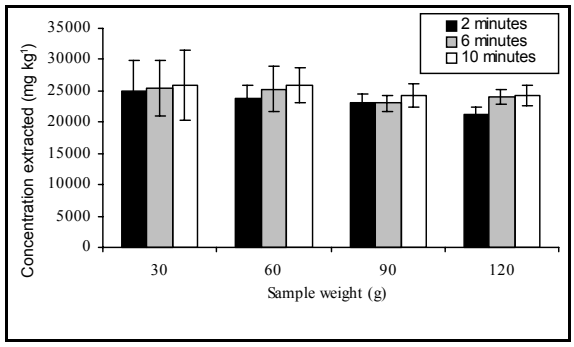


*Figure 1. Total extractable material after first extraction using a wrist action shaker and ethyl acetate as solvent for different sample weights and extraction times*

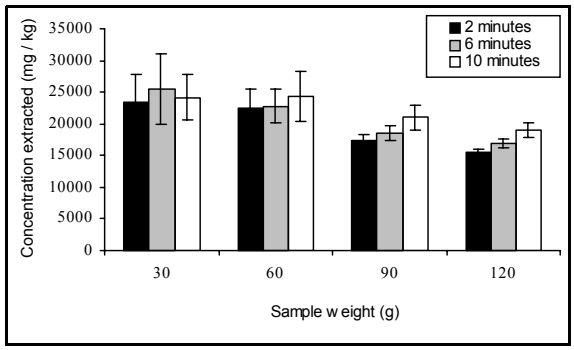


*Figure 2. Total extractable material after first extraction using ultrasonic bath and ethyl acetate as solvent for different sample weights and extraction times*

minutes, 11.8 and 4.0%, respectively. For larger 120 g sample sizes, the percentage extracted for ten minutes dropped to 16.8 and 3.3% while for two minutes the values were as low as 25.6 and 5.9%, respectively. In the case of mechanical agitation, increasing the weight of sample had a less pronounced effect on contaminant recovery. With the exception of the 120 g sample sizes extracted for two minutes, nearly 90% of the total amount extracted was removed after the first cycle. This apparent difference in extraction efficiency for sonication compared to mechanical shaking probably results from the ability of the solvent to wash over the ballast providing a more intimate contact between solvent and stones compared to sonication. In terms of reproducibility, a significant improvement was observed moving to larger weights with RSD values typically dropping to between 5 and 10%. The RSD reduced to just 3.3% for six minutes sonication for the 120 g ballast samples.

An increase in sample mass to 150 g or greater for mechanical shaking might lead to cracking or breaking of the extraction flask. It is anticipated the extraction efficiency for mechanical shaking will also begin to deteriorate, even for an extraction time of ten minutes due to incomplete coverage of the sample.

Soxhlet extraction yielded 26 900 ± 2000 mg $kg^{-1}$ ballast for a triplicate extraction of 60 g sample sizes. For mechanical shaking and sonication, total amounts of 25 920 ± 2500 and 24 430 ± 3900 mg $kg^{-1}$, respectively, were removed from a 60 g sample of ballast for an extraction time of ten minutes, representing no significant difference compared to Soxhlet (P = 0.05). For the 120 g sample sizes extracted using the wrist action shaker, 24 200 ± 1610 mg $kg^{-1}$ was removed. These results indicate that even for the larger sample mass, 100 ml of solvent was able to extract 85–90% of the amount extracted using Soxhlet.

## CONCLUSIONS AND FUTURE WORK

Mechanical shaking (e.g. wrist action shaking) and sonication (e.g. ultrasonic bath) have the potential to extract samples containing larger fragments. Both utilise relatively inexpensive equipment commonly used in an analytical laboratory, are straightforward to operate and, with appropriate glassware, can handle larger sample masses without a corresponding increase in solvent volume. In addition, contaminant recoveries compared favourably to Soxhlet extraction for the equivalent amount of processed ballast (60 g) while requiring only a fraction of the solvent and processing time to effect extraction.

Multiple extractions with ethyl acetate over different time periods and sample masses up to 120 g showed that 90% of the total amount of contaminant present in the samples was removed after the first extraction cycle for mechanical shaking. However, a mass effect was observed for sonication as lower recoveries were obtained for 90 and 120 g even for extraction times of ten minutes. Overall, sample precision improved for both methods with increasing sample weight. In summary, these results show that practical assessment of contamination on railway ballast can be achieved using ethyl acetate as an extractant in the ratio of at least 100 ml solvent to 120 g of ballast.

Future analytical work will involve the development of clean-up methods for fractionating extracts into compound classes to allow identification and quantification (both semi- and full) of individual constituents by GC-MS. Ballast column experiments are also being developed to assess the increase in mobility of specific fractions into the environment following cleaning of ballast with a variety of widely used solvents.

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## Appendix C. Anderson *et al*. (2002)

Anderson, P., **Cunningham, C.J.**, Barry, D.A. (2002). Efficiency and Potential Environmental Impacts of Different Cleaning Agents used on Contaminated Railway Ballast. *Land Contamination & Reclamation.* 10 (2), 71-77.

### *C.1 Declaration of contribution*

The work on ballast cleaning was a follow up to the previous paper and the author effectively drove the project that was heavily informed by the observations made by the author while attending the demonstration of a track-mounted rail ballast-cleaning machine. The author was involved in undertaking the laboratory work and also closely involved in the interpretation of results and at all stages of writing the paper.

*Land Contamination & Reclamation*, 10 (2), 2002 

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# Efficiency and potential environmental impacts of different cleaning agents used on contaminated railway ballast

P. Anderson, C. J. Cunningham and D. A. Barry

Abstract

*Railway ballast consists of crushed aggregate and serves as foundation and drainage for railway tracks. Over time, ballast loses its geotechnical properties and cannot be reused within the rail industry but can be sold on to other users to be utilized as a recycled engineering fill. However, it is often contaminated with diesel, grease, lubricating oils, and other deposits from locomotives and carriages. Its reuse generally involves cleaning at a specialist plant. Such contamination may also be removed from geotechnically sound ballast returned to the track where the appearance of dirty ballast is considered unsightly, e.g. in railway stations, and a potential health hazard. Cleaning of the ballast generally involves the use of solvent or surfactant cleaning agents, each with different efficiencies and potential environmental impacts. In this study, the efficiency of three cleaning agents, two terpene-based organic solvents and a surfactant-based system were tested on heavily contaminated ballast using a laboratory-scale cleaning system. The solvents used, both derived from oranges, reduced contamination by 96% or 98%. The surfactant-based cleaning removed 93%. Environmental impacts of residual contamination, solvent or surfactant are discussed and consideration given to the overall sustainability of the approach including disposal of wastewater.*

Key words: cleaning agents, railway ballast, solvent, surfactant, sustainability

## INTRODUCTION

Railway ballast provides both the foundation and drainage for railway track (Selig and Waters 1994). Ballast deteriorates over time due to the accumulation of fines in the voids of the normally open structure as a result of oil and grease leaking onto the ballast. This deterioration results in reduced stability leading to an inability of the tracks to maintain their required geometry and drainage properties (Awoleye 1998). Throughout the UK's rail network, around £30m is spent annually on three million tonnes of stone ballast, with a further estimate of £15m to transport the ballast to its final destination. From this tonnage, 60% is used for renewing fouled ballast, 23% is used for maintenance operations, i.e. packing medium after tamping operations, and 17% is used for major projects involving changes to main line junctions or stations (Collinson 1998).

Despite advances in ballastless track technology in Europe and Japan, with advantages in terms of low maintenance requirements, alignment quality and high speed performance, conventional railway tracks continue to produce large amounts of 'spent' ballast. In many instances, this may be suitable for recycling as an engineering fill material. However, contamination by fuels and oils necessitates cleaning before reuse (Ceney 2001). Contaminated but geotechnically sound ballast is also cleaned *in situ* and returned to the track. In any event, disposal to landfill of contaminated ballast is an unsustainable option and recycling has gained priority over disposal (Tiefel *et al.* 1994).

In May 1999, the Department of the Environment, Transport and the Regions (DETR) included greater use of recycled and waste materials and more efficient usage of primary aggregates as part of the overall strat-

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egy for sustainable development in the UK (DETR 1999). The landfill tax, introduced in 1996, already went some way towards making 'dig and dump' of solid wastes a less economically attractive route for materials that may be recycled. A further green tax of £1.60 per tonne is due to be levied on primary aggregate quarrying as of April 2002, providing further stimulus for reuse (HMSO 2001).

Contaminated ballast may be excavated for treatment at a processing facility or removed by a track-mounted mobile washing system (Tiefel *et al.* 1994). The use of solvents or surfactants increases the risk of residual material being mobilised when cleaned ballast is returned to the track or used as a fill material. Surfactant-based cleaning processes may represent the highest risk of contaminant mobility but may also be non-toxic and potentially enhance biodegradation of organic contaminants. Disposal or treatment of 'wastewater' produced from the cleaning process is a further consideration in the overall sustainability of a ballast cleaning approach.

Previously (Anderson *et al.* 2000), we considered ballast contamination in the context of modified soil analytical methodologies to assess initial and post clean-up levels of contamination. In this study, we evaluated the efficiency and potential environmental impact of two organic solvents and a water-based surfactant on diesel contaminated railway ballast.

## EXPERIMENTAL DETAILS

### Contaminated ballast

Contaminated ballast was obtained from the Haymarket Sprinter Depot, Edinburgh.

### Cleaning agents

Three cleaning agents, Terpene, Pronatur and Biosolve, were used to wash the ballast. Terpene (TP), a clear, odourless solvent with a citrusy organoleptic quality, was obtained from Bush Boake Allen Ltd., London. Pronatur (PN), an orange-coloured proprietary blend of orange oils, fully de-aromatised mineral spirits and anti-oxidants was supplied by Orapi Ltd., Liverpool. Biosolve™ (BS), a pinkish, water-soluble surfactant was obtained from Cygnus Technologies, Aberdeen.

### Laboratory-scale cleaning tests

An electric cement mixer was used to simulate the commonly employed rotary tumbling action of commercial ballast cleaners. Liners, made from 14-l PVC buckets modified by the addition of two 130 mm lengths of narrow PVC guttering, drilled to reduce resistance and glued longitudinally inside, were fitted inside the mixer. The mixer was operated at an incline of 50° to the horizontal, at a speed of 24 rpm for 15 min. Triplicate 1 kg samples of contaminated ballast were cleaned in 1 L of water; 6% BS, 10% BS, TP and PN. These were compared against three untreated control samples. At the end of each 15 min cycle, washed ballast was transferred to a clean liner and 1 L of water added. To simulate rinsing of the washed ballast, this was placed into the mixer for a further two minutes. After rinsing, the ballast was sieved (10 mm) to remove fines and the level of contamination determined.

### Determination of ballast oil contamination

The efficiency of the different cleaning agents was assessed according to the gravimetric procedure previously described (Anderson *et al.* 2000). Briefly, triplicate 200 g samples were subjected to three successive extractions in 500 mL glass beakers using 200 ml of ethyl acetate (Rathburn Chemicals, Walkerburn, Peebleshire, UK) and treated in an ultrasonic bath (Grant XB14 model, Grant Instruments Ltd., Cambridge, UK) for 15 min. Extracts were decanted into Teflon tubes, centrifuged for 5 min at 4500 rpm (MSE Mistral 1000 model), filtered through 150 mm diameter Whatman No. 1 filter paper under vacuum and transferred into 500 mL round-bottomed flasks, pre-weighed using a four-point balance (AND HR-200 model). Extracts were rotary evaporated to near dryness at 40°C (Heidolph Laborate 4000 model, Germany) and finally left to evaporate in a fume cupboard overnight. The amount of oil contamination as solvent extractable material (SEM) was determined gravimetrically.

### Analysis of extracts by gas chromatography

Extracts were re-suspended in dichloromethane for examination by gas chromatography (GC) using a Hewlett Packard HP5890 gas chromatograph equipped with a flame ionisation detector (FID). A 30 m, HP-5 column with 0.32 mm inside diameter and 0.25 μm film thickness was used to effect separation. All analyses were carried out in splitless mode at a flow rate of 30 mL $min^{-1}$ with the purge valve time set at 1.5 minutes. Helium was used as the carrier gas and was set at a flow rate of 2.5 mL $min^{-1}$. A linear temperature gradient was employed, the column temperature being held at 50°C for 2 min following injection, ramped at 10°C $min^{-1}$ to 320°C, then held at this temperature for a further 10 min. The injector and detector temperatures were set at 285 and 315°C, respectively. Sample volume of 3 μL aliquot was injected using an auto-sampler.

## RESULTS AND DISCUSSION

The results for each of the cleaning agents are summarised in Figure 1 as mg $kg^{-1}$ solvent extractable material (SEM). Compared to the untreated control, water alone removed 62% of contamination from a mean of 11450 ±1210 mg $kg^{-1}$ to 4360 ± 190 mg $kg^{-1}$. This reduction in contamination was most likely due to the physical removal of particulates through agitation with associated removal of oil and grease. The BS surfactant system reduced contamination by 91% to 990 ±110 mg $kg^{-1}$ and by 93% to 790 ± 40 mg $kg^{-1}$ at concentrations of 6% and 10% respectively. Both organic solvent-based systems showed even greater cleaning efficiencies. TP reduced contamination by 98% to 250 ± 5 mg $kg^{-1}$ and PN by 96% to 480 ± 10 mg $kg^{-1}$. There was no significant difference in the cleaning efficiency for the three cleaning agents.

The chromatograms obtained from the GC-FID analysis of the extracts are shown in Figure 2 (a)–(d) for ballast treated with 10% BS, TP and PN in addition to untreated ballast. The chromatogram obtained for untreated ballast (Figure 2 (a)) exhibited a large 'hump' between 10 and 40 minutes. This is primarily composed of petroleum hydrocarbons comprising many compounds and associated isomers, especially those above about $C_8$. Isomers of nearly the same boiling point co-elute giving rise to what is known as an unresolved complex mixture (UCM) (TPHCWG 1998).

In Figures 2 (b)–(d), the presence of UCM is considerably reduced for BS, TP and PN and reflects the results obtained from the gravimetric analysis, i.e. lower SEM values shown in Figure 1. However, a

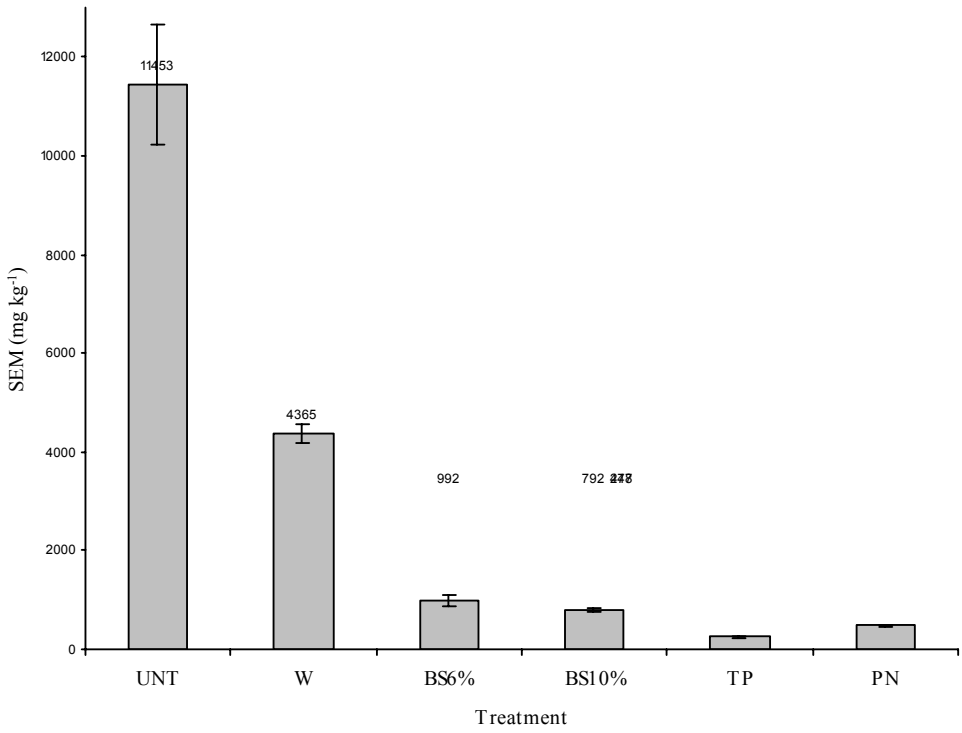


*Figure 1. Cleaning efficiency of the different ballast-cleaning agents, TP, PN and BS compared to treatment with water (W) and untreated ballast (UNT). Error bars represent standard error of the mean (number of samples is 3 in each case)*

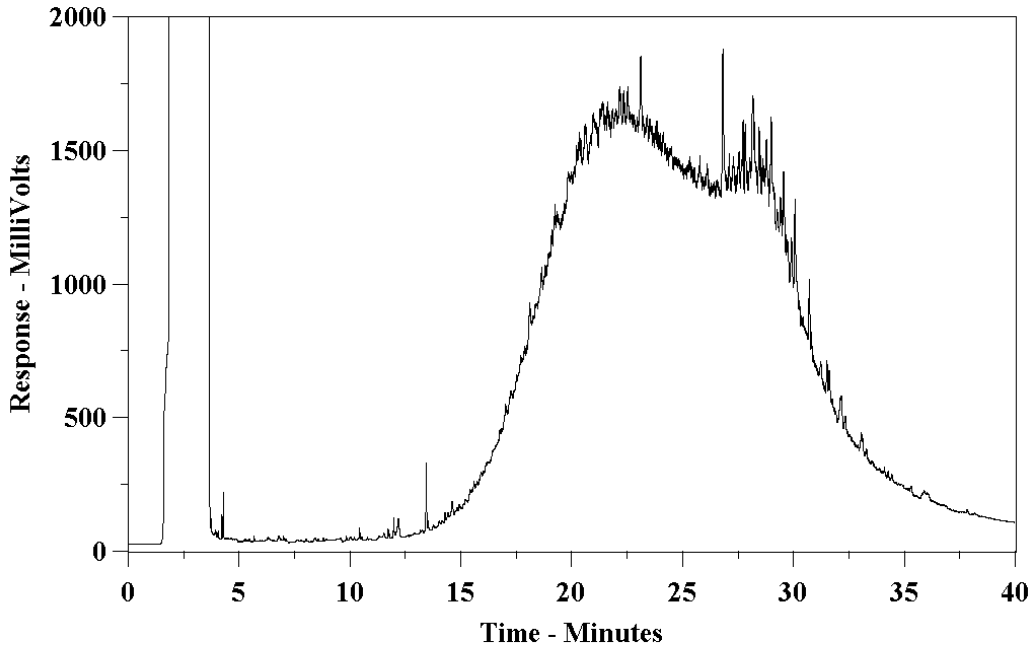


*Figure 2 (a)*

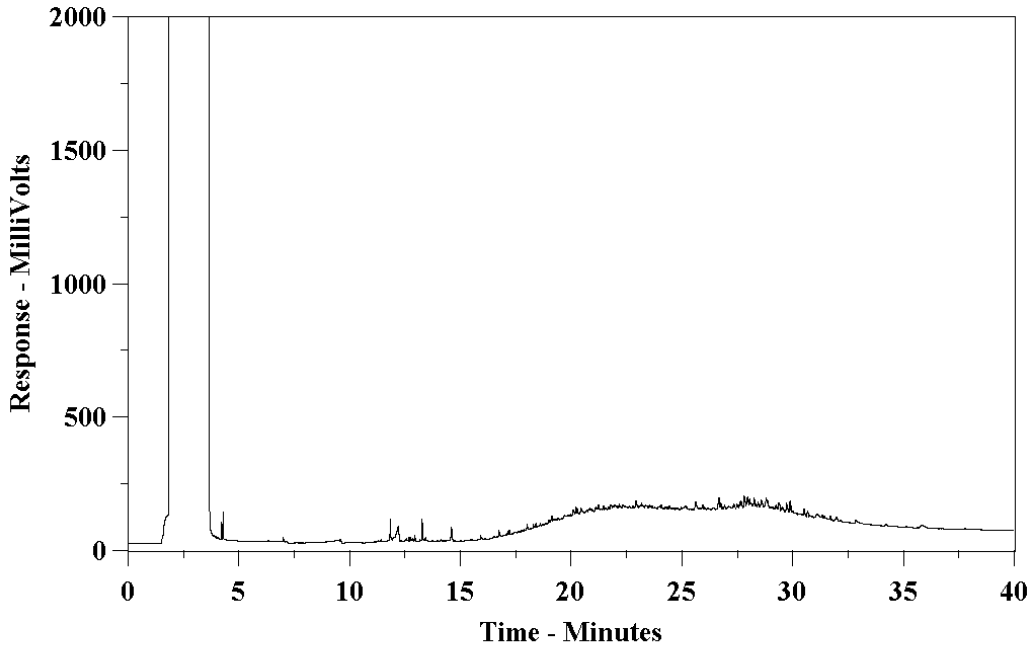


*Figure 2 (b)*

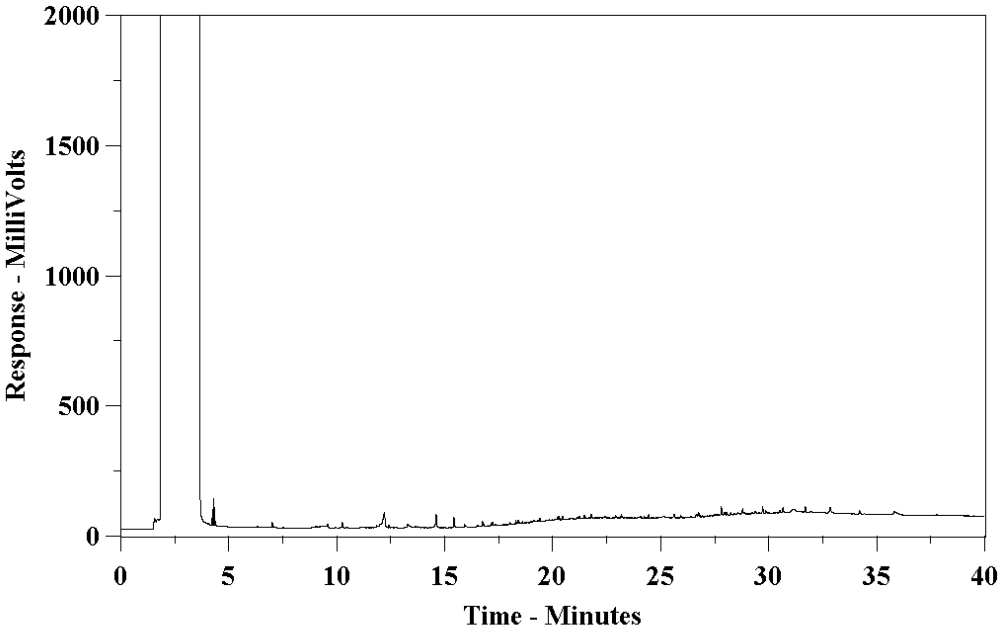


*Figure 2 (c)*

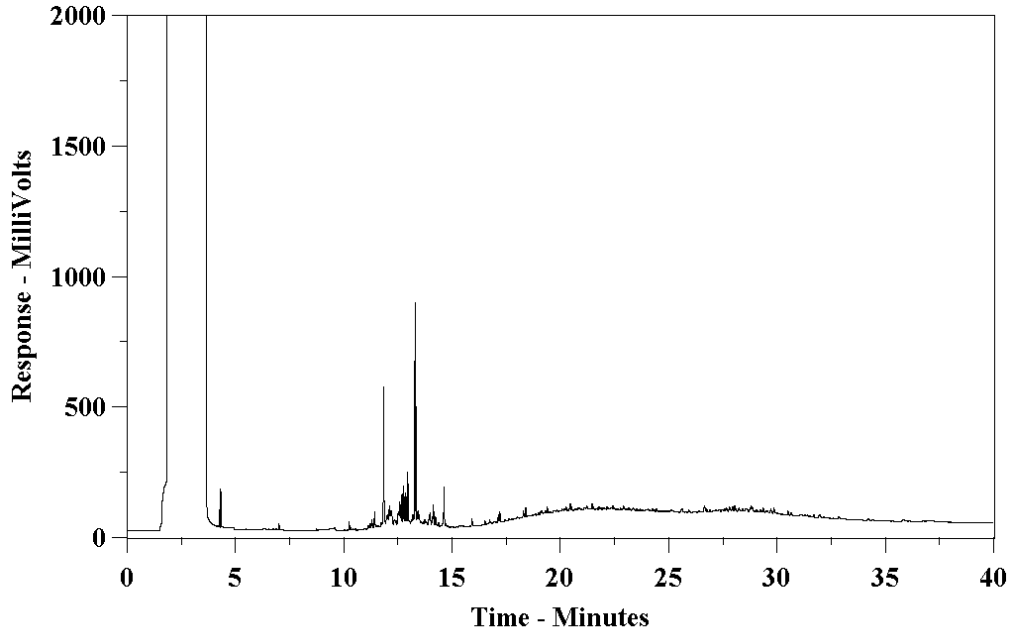


*Figure 2 (d)*

*Figures 2 (a) – (d). Gas chromatograms of extracts obtained from solvent extraction of ballast subjected to the different treatments: (a) untreated ballast, (b) 10% BS, (c) TP and (d) PN.*

number of additional peaks, not present in the chromatograms for the other treatments, appeared in the chromatogram between 10 and 15 minutes for the extract obtained after treatment with PN. It is thought these peaks represent trace amounts of PN left on the surface of the ballast after rinsing. Therefore, the SEM value obtained for PN may be a slight overestimate as this value has contributions from both the oil contamination and residual solvent.

Residual solvent raises concerns over potential adverse effects cleaning agents may have on the environment. Leaching may result in the mobilisation of contaminants. This is problematic both in the case of cleaned ballast returned to the track and material produced at a treatment plant for use as a fill material. However, the rinsing procedure used by a commercial washing system will be more vigorous in eliminating or reducing levels of residual contamination.

According to the suppliers, Orapi Ltd., PN is fully biodegradable in 30 days and degradation rates could be increased if microbes are added either (a) to the rinse water prior to rinsing the treated ballast, or (b) sprayed onto the treated ballast after it has been returned to the track.

Another potential environmental impact is disposal of waste from the washing process and the effect on the overall sustainability of the cleaning programme. Although the cleaning agents are not classed as hazardous, after use they might not be suitable for disposal to foul sewer untreated and could be considered as special waste. For example, TP or PN waste discharged to sewer represent high biological and chemical oxygen demands (BOD/COD) (State of Florida Department of Environmental Protection 1995). Irrespective of the cleaning system adopted and on the waste produced, advice must be sought from the local water authority before disposing of the waste in this way. If disposal to sewer is not permitted, other alternatives have to be considered. TP has a very high BTU (British Thermal Unit) value as a fuel source, so incineration as waste oil is an option. However, vacuum distillation of TP waste offers the possibility of reclaiming this solvent, negating the need to replenish solvent stocks for cleaning (S. Perry, Bush Boake Allen Ltd., pers. comm. 2002). In addition, a significant amount of fines will be removed from the ballast during the washing process and, if collected with the wastewater, allowed to settle and separated from the waste liquor, can be disposed of separately.

Another consideration in selection of a suitable cleaning agent for railway ballast is the cost. The surfactant based BS is currently 50% of the cost of TP at £0.32 per litre compared to £0.65. It is only 5% of the cost of PN, costing £7.00 per litre. The cost of applying these solvents will be affected by the concentrations used (e.g. less than 6% for BS) or if a means of recycling or reclamation has been implemented.

## CONCLUSIONS AND FUTURE WORK

The main objective of this study was to compare the efficiency of three cleaning agents applied to contaminated railway ballast. From the gravimetric assessment data little difference between the cleaning systems was observed as each removed over 90% of the contamination present on the ballast. However, from the chromatographic data obtained for the extracts produced after treatment with PN, there was evidence of residual solvent remaining on the ballast. Although only present for PN, rinsing of ballast will be an important consideration when assessment of cleaning agents is made at full scale. Additional rinse cycles or the incorporation of a biological treatment could help prevent cleaning agent from leaching into the environment. Improvements to the cleaning process will also be considered taking into account the concentration and temperature of the cleaning agent and application of abrasives to aid in the removal of fines.

Careful consideration should also be given to the disposal of the waste produced after cleaning. Incineration, landfilling or recycling of the waste are the options available, the choice depending on the solvent system and on the nature of the waste (hazardous, containing fines, etc.). The cost of BS is half that of TP and only a fraction of the cost of PN and although recycling solvents by distillation is an option, albeit expensive, application of BS represents the cheapest treatment and cleans equally efficiently.

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## Appendix D. Anderson *et al*. (2003)

Anderson, P., **Cunningham, C.J.**, Hearnden, R., Barry, D.A., Philp, J.C. (2003). Optimisation and Assessment of Different Railway Ballast Cleaning Systems. *Land Contamination & Reclamation.* 11 (4), 1-7.

### *D.1 Declaration of contribution*

This work on ballast cleaning was also a follow up to the previous paper and the author again drove the project and was involved in undertaking the laboratory work and also closely involved in the interpretation of results and at all stages of writing the paper.

*Land Contamination & Reclamation*, 11 (4), 2003 

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# Optimisation and assessment of different railway ballast cleaning systems

P. Anderson, C.J. Cunningham, R.A. Hearnden, D.A. Barry and J.C. Philp

Abstract
*Spent railway ballast is a source of recycled aggregate. Recycling of aggregates contributes to sustainable development by reducing the volume of construction waste going to landfill, reducing transportation and reducing the impact of primary mineral extraction supplying primary aggregates. Railway ballast is renewed when it loses its geotechnical properties and is no longer able to support the track adequately and provide drainage. Alternatively, ballast is removed from locations where contamination, primarily by diesel, is unsightly and adds to the characteristic smell of a UK railway station. In this case ballast must first be cleaned before reuse as aggregate. Track-mounted systems exist to remove the ballast by vacuum and return it to the track after processing. Off-site systems are similar to traditional soil- and gravel-washing plants. An optimised cleaning system can represent savings in both time and money, producing less waste for processing and disposal and returning more materials to the marketplace. Such an approach is in keeping with the overall thrust of sustainable engineering. In this study, the primary factors of contact time, cleaner concentration and abrasive action were investigated for a surfactant-based cleaning agent (Biosolve®), applied to contaminated railway ballast using a laboratory-scale cleaning system. It was found that a 15-minute wash cycle incorporating a 1% surfactant solution concentration with abrasive action gave the optimum cleaning efficiency, reducing contamination by 86% from 17510 ±445 to 2525 ±345 mg $kg^{-1}$. Several batches of contaminated ballast could be cleaned before significant reduction in cleaning efficiency was observed. Potential environmental impacts of surfactant and hydrocarbon residues were considered. The metal content and the biodegradability, with respect to the biochemical oxygen demand (BOD), of wastewaters generated were also measured.*

Key words: cleaning, contamination, environmental impact, optimisation, railway ballast

## INTRODUCTION

Spent railway ballast is a source of recycled aggregate. Recycling of aggregates contributes to sustainable development by reducing the volume of construction waste going to landfill, reducing transportation and reducing the impact of primary mineral extraction supplying primary aggregates. Railway ballast is renewed when it loses its geotechnical properties and is no longer able to support the track adequately and provide drainage (Awoleye 1998). Alternatively, ballast is removed from locations where contamination, primarily by diesel, is unsightly and adds to the characteristic smell of a UK railway station. In this case ballast must first be cleaned before reuse as aggregate. One track-mounted system developed in the UK lifts contaminated ballast from the track by vacuum. Ballast then passes through two consecutive rotating drums containing a cleaning agent (terpene solvent) and then a water rinse. The system is capable of cleaning a 300 m length of track to a sleeper depth of 150 mm in 12 hours, returning the ballast to the track. Oil-containing waste solvent can be processed as a low-grade fuel, or disposed of (Monbiot 1999). Off-site systems are similar to traditional soil- and gravel-washing plants. These

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may potentially employ a combination of mechanical screening, physical, chemical and biological treatment (Tiefel *et al*. 1994).

Recent figures indicate an increase in the use of recycled aggregates in the UK. The total arisings of spent railway ballast in England and Wales during 2001 were reported as 1.3 Mt $yr^{-1}$ with 1.24 Mt $yr^{-1}$ used as aggregates (ENDS 2002). However, this report also raised a key question regarding the nature of the recycling of aggregates and the extent to which high-value end uses exist or whether they were being used simply as 'fill' material. In order to ensure the highest-value end use diesel contaminated railway ballast must be cleaned sufficiently to remove odour and contamination. In a previous study, Anderson *et al.* (2002) reported on the cleaning efficiency and environmental impacts of different cleaning agents for contaminated railway ballast. We found that the commercially available surfactant blend, Biosolve® (BS), was the most cost-effective and environmentally sustainable option. In this study, we attempted to optimise the factors affecting the cleaning process: mode of action, concentration and wash cycle time, and examined recycling of BS to clean fresh batches of contaminated ballast.

Potential environmental impacts of the cleaning process, whether applied by a track-mounted system or *ex situ* at a washing plant, mainly involve the release of residual contamination mobilised by the addition of surfactant and disposal of wash and rinse waters. We therefore also investigated the biodegradability of liquid wastes and leachate from the cleaning process by measuring the biochemical oxygen demand (BOD) and the potential for metal release by analysing waste rinse and leachates by ICP-AES (inductively coupled plasma atomic emission spectrometry). The cleaning process used in this work is illustrated in Figure 1.

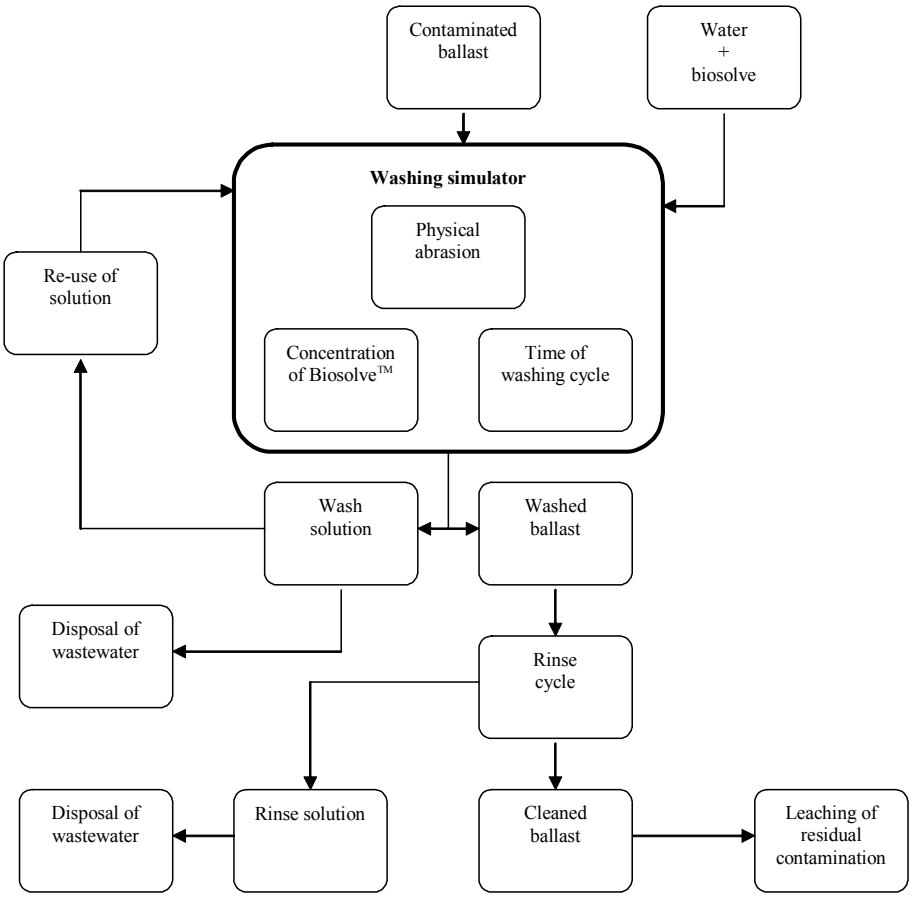


FIGURE 1. FLOW CHART ILLUSTRATING THE FACTORS AFFECTING THE PROCESS OF CLEANING CONTAMINATED RAILWAY BALLAST

## EXPERIMENTAL DETAILS

### Materials

Contaminated ballast was obtained from the Haymarket Sprinter Depot, Edinburgh. Biosolve® was obtained from Cygnus Technologies, Aberdeen, UK. All other laboratory reagents used were of analytical grade (Rathburn, Peebles, UK). Metal standard solutions were prepared from 1000 μg $mL^{-1}$ spectrosol stock solutions (Merck, Poole, Dorset, UK).

### Ballast cleaning process

The ballast-cleaning simulator was set up as described by Anderson *et al.* 2002. Briefly, an electric cement mixer operated at a speed of 24 rpm and an incline of 50° to the horizontal was used to simulate the full-scale washing action. Ballast was cleaned in 14 L PVC buckets modified with perforated, longitudinal fins to facilitate mechanical agitation or with no fins but lined with Astroturf®. Each wash cycle used 2 kg of contaminated ballast with 2 L of cleaning solution. Samples were washed for a set period of time at the end of which treated ballast was transferred to a similar clean bucket, with 1 L of water and rinsed in the mixer for 2 minutes. Wash and rinse samples were collected and the level of contamination remaining on the treated ballast was then determined.

Three samples were cleaned in the finned and Astroturf®-lined system as described above in 6% BS for 15 minutes. The effects of 1%, 3% and 6% concentrations of BS were then assessed with a wash-cycle time of 15 minutes. Finally, the effect of wash-cycle time was assessed for five, ten and 15 minutes using 1% and 6% concentrations of BS cleaning solution. Samples for BOD determination of the wash and rinse waters were taken from a 15-minute wash using 6% BS in the Astroturf®. Circulating 1 L of tap water through 1 kg of the freshly cleaned ballast three times then generated the leachate.

### Determination of ballast oil contamination

The cleaning efficiency of the washing process was assessed using the gravimetric procedure previously described (Anderson *et al.* 2000). Triplicate 200 g samples of ballast were subjected to three consecutive extractions in 500 mL beakers using 200 mL of ethyl acetate and agitated in an ultrasonic bath (Grant XB14 model, Grant Instruments Ltd, Cambridge, UK) for 15 minutes. Extracts were transferred into teflon tubes, centrifuged for two minutes at 4500 rpm (MSE Mistral 1000 model), filtered under vacuum through 150 mm diameter Whatman No. 1 filter paper and decanted into 500 mL round-bottom flasks, pre-weighed using a four-point balance (AND HR-200 model). Extracts were evaporated to near dryness using a rotary evaporator (Heidolph Laborate 4000 model, Germany) operated at a temperature of 40 °C and left to evaporate overnight in a fume cupboard. Flasks were reweighed and the total oil contamination, as solvent-extractable material (SEM), was determined gravimetrically. To appraise the results statistically and compare treatments and cleaning systems a Tukey's pair-wise comparison was carried out using Minitab.

### Determination of five-day biochemical oxygen demand ($BOD_5$) in leachate, wash water and rinse samples

Five-day Biochemical Oxygen Demand ($BOD_5$) was measured using a WTW Oxi-Top® (Oxi-Top, Germany) bottle system based on pressure measurement using a modification of a standard $BOD_5$ test (US EPA 1995). Range finding was done by dilution of test samples in dilution water and commercial inoculum of microorganisms applied according to manufacturer's instructions (Polyseed, Interbio, Texas, US). A capsule was added to 500 mL of dilution water, which was then aerated for 60 minutes. The standard used was glucose-glutamic acid. Dissolved oxygen (DO) was measured at the beginning and after five days of incubation at 20 °C, and $BOD_5$ was calculated after correction for the seed blank (See Equation 1). Dilution water consisted of (g $L^{-1}$ in air-saturated distilled water): $KH_2PO_4$, 0.085; $Na_2HPO_4.2H_2O$, 0.334; $NH_4Cl$, 0.005; $CaCl_2.2H_2O$, 0.364; $MgSO_4.7H_2O$, 0.225; $FeCl_3.6H_2O$, 0.0025, with pH adjustment to pH 7.4.

$$BOD_5 = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

Equation 1 (Crities and Tchobanoglous 1998)

Where:

$D_1$ = Dissolved oxygen of diluted sample immediately after preparation, mg $L^{-1}$

$D_2$ = Dissolved oxygen of diluted sample after five days incubation at 20 °C, mg $L^{-1}$

$B_1$ = Dissolved oxygen of seed control before incubation, mg $L^{-1}$

$B_2$ = Dissolved oxygen of seed control after incubation, mg $L^{-1}$

f = Fraction of seeded dilution water volume in sample to volume of seeded dilution water in seed control

P = Fraction of wastewater sample volume to total combined volume

### Determination of metal content of rinse and leachate samples

Total concentrations of metals in leachate and rinse samples were determined by ICP-AES using a TJA IRIS instrument (ThermoElemental, USA) at 1350 W and with coolant, auxiliary and nebuliser argon gas flows of 15, 0.5 and 0.7 mL $min^{-1}$ respectively and a pump flow rate of 1 mL $min^{-1}$. Multi-element calibration standards in the concentration range 1–10 mg $L^{-1}$ were used and the emission intensity measured at two different wavelengths for each element. For all elements, analytical precision (RSD) was typically 5–10% for individual aliquots (n = 3).

## RESULTS AND DISCUSSION

An increased abrasive effect was observed in the Astroturf® (AT) system as shown in Table 1 below. Total contamination as measured by SEM was reduced from 17510 ± 445 mg $kg^{-1}$ by 85% to 2615 ± 398 mg $kg^{-1}$ and by 93% to 1245 ± 134 mg $kg^{-1}$ using the finned and AT-lined buckets, respectively. The AT system removed significantly more contamination from the ballast than the plain liner (P = 0.05) and was subsequently used to study effects of BS concentration and wash-cycle time on cleaning efficiency.

The effect of applying different concentrations of BS on cleaning efficiency is shown in Table 2. As the concentration was increased the SEM levels measured were reduced by 86% to 2525 ± 345 mg $kg^{-1}$, by 91% to 1680 ± 214 mg $kg^{-1}$ and by 93% to 1245 ± 134 mg $kg^{-1}$ for 1%, 3% and 6% respectively. No significant differences (P = 0.05) were observed comparing concentrations of BS for a wash-cycle time of 15 minutes. Indeed, without any liquid in the system, the dry AT was able to reduce the SEM by 80%. However, this was significantly lower (P = 0.05) when compared to the application of BS at concentrations used.

**Table 1. Solvent extractable material (SEM) measured with 6% BS using finned and Astroturf®-covered liners and a wash time of 15 minutes (n = 6)**

| Cleaning system | Solvent-extractable material (mg $kg^{-1}$) ± standard error of mean | Contamination removed (%) |
|---|---|---|
| Untreated | 17510 ± 445 | – |
| 6% BS, finned | 2615 ± 398 | 85 |
| 6% BS, Astroturf® | 1245 ± 134 | 93 |

**Table 2. SEM measured for different cleaning systems using Astroturf®-covered liner and a wash time of 15 minutes (n = 6)**

| Cleaning system | Solvent-extractable material (mg $kg^{-1}$) ± standard error of mean | Contamination removed (%) |
|---|---|---|
| Untreated | 17510 ± 445 | – |
| Dry, Astroturf® | 3470 ± 419 | 80 |
| 1% BS, Astroturf® | 2525 ± 345 | 86 |
| 3% BS, Astroturf® | 1680 ± 214 | 91 |
| 6% BS, Astroturf® | 1245 ± 134 | 93 |

The influence of wash-cycle time, for BS concentrations of 1% and 6%, is shown in Table 3. For 1% BS, the residual SEMs were measured as 4532 ± 97 mg $kg^{-1}$, 3673 ± 562 mg $kg^{-1}$ and 2525 ± 345 mg $kg^{-1}$ for 5, 10, and 15 minute cycles, respectively. For 6% BS, the corresponding values were measured as 3073 ± 85 mg $kg^{-1}$, 2600 ± 556 mg $kg^{-1}$, and 2310 ± 415 mg $kg^{-1}$ for 5, 10 and 15 minutes. Due to the high variability in some of the results, the only significant differences (P

**Table 3. SEM measured for different cleaning systems using Astroturf®-covered liner for wash times of 5, 10 and 15 minutes**

| Cleaning system | Wash-cycle time (mins) | Solvent-extractable material (mg $kg^{-1}$) ± standard error of mean | Contamination removed (%) |
|---|---|---|---|
| Untreated (n = 6) | – | 17510 ± 445 | – |
| 1% BS, Astroturf® (n = 6) | 5 | 4532 ± 97 | 74 |
| | 10 | 3673 ± 562 | 79 |
| | 15 | 2525 ± 345 | 86 |
| 6% BS, Astroturf® (n = 3) | 5 | 3073 ± 85 | 82 |
| | 10 | 2600 ± 556 | 85 |
| | 15 | 2310 ± 415 | 87 |

**Table 4. $BOD_5$ levels measured for wash, rinse and leachate waste produced from treating railway ballast with 6% BS and wash time of 15 minutes (n = 3)**

| Sample type | Initial dissolved oxygen (mg $L^{-1}$) | 5-day dissolved oxygen (mg $L^{-1}$) | Fraction of seed in sample to seed in control (f) | Fraction of wastewater to total sample volume (P) | $BOD_5$ (mg $L^{-1}$) ± standard error of mean |
|---|---|---|---|---|---|
| Control | 10 | 50 | – | – | – |
| Wash | 1130 | 2230 | 0.008 | 0.45 | 4890 ± 156.5 |
| Rinse | 20 | 50 | 0.008 | 0.99 | 50 ± 1.0 |
| Leachate | 1 | 3 | 0.008 | 0.99 | 3 ± 0.8 |

= 0.05) observed were between the system using 1% BS and wash-cycle time of five minutes with those using BS concentrations of 1% and 6% and wash-cycle times of 15. Interestingly, when wash water was re-applied to a fresh batch of contaminated ballast, the contamination was reduced to 1490 ± 30 SEM mg $kg^{-1}$ from 17510 ± 445 mg $kg^{-1}$ representing a clean-up of 92%. Comparing the results with washing contaminated ballast with fresh 6% BS (2310 ± 410 SEM mg $kg^{-1}$, representing a clean-up of 87%) showed no significant differences (P = 0.05).

$BOD_5$ levels measured for wash, rinse and leachate waste produced from treating the ballast with 6% BS along with values for control are summarised in Table 4. The $BOD_5$ values measured for the waste washes (approximately 5000 mg $L^{-1}$) would not allow for direct discharge to sewer without incurring a treatment charge. Biochemical oxygen demand for domestic sewage is approximately 300 mg $L^{-1}$ (Lester 1990). Although the rinses could be discharged to sewer, the $BOD_5$ levels are in excess of the target for a Class D river and would cause 'serious pollution' (SEPA 1997).

The metal contents of the rinse and leachate samples are shown in Table 5. Many of the analytes determined were below the detectable limit of the ICP-AES instrument as indicated by <DL. Although concentrations of some metals would not meet stringent drinking water guidelines the levels are not high enough to prevent discharge to sewer.

The main objective of this study was to optimise the process of cleaning contaminated railway ballast using a water-based surfactant considering the effects of concentration, wash-cycle time and mode of action. Introducing an abrasive surface to the plain liner significantly improved cleaning efficiency although a more robust material than AT would be required to withstand the rigours of an industrial-scale process. Reducing the wash-cycle time while using the recommended BS concentration of 6% did not impair cleaning efficiency, whereas reducing the cleaner concentration (to 1%) and wash-cycle time (to five minutes) simultaneously had a detrimental effect. Reusing BS wastewater on fresh railway ballast gave a comparable cleaning efficiency, raising the prospect of not having to separate BS from the wastewater for reuse. Thus, a decision on the optimum conditions for ballast washing becomes mainly economic: is it cheaper to use a more diluted solution of cleaner or to spend less time cleaning the ballast? In addition, if the temperature of the cleaner could be raised during the wash cycle (e.g. using heat generated from other on-site processes) further improvements to cleaning efficiency would be expected. Furthermore, a system employing an abrasive surface to aid cleaning might also have the desired effect of allowing reduced volumes of cleaner to be used whilst not impairing cleaning efficiency. Again the economics of these approaches must be taken into account. However, using the least amount of cleaner (1% BS), while maintaining an acceptable level of cleaning (e.g. wash cycle of 15 minutes), will also reduce the likelihood of residual surfactant, on the surface of cleaned ballast, being returned to the track. Thus, there will be minimal potential for mitigation of remaining contamination, although any minute amounts of residual BS remaining will be quickly weathered.

If the geotechnical properties of the cleaned material had deteriorated to the extent that it was no longer suitable as railway ballast, alternative end uses could be considered. For example, it could be used as a fill material (e.g. in laying of road surfaces), in the preparation of concrete or even as an additive to soil for landscaping. However, for any future potential application it is imperative that the cleaned material meets certain performance targets and specifications, e.g. physical characteristics, chemical composition, leachability, as laid down by the industrial end-users and environmental regulators.

Handling of waste wash and rinse liquors and of the by-products, i.e. separated fines and organic waste, generated from the cleaning process, is important when considering a more sustainable approach to cleaning

**Table 5. Metal concentrations measured in rinse and leachate wastewaters, and instrumental detection limits for each**

| | Concentration (mg $L^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Aluminium** | **Barium** | **Boron** | **Cadmium** | **Calcium** | **Chromium** | **Cobalt** | **Copper** |
| Rinse | 0.2 | < DL | 0.1 | < DL | 7.5 | < DL | < DL | 0.1 |
| Leachate | 0.08 | < DL | 0.03 | < DL | 0.26 | < DL | < DL | 0.01 |
| DL | 0.2 | 0.02 | 0.02 | 0.01 | 0.03 | 0.09 | 0.03 | 0.03 |
| | **Iron** | **Lead** | **Magnesium** | **Manganese** | **Nickel** | **Selenium** | **Tin** | **Zinc** |
| Rinse | 0.3 | < DL | 1.4 | 0.1 | 0.1 | < DL | < DL | 0.1 |
| Leachate | 0.01 | < DL | 0.05 | 0.01 | 0.002 | < DL | < DL | 0.01 |
| DL | 0.02 | 0.07 | 0.01 | 0.01 | 0.02 | 0.3 | 0.5 | 0.01 |

DL: detection limit, defined as three times the standard deviation of a blank analysed ten times by ICP-AES

railway ballast. Once separated from the waste liquor, the fines could be incorporated into a composting process or blended as part of manufactured topsoil. The wash produced a $BOD_5$ which was nearly 100 times greater than the rinse solution and in excess of 1600 times that of the leachate. The $BOD_5$ for the waste wash water (approximately 5000 mg $L^{-1}$) would not allow for direct discharge to sewer without incurring a treatment charge and the rinses (50 mg $L^{-1}$) would not meet the target for discharging to a watercourse. Low metal concentrations detected in the rinse and leachate samples would not present disposal problems. At dedicated treatment facilities conducting ballast cleaning, one sustainable option may be to direct the wastewaters for treatment in a constructed wetland. In recent years constructed wetlands have been used to remove contaminants from wastewater, whether it is effluent from municipal or private waste systems, industrial or agricultural wastewater, or acid mine drainage (Cooper *et al*. 1996). If developed, a constructed wetland system offers the potential as a low-cost approach for the treatment of waste liquor from washing railway ballast.

## CONCLUSIONS AND FUTURE WORK

Use of abrasive action in the form of an Astroturf® covered liner removed significantly more hydrocarbon contamination from railway ballast compared to a non-abrasive design. Using this abrasive design and maintaining a wash cycle in excess of five minutes allowed a concentration as low as 1% BS to remove up to 90% of the contamination. Future work will include investigating scale-up of the cleaning process taking into account the efficacy of temperature-controlled cleaning and the volume of cleaner giving an optimum cleaning efficiency. Measured levels of BOD in the wastewaters were higher than the target for discharge to watercourses, thus requiring further treatment or dilution prior to disposal. Future work will therefore also include consideration of wastewater treatment from the ballast cleaning process, possibly utilising constructed wetlands.

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## Appendix E: Cunningham & Philp (2000)

**Cunningham, C.J.**, Philp, J.C. (2000). Comparison of Bioaugmentation and biostimulation in *ex situ* Treatment of Diesel Contaminated Soil. *Land Contamination & Reclamation*. 8 (4), 261-269.

### *E.1 Declaration of contribution*

The author was heavily involved in all aspects of the project from the initial design and site investigation. The author carried out all of the laboratory work with the exception of the determination of metals by ICP-MS which was carried out by an external laboratory and was closely involved in the interpretation of results and at all stages of writing the paper.

 

# Comparison of Bioaugmentation and Biostimulation in *ex situ* Treatment of Diesel Contaminated Soil

C. J. Cunningham and J. C. Philp

Abstract

*A bioremediation programme was designed to investigate several factors that may influence the rate of diesel removal in an* ex situ *treatment of a contaminated soil. These were bioaugmentation; biostimulation via inorganic fertiliser (NPK) or manure as an organic source of nutrients; and bulking agents added to improve aeration within the systems. From a high initial level of diesel, removal/degradation proceeded rapidly in all but the non-amended control. In non-augmented systems, diesel removal in windrows proceeded significantly more rapidly than in biopiles. However, the most rapid remediation occurred in bioaugmented systems, where the inoculum consisted of laboratory enrichments of diesel-degrading microorganisms, with soil from the contaminated site as initial inoculum. All such systems reached the remediation end-point within one week, and no difference in rate due to windrows, static biopiles, or source of nutrients could be discerned.*

Keywords: bioaugmentation, bioremediation, biostimulation, biopile, windrow, contaminated land

## INTRODUCTION

Bioremediation may be defined as the use of microorganisms to degrade pollutants (Atlas and Bartha 1998). This approach to the restoration of contaminated environments exploits the metabolic diversity and adaptability of microorganisms to degrade or transform a wide range of organic and inorganic contaminants. As a treatment technology, bioremediation has been most widely applied for degradation of petroleum hydrocarbons including petrol, diesel, jet fuel, and heating oils.

In most cases, the treatment of oil contaminated environments has involved biostimulation – the addition of nutrients to stimulate the indigenous microbial population (Bartha 1986; Leahy and Colwell 1990; Morgan and Watkinson 1989). Rosenberg and Gutnick (1986) proposed that approximately 150 mg nitrogen and 30 mg phosphorus are required for metabolism of 1 g of hydrocarbon substrate. However, there has been considerable debate over the efficacy of bioaugmentation (*e.g.* Atlas 1991; Pritchard 1992; Vogel 1996), the addition of dried or liquid cultures of either indigenous or exogenous microorganisms to expedite the remediation process.

Diesel is largely comprised of simple un-branched *n*-alkanes, with only around 4% of polyaromatic compounds (Heath *et al*. 1993). Although metabolism of *n*-alkanes from $C_6$ to $C_{12}$ is possible (Chakrabarty 1973) these may however act as solvents, permeabilising cells by partial solubilisation of membrane phospholipids (Sikkema *et al*. 1995) and are therefore toxic to many microorganisms. The initial enzymes required for alkane metabolism are mono-oxygenases. Meta-cleavage dioxygenases are key enzymes in the degradation of aromatic compounds (Daly *et al*. 1997). Polycyclic aromatic hydrocarbons (PAHs) such as naphthalene and phenanthrene are readily biodegradable; however, PAHs with more than five rings may be recalcitrant (Allard and Neilson 1997). As these enzymes consume oxygen, it must be available in sufficient quantities to prevent limitation of hydrocarbon

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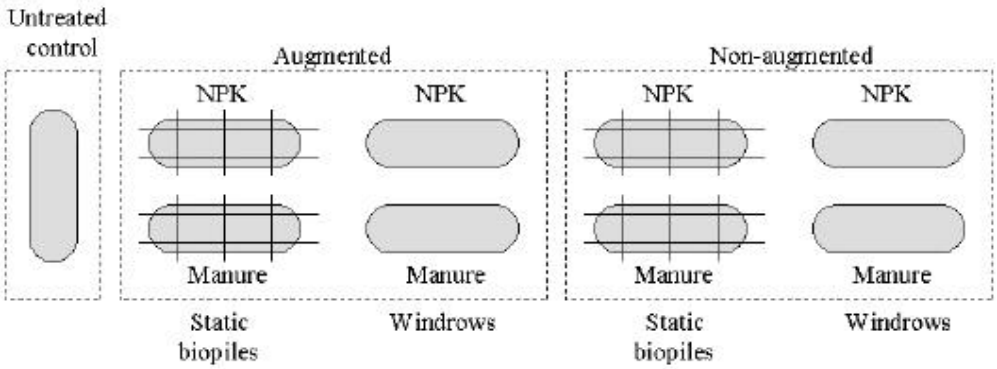


*Figure 1. Site layout*

degradation. One approach to the enhancement of oxygen transfer in constructed windrows or biopiles is the addition of bulking agents such as wood chips, sawdust, leaves or shredded rubber tyres (Cookson 1995) to improve the porosity of soils.

Other factors limiting microbial biodegradation include temperature, soil moisture, and pH. Within the range of 10°C to 45°C, the rate of microbial activity typically doubles for every 10°C increase in temperature (Atlas and Bartha 1998). Temperature will also influence the physical nature of hydrocarbons. For example, short chain alkanes will be more readily volatilised at higher temperatures (van Deuren *et al.* 1997). Water availability in contaminated soils may limit microbial activity and growth. However, excessive water may result in blockage of soil pores and therefore limit oxygen transfer. During treatment, water content is typically retained at 50-80% of soil water holding capacity (Cookson 1995). The optimum pH range for hydrocarbon degradation in soil has been commonly reported as being between 6.5-8 (Morgan and Watkinson 1989). Dibble and Bartha, (1979) concluded that pH 7.7-7.8 was optimal for hydrocarbon degradation and suggested that lower values may result in partial inhibition of degradation.

The present study examined the efficacy of bioaugmentation at a railway siding where contamination, principally by diesel fuel, had occurred over several decades due to leakage from stabled Diesel Motor Unit (DMU) sets. Soil bioremediation may be broadly divided into *in situ* and *ex situ* strategies. *In situ* bioremediation refers to treatments not requiring the excavation of contaminated soil prior to treatment. Common *ex situ* treatments include landfarming, windrows and biopiling. This study also compared the choice of engineered *ex situ* treatment using both windrow and biopile systems with either NPK fertiliser or horse manure as the source of nutrients.

There have been numerous reported studies on bioremediation of hydrocarbon contaminated soils. In a relevant example, Balba *et al*. (1991) studied the bioremediation of contaminated soils from railway maintenance yards, where the contamination consisted of diesel and heavy motor oil, and varied between 5000 and 60 000 mg $kg^{-1}$ dry weight soil of Total Petroleum Hydrocarbon (TPH). These were mostly linear and branched alkanes of $C_{22}$ and above. In 500 g microcosms at 15°C, they found up to 94% removal of TPH in less than 16 weeks. In the next phase of the work field trials of 40 m x 4 m beds were constructed. This soil had over 100 000 mg $kg^{-1}$ dry soil of TPH. More than 85% degradation was achieved in less than 28 weeks.

## MATERIALS AND METHODS

### Site assessment

The site consisted of waste ground with large amounts of clinker and ash on top of a layer of clay approximately 3 m below ground level. Samples were taken for gravimetric total oil and grease (O and G) analysis. Heavy metals analysis was performed by inductively coupled plasma/mass spectrometry (ICP-MS). Soil pH was also measured.

### Bioaugmentation cultures

A composite sample was obtained from various locations around the site at depths of up to 30 cm. Microorganisms were desorbed from the soil in a 0.85% (w/v) NaCl and 0.2% (w/v) tetra-sodium pyrophosphate ($Na_4P_2O_7$) solution, including sonication for 1 min. Serial enrichment of duplicate isolated cultures was carried out in a defined mineral medium prepared from 10 ml of salt solution A; 990 ml distilled water; $(NH_4)_2SO_4$, 1 g; $K_2HPO_4$, 1 g. Salt solution A con-

tained ($l^{-1}$ of 0.1M HCl): $MgSO_4.7H_2O$, 25 g; $FeSO_4.7H_2O$, 0.28 g; $MnSO_4$. $H_2O$, 1.70 g; NaCl, 0.60 g; $CaCl_2.2H_2O$, 0.10 g; $MoNa_2O_4.2H_2O$, 0.10 g; $ZnSO_4.7H_2O$, 0.06 g (Goodhue *et al*. 1986).

Filter sterilised diesel (aerated for 48 hours to remove volatile components) was added at 2% (v/v) at the start of each enrichment as the sole carbon source. After three enrichment steps with one week incubation periods (shaking incubator at 200 rpm and 27°C), a 20-litre aspirator containing 15 litres of mineral medium was inoculated with 10 ml from each of the duplicate serial enrichment cultures. The batch culture, consisting of a mixed consortium of bacteria, fungi and yeast was grown with 2% (v/v) diesel (treated as above) for ten days, and aerated using a small air pump. The culture was centrifuged in batches, the supernatants of which were discarded and the pellets resuspended in the same volume of sterile 0.85% NaCl. This removed inorganic nutrients and residual diesel from the inoculum.

**Site development**

Contaminated soil was excavated to a depth of approximately one metre using a mini-excavator. Biopiles and windrows of approximately 1.5 m x 0.5 m x 0.5 m were constructed. Coarse wood chips were used as a bulking agent, in the ratio of five parts wood chips: five parts top soil: ten parts contaminated soil. The use of horse manure, which also imparts considerable bulking, was compared with NPK fertiliser (7% each of N, P and K). The NPK application rate was one kilogram per $m^3$ (5 litres $m^{-3}$ of soil of an inoculum containing approximately $10^8$ CFU $ml^{-1}$ of hydrocarbon-oxidising bacteria). Sufficient quantities of liquid enrichment culture were added to raise the total microbial population, as Colony Forming Units per gram (CFU $g^{-1}$) by approximately one order of magnitude. Thorough mixing of all materials was achieved using an electric cement mixer. Static biopiles were aerated using 10 cm diameter slotted PVC pipe. Windrows were thoroughly mixed once weekly using a garden fork, all plots were watered once weekly as required. Two biopiles and two windrows with either NPK or manure were constructed for bioaugmentation and biostimulation alone. A control plot received no nutrients or bioaugmentation and although watered along with other plots, received no additional treatment.

**Enumeration of microorganisms**

Hydrocarbon-oxidising microorganisms were enumerated according to the 96-well microtitre plate Most Probable Number (MPN) procedure of Wrenn and Venosa (1996), modified by the addition of aerated diesel as the sole carbon source. MPN methods for enumerating hydrocarbon-oxidising bacteria have evolved from 1990 (Brown and Braddock 1990) due to a general dissatisfaction with the existing methods. Traditional plate counts based on agar media suffer from the fact that the oil (substrate) is insoluble in the highly hydrophilic agar matrix. Attempts to remedy this

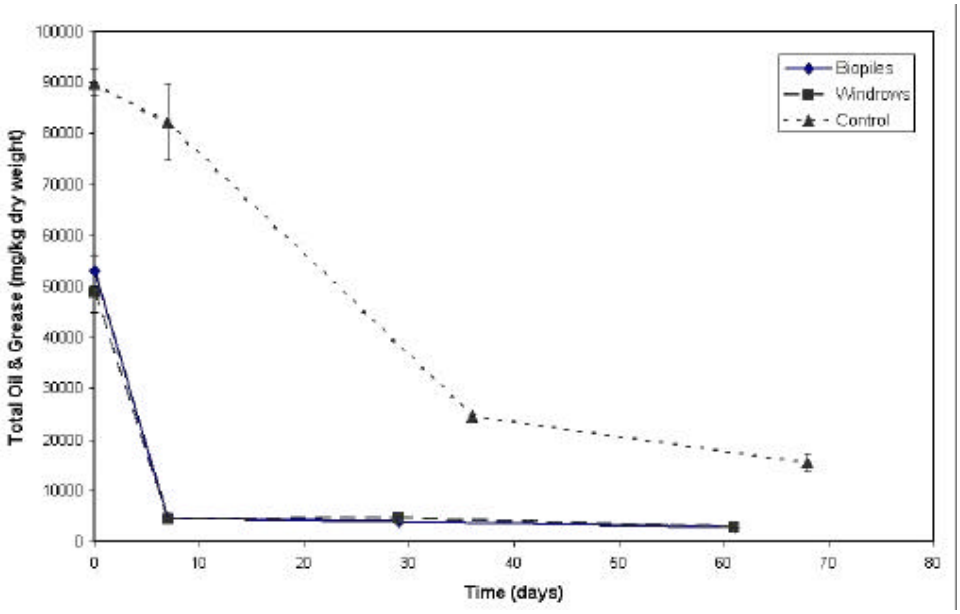


*Figure 2. Reduction in total O and G levels in bioaugmented piles*

include the use of emulsifiers to incorporate the oil into the medium. However, emulsifiers are often toxic to microbes. Alternatively, microbial growth may be at the expense of the emulsifier and not the oil. Another method is to supply the oil in the vapour phase to the surface of the agar plate (Rosenberg and Gutnick 1986; Kleinheinz and Bagley 1997). This will underestimate hydrocarbon-oxidising populations as it selects for the strains which can grow on volatile components: for example, those strains capable of growth on *n*-alkanes from $C_{12}$ upwards may not be counted.

### Determination of oil content

Triplicate 10 g soil samples were placed in a solvent system of 50 ml methanol/60 ml dichloromethane (DCM), and extracted in an ultrasonic bath at 25 MHz for 15 minutes. The solvent was decanted and filtered through Whatman GF/C filters into glass separating funnels containing 100 ml pentane-extracted water. The remaining sediment was re-extracted in 50 ml DCM. The solvent phase was filtered as described above. The DCM fraction was then collected in a pre-weighed 250 ml round-bottomed flask, rotary evaporated at 40°C, and displaced under a stream of moisture-free nitrogen. The O and G was then determined gravimetrically. Soil dry weight was determined by the method of Topp (1993).

## RESULTS

Initial characterisation of the site revealed O and G levels of almost 90 000 mg $kg^{-1}$. Due to the presence of ash and clinker from the made-ground, metals concentrations were determined to investigate their possible influence on microbial metabolism (Table 1). For this reason, the contaminated soil on the site was diluted with clean topsoil. This and the other amendments lowered the initial mean pH of around 8.1 to between 7.0 and 7.5 in the augmented and non-augmented treatment piles and no further adjustment was required during the course of the study.

**Table 1. Metal concentrations in the contaminated soil**

| Metal | Concentration (mg $kg^{-1}$) |
|---|---|
| Cadmium | 0.6 |
| Chromium | 57.4 |
| Copper | 191.0 |
| Lead | 337.0 |
| Nickel | 105.0 |
| Zinc | 323.0 |

Bioaugmented treatment systems were considered to be more successful, in terms of the time required for treatment. All treatment systems were seen to perform equally where bioaugmentation was applied. Averaged over all treatments, contamination was reduced by 91.2% from 50 990 (±3400) mg $kg^{-1}$ to 4500 (±420) mg $kg^{-1}$ in seven days (Figure 2). No distinction could be made between the source of nutrients (NPK or

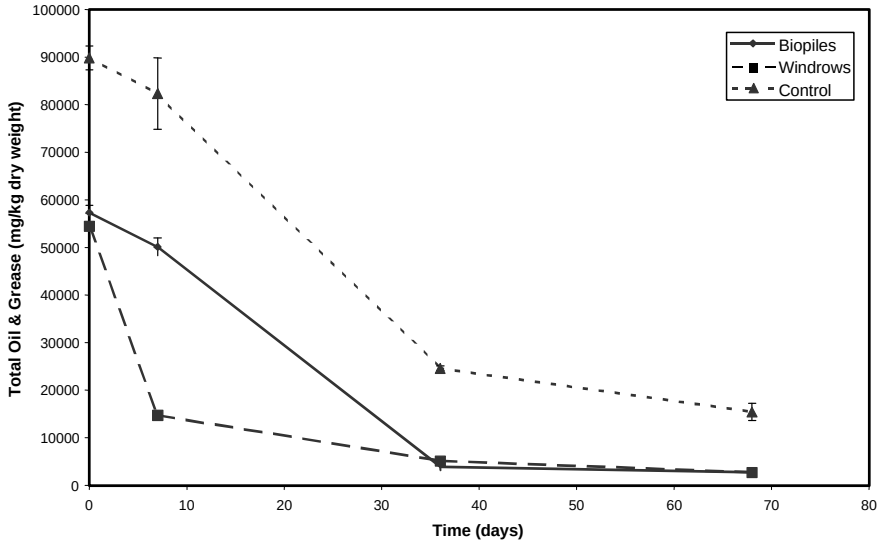


*Figure 3. Reduction in total O&G levels in non-bioaugmented piles*

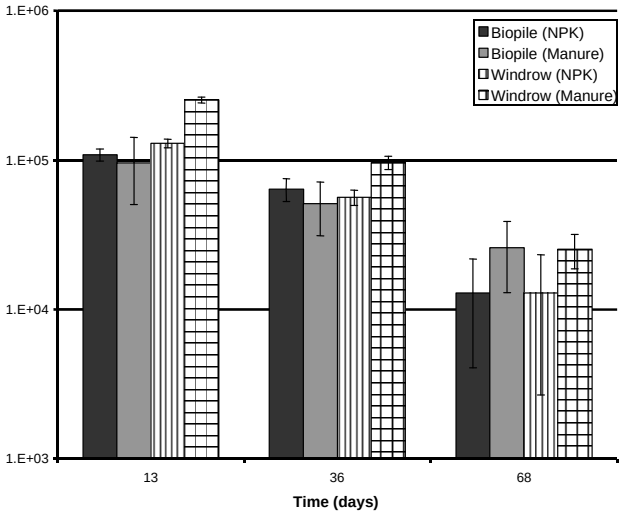


*Figure 4. Hydrocarbon oxidising bacteria in bioaugmented piles*

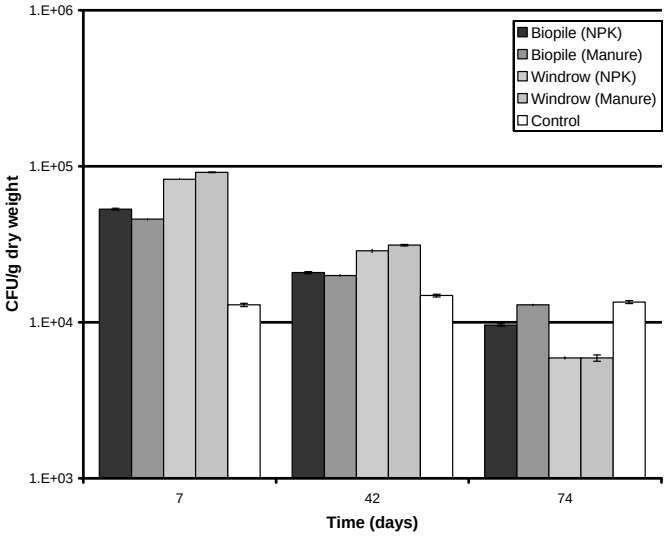


*Figure 5. Hydrocarbon oxidising bacteria in non-bioaugmented piles*

manure) or the engineered soil pile design (biopile or windrow). After this initial rapid decline, further reduction in O and G levels proceeded to an average endpoint of 2900 (±240) mg $kg^{-1}$. This represented a reduction of 94.3% for all bioaugmented treatment systems after 60 days.

Using biostimulation alone, a clear distinction was apparent between the biopile and windrow systems (Figure 3). As in the augmented treatments, no distinction could be drawn between NPK and horse manure as sources of nutrients. After seven days, contamination had reduced in windrow systems by 72.9% from an average of 54 500 (±2640) to 14 780 (±820) mg $kg^{-1}$. The corresponding figure for the non-augmented biopile systems was 12.6%, an average reduction of 57 350 (±1490) mg $kg^{-1}$ to 50 130 (±1920) mg $kg^{-1}$.

However, despite the initially higher performance of the windrow systems, after 36 days all non-augmented treatments had produced roughly equal degradation (Figure 3). After 68 days the average reduction for all non-augmented systems was 95.1%. Only 8.4% reduction was observed in the control plot (Figure 3) in the first 7 days from 89 870 (±2510) mg $kg^{-1}$ to 82 340 (±7480) mg $kg^{-1}$. These data report one method of assessing diesel levels in soil, i.e. O and G extraction in DCM and methanol. Total solvent extractable material from the uncontaminated topsoil used was also of the order of 2700 (±270) mg $kg^{-1}$. The overall hydrocarbon removal in the treatments were therefore higher than suggested by these data.

As would be expected, the diesel-degrading microorganism counts in the augmented systems were at a higher level in the early stages of remediation (Figure 4). The inoculation level was intended to raise the microbial population by approximately one order of magnitude, but the day seven counts show that the level of augmentation was lower than this.

However, the pattern of hydrocarbon-oxidiser counts in both augmented and non-augmented systems was roughly the same. In all except the control pile, there was a rapid decline in numbers between 7 and 42 days, with a less marked decline to day 74. In the control pile the level remained almost constant throughout the test period (Figure 5). These observations are consistent with the removal of oil from the various systems. The pattern in the non-augmented systems was of a more rapid decline in oil concentrations from the start of the test until day 36 (Figure 3), followed by a less rapid decline. By day 36 the end-point had been reached in all non-augmented piles.

## DISCUSSION

This study provided evidence for the efficacy of bioaugmentation for *ex situ* treatment of diesel contaminated soil. These data demonstrated one of the important benefits of bioaugmentation as a remediation strategy. Rapid mineralisation of diesel was achieved using static biopiles. In contrast to windrow systems, these are less labour intensive and do not require specialist soil turning equipment and associated staff on site to carry out a full-scale remediation project. It was not possible to detect any significant difference between the use of commercial NPK fertiliser and horse manure, which has been reported as having potential application for degradation of oil wastes when used in a composting system (Kirchmann and Wasiyhun 1998).

The augmentation was calculated to raise the numbers of bacteria by approximately one order of magnitude from the indigenous population at the site. There are various fields of application where deliberate introduction of microorganisms to soils has been practised *e.g.* enhancement of crop growth or the use of biocontrol agents for crop protection. It has often been observed that when microorganisms are added to soils as an inoculum, there is a rapid decline in inoculum population activity (Edgehill 1992). The possible reasons for these observations have been extensively reviewed recently (van Veen *et al.* 1997). Factors involved are both biotic (predation by protozoa, competition from other soil microorganisms) and abiotic (clay minerals, water tension, quality of organic carbon, nutrients, pH, temperature, toxic chemicals) (Pritchard 1992). One possible factor in addition to those identified by van Veen *et al.* (1997) is that the level of substrate is so reduced over time that the hydrocarbon-oxidising populations declined to background levels. Alexander (1999) discussed the phenomenon of threshold, whereby substrate levels become so low that the microorganisms are only able to fulfil the requirements of maintenance and are unable to grow and divide. Once growth and division through hydrocarbon oxidation has been completed upon depletion of the substrate source, and the microbial population has equilibrated through the various forces which tend to lower the biomass, organic nutrition will revert to non-hydrocarbon sources, and the population is likely to stabilise through nutrient limitation.

The efficacy of bioaugmentation is demonstrated by comparing Figures 2 and 3. By day 7 all augmented biopiles and windrows had reached the same end-point. *Ex situ* techniques must account for the losses through volatilisation (Heitzer and Sayler 1993; Arthurs *et al.* 1995). The levels in the non-augmented and control piles suggest that this rapid removal rate cannot be accounted for by volatilisation alone, and that microbial degradation was enhanced by bioaugmentation. In most cases, the rapid oil removal in augmented piles is not reflected in declining hydrocarbon-oxidiser counts. One way to circumvent reliance on microbial counts is to measure microbial activity directly using respirometry, preferable by $CO_2$ evolution. However, performing this in the laboratory is a gross modification of conditions, and site security conditions were not conducive to respirometry. Respirometry should provide direct evidence for the rate of bioremediation. Recent developments in molecular microbiology have produced genetically modified biomarkers containing bioluminescent reporter genes such as *luc*, *lux* and *gfp*, for use in bioremediation trials (Jansson *et al.* 2000). However, strict legislative controls on the release of genetically modified organisms will preclude their application in the field for the foreseeable future.

The delayed growth observed in the control pile may be a result of slow stimulation due to aeration during soil excavation, with concomitantly increased availability of oxygen in the non-amended pile, compared to the lower availability in the compacted soil before excavation. However, viable counting by agar or MPN techniques are likely to underestimate grossly the true numbers of bacteria present. Also, the numbers of viable, non-culturable microorganisms are not accounted for. The uncertainties in hydrocarbon-oxidiser counts are exacerbated by the growth substrate being insoluble. Growth limitation may result from limitation of carbon mass transfer from the oil phase to the aqueous phase.

Significantly, the initial rate of oil removal was much greater in both non-augmented windrows than in the biopiles, which may reflect better aeration and/or break-up of soil clumps. The latter is not achieved in static biopiles. By breaking up clumps, both improved aeration and a larger surface area for microbial attack (improved bioavailability) are achieved. Static biopiles would only offer increased aeration. Redistribution of moisture is also better in windrows as water movement in biopiles would occur by natural infiltration only (Rhykerd *et al*. 1999). The surface of biopiles dries rapidly, and the interior moisture content is likely to follow a gradient. More uniform water distribution is likely in windrowing operations as a result of soil turning.

Individual metal concentrations (Table 1) suggested there would be no microbial inhibition, given known values for metal resistances in bacteria. However, this did not account for a possible cumulative effect of several metals at elevated concentrations. von Fahnestock *et al*. (1998) stated that total toxic metal levels above 2500 mg $kg^{-1}$ may inhibit microbial growth. The data in Table 1 indicate that the total concentration was around 1000 mg $kg^{-1}$; however, levels of total iron were very high at 32 800 mg $kg^{-1}$. This analysis did not reflect the bioavailability, which has a crucial influence on metal toxicity (Selifonova *et al*. 1993). Many physicochemical factors may affect bioavailability, especially pH but also others such as adsorption to clay minerals in soil. Topsoil was therefore used as a means of buffering against any inhibition of microbial activity by heavy metals. It would also have acted as a further source of inoculum. However, in full-scale treatment topsoil is unlikely to be a sustainable option in most situations, although local topsoil excesses are sometimes stockpiled or landfilled. Other options as replacements for topsoil include clay minerals and waste materials. Clay minerals have cation exchange capacity that would help sorb heavy metals, but would add little as inoculum. Compost is a feasible option, but its availability is location-specific. The UK has relatively few composting facilities, but the recent EU Landfill Directive may make composting more popular, as there is a drive to reduce levels of biodegradable material going to landfill. Poultry waste is available as a waste product, and would provide inoculum, and an extraneous source of inorganic nutrients.

Horse manure not only acts as a source of nutrients, but due to the high percentage of hay, acts as a further source of bulking. A priming effect on microbial populations due to the addition of hay as a bulking agent has been observed (Rhykerd *et al*. 1999), where a greater consumption of oxygen than could be attributed to hay decomposition was noted. In the present study, such an effect in non-augmented systems was not observed. Rather, the defining difference was between windrowing and biopiling, with windrows being considerably more efficacious.

There remains confusion over the efficacy of bioaugmentation for remediation of contaminated soil. Following the *Exxon Valdez* clean up, many commercial bioaugmentation products were oversold. It has generally been stated that bioaugmentation is best applied in cases where intrinsic bioremediation or biostimulation does not work because of insufficient or non-acclimatised bacterial populations (Pritchard 1992). This is generally the case for only very recalcitrant chemicals e.g. pentachlorophenol (Otte *et al*. 1994). In most cases, this would not apply to diesel-contaminated soils. The bioaugmentation experience with diesel-contaminated soils is contradictory; however several parallels exist with other contaminants. For example, MendozaEspinosa and Stephenson (1996) demonstrated that natural activated sludge microorganisms performed as well in grease degradation as a commercial bioaugmentation product. In none of the five soils studied by Margesin and Schinner (1997) did biostimulation and bioaugmentation result in higher diesel decontamination than by biostimulation alone. Indeed, some authors have reported negative effects on diesel-contaminated soil bioremediation, either using acclimatised indigenous populations (Demque *et al*. 1997) or commercial bioaugmentation products (Moller *et al*. 1995). Radwan *et al*. (1997) described a feasibility study in which both exogenous and indigenous cultures were introduced to sand cores artificially contaminated with weathered crude oil. They concluded that, in the case of terrestrial oil spills, management of environmental conditions to stimulate the natural indigenous microbial population was likely to produce better results than bioaugmentation, especially immediately after the spill. They suggested that inability of introduced cultures to compete effectively with indigenous populations was the reason for the failure.

The implication is that bioaugmentation has to be judged as a treatment on a case-specific basis. Despite

the widespread availability of commercial bioremediation cultures, much research effort is required before use of bioaugmentation reaches greater levels of predictability. What is suggested by the literature to date and by this study is that bioaugmentation, where possible, should proceed using the indigenous microorganisms, cultured as a balanced population in the laboratory and reapplied to the soil. The ability of a commercial preparation obtained from another location to compete in a new contaminated site is not predictable at present. This would help to explain some apparent failures in bioremediation trials using bioaugmentation to treat hydrocarbon-contaminated soils.

Windrow turning involves a cost, as a capital/maintenance cost for this specialised equipment or for its rental and a labour cost. In either case, the equipment is used intermittently, so there can be high idle time. As a high level of clean-up was eventually achieved in the control pile, it may be argued that there is no need for windrows or biopiles. It should be borne in mind that the experimental piles used in this study were very small, and in full-scale piles, treatment of the order of hundreds or thousands of tonnes is normal. In such instances, after initial mixing and pile construction, the principle-limiting factor is likely to be oxygen availability. At the full-scale, if there is no amendment to improve oxygen supply, then it is conceivable that under microaerophilic/anoxic conditions the rate of oil removal would drop to zero. It can be assumed that the ten-day time saving seen experimentally would be greater in the full-scale.

If the site being treated is an inner city brownfield site, the stimulus for remediation is likely to be near-future land development. It can be assumed that even a ten day saving on-site is a significant cost saving, but that time is more important under these circumstances. The landfill option is the main competing technique, and offers an advantage of speed. For bioremediation to be accepted as a mainstream technique it must compete on a cost-per-tonne basis and treatment time is a crucial factor.

## ACKNOWLEDGEMENTS

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## Appendix F. Li *et al*. (2004)

Li, L., **Cunningham C.J.**, Pas, V. Philp, J.C., Barry, D.A., Anderson, P. (2004) Field trial of a new aeration system for enhancing biodegradation in a biopile. *Waste Management*. 24 (2), 127-137.

### *F.1 Declaration of contribution*

The observation by the author of the random placing of aeration pipes when visiting sites undergoing remediation using biopiles was a key driver for the study. Serendipity then played a role as the author noticed sunlight reflecting from a chimney cowl rotating in the wind whilst driving back from a site visit and this drove the project. To assess the efficacy of this approach required collaboration with Li and Barry who provided modelling expertise. The author was involved in undertaking the laboratory work and also closely involved in the interpretation of results and at all stages of writing the paper.

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# Field trial of a new aeration system for enhancing biodegradation in a biopile

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**Abstract**

The influence of a new aeration system on the biopile performance was investigated. The purpose was to increase biodegradation efficiency by optimising airflow through the pile. During a 1-month field trial, the performance of a new system using two perforated vertical pipes with wind-driven turbines was compared with that of a standard pile configuration with two horizontal perforated pipes. Both piles were composed of a similar mix of diesel-contaminated soils, woodchips, compost and NPK fertiliser. Hydrocarbons were recovered using solvent extraction, and determined both gravimetrically and by gas chromatography. Total heterotrophs, pH and moisture content were also assessed. Air pressure measurements were made to compare the efficiency of suction in the pipes. Results at the end of the experiment showed that there was no significant difference between the two piles in the total amount of hydrocarbon biodegradation. The normalised degradation rate was, however, considerably higher in the new system than in the standard one, suggesting that the vertical venting method may have improved the efficiency of the biological reactions in the pile. The pressure measurements showed a significant improvement in the suction produced by the new aeration system. However, many factors other than the airflow (oxygen supply) may influence and limit the biodegradation rates, including moisture content, age of contaminants and the climatic conditions. Additional experiments and modelling need to be carried out to explore further the new aeration method and to develop criteria and guidelines for engineering design of optimal aeration schemes in order to achieve maximum biodegradation in biopiles.



## 1. Introduction

Bioremediation may be defined as the use of microorganisms to degrade pollutants (Atlas and Bartha, 1998). Treatments may be broadly divided into in situ and ex situ techniques. In situ bioremediation refers to treatments not requiring the excavation of contaminated soil prior to treatment. Biopiles have become an increasingly common ex situ treatment technology, especially for petroleum hydrocarbon-contaminated land (Cookson, 1995; Martin and Bardos, 1996; Patterson et al., 1999). Contaminated sites are often polluted by a wide variety of petroleum products. Diesel is a common soil contaminant. Although diesel is largely comprised of simple unbranched *n*-alkanes in the range of $C_{10}$–$C_{22}$ (Heath et al., 1993), it also contains more recalcitrant and less bioavailable hydrocarbons. Sufficient oxygen must be available to prevent limitation of aerobic hydrocarbon degradation.

Biopiles are constructed by forming excavated contaminated soils into piles or cells above ground (Fig. 1), with the aim of enhancing conditions for biodegradation. These piles may be placed on an impermeable membrane or clay layer to prevent contamination of the surrounding area by leachate. Inside the biopile, microbially mediated reactions result in degradation of the organic contaminants. By suitable enhancement of the conditions within the biopiles, degradation rates and

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**Nomenclature**

| | |
|---|---|
| $b$ | degradation rate in the first-order model ($day^{-1}$) |
| $f$ | degradation rate coefficient in the zero-order model (mg/kg/day) |
| $H$ | oil content in soil sample (mg/kg) |
| $p$ | air pressure (Pa) |
| $p_{vp}$ | air pressure in the vertical pipe (Pa) |
| $p_\infty$ | ambient air pressure (Pa) |
| $r$ | radial coordinate (m) |
| $R$ | radius of the pile (m) |
| $R_p$ | radius of the pipe (m) |
| $t$ | time (day) |
| $U$ | wind speed (m/s) |
| $w$ | complex variable for the physical plane (m) |
| $x$ | coordinate in the $x$ direction (m) |
| $z$ | coordinate in the vertical direction (m) |
| $\eta$ | complex variable for the mapped plane (m) |
| $\phi$ | velocity potential ($m^2/s$) |
| $\rho$ | air density ($kg/m^3$) |
| $\theta$ | inclination angle from the ground (Rad) |

the degree of degradation can be increased. In particular, natural and forced aeration (blowing or extracting air through the pipes) can be introduced to enhance soil venting in order to provide oxygen for the bioreaction in the pile. Extracted air can be treated to remove volatile organic compounds (VOC) using a filtration system such as activated charcoal (Van Deuren et al., 1997).

The function and performance of a biopile are affected by interacting physical and biological processes: transport of oxygen, moisture and heat due to airflow and diffusion, consumption of oxygen and water by microorganisms and heat generated by bioreactions. As the oxygen supply is increased by enhanced airflow, water and heat loss will be intensified. The latter two factors are also important for biological reactions. Therefore, overly stimulated airflow will not necessarily lead to improved biodegradation in conditions where the ambient temperature is less than that needed for optimal biodegradation within the pile. In order to optimise the remediation system, one must understand how the increased airflow will change the internal moisture content and temperature of the pile. In turn, the moisture content and temperature are also affected by microbial activity. So far, there has been no study on how these processes interact with one another. In this paper, one factor, the airflow within the pile, is investigated.

Natural airflow in a non-engineered pile is driven mainly by wind-induced pressure gradients. These gradients are non-uniform and particularly weak in the central region near the base of the pile, possibly resulting in a local oxygen deficit (Kodres, 1997). Passive and active soil venting has been introduced to overcome this deficiency. Most venting schemes are based on horizontal perforated pipes placed in a random fashion. A simple alternative venting method is to use a vertical perforated pipe that penetrates the centre of the pile and has a wind-driven extraction fan on top. This system has the advantage that it is easier to install than the horizontal pipe system and can be retrofitted. Also, it offers the potential to more efficiently aerate basal areas of the pile where oxygen supply may be limiting. The purpose of our field study was to compare the efficiency of this vertical venting method with the perforated-pipe approach.

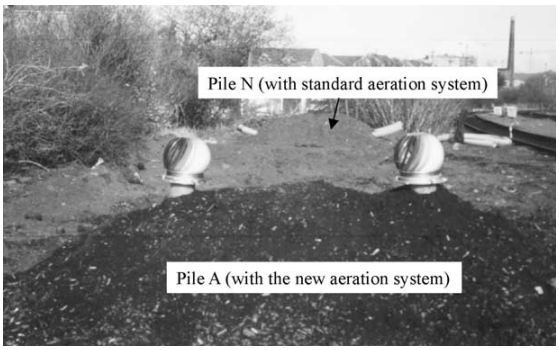


Fig. 1. Setup of biopiles with standard and new aeration systems.

This paper is organised as follows: Section 2 presents simulation results of airflow in biopiles under natural, horizontally and vertically venting conditions; Section 3 describes the experiment set-up; Section 4 presents and discusses the results; and Section 5 draws conclusions.

## 2. Simulation of airflow in biopiles under natural, horizontally vented and vertically vented conditions

The airflow in the biopile is assumed to be two-dimensional and the geometry of the pile is approximated by a half circle (Fig. 2). Because the pressure gradients are relatively low, the air compressibility is neglected. Flow in the interior of the biopile is driven by the wind-induced pressure difference around it. Based on the potential flow theory, the pressure difference can be quantified as (Kundu and Cohen, 2001):

$$2(p - p_\infty) = \rho U^2\left(1 - 4\sin^2\theta\right), \tag{1}$$

where $p_\infty$ is the ambient pressure (at a distance from the pile), $U$ is the wind speed, $p$ is the pressure on the surface of pile ($r = R$), $\rho$ is the air density and $\theta$ is the angle shown in Fig. 2. The notation list gives the units of all the symbols used.

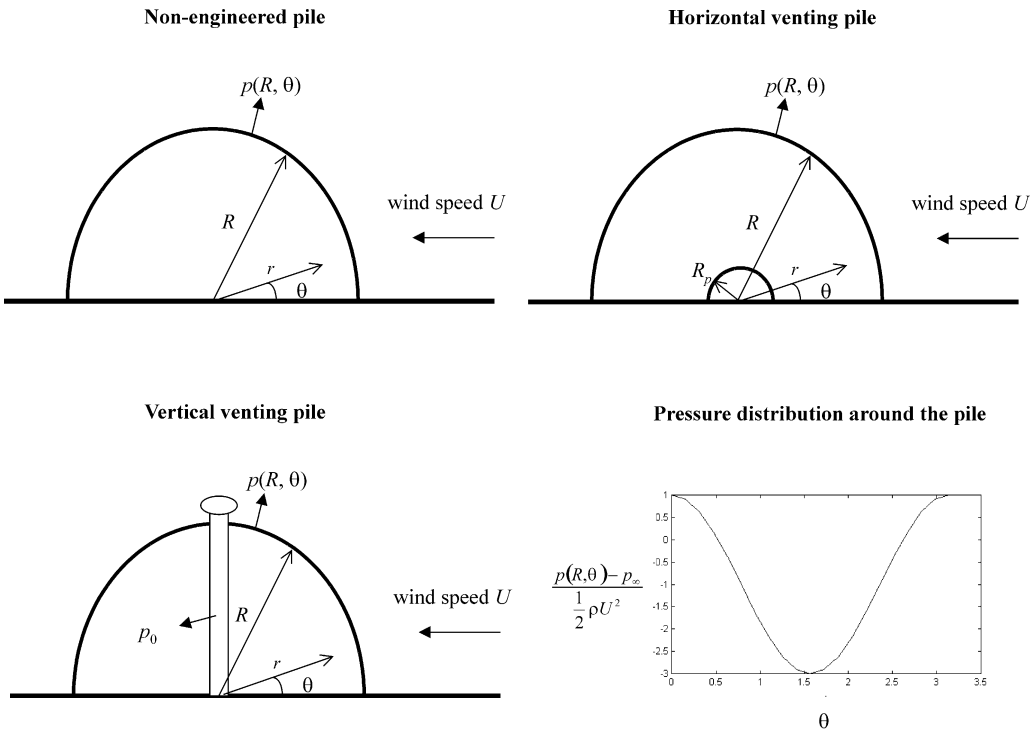


Fig. 2. Biopiles modelled as half circles and the pressure conditions at the boundaries (the surface of the piles and the vertical venting pipe).

With the air compressibility neglected, the governing equation of the airflow is Laplace's equation:

$$\frac{\partial^2\phi}{\partial x^2}+\frac{\partial^2\phi}{\partial z^2}=0, \tag{2}$$

where $\phi$ is the velocity potential. Under natural conditions, i.e., with the boundary condition as described by (1), the solution to (2) is (Carrier and Pearson, 1988):

$$\phi(r,\theta)=\frac{R^2-r^2}{2\pi}\int_0^{2\pi}\frac{\phi(R,\vartheta)}{R^2+r^2-2r\cos(\theta-\vartheta)}d\vartheta, \tag{3}$$

where $\phi(R,\vartheta)$ is given by the boundary condition described by (1). Note that the solution is expressed in the polar coordinates (Fig. 2).

The configuration of a horizontal venting pile is simplified as shown in Fig. 2 to permit a simple analytical solution. The horizontal venting pipe is approximated by an inner half circle, i.e., $r=R_p$, where the pressure is specified as a constant. The resulting solution is,

$$\phi(r,\theta)=\frac{1}{2}a_0(r)+\sum_{n=1}^{\infty}[a_n(r)\cos(n\theta)+b_n(r)\sin(n\theta)] \tag{4a}$$

with

$$a_0(r)=\frac{\ln(R_p/r)}{\ln(R_p/R)}a_{nR}-\frac{\ln(R/r)}{\ln(R_p/R)}a_{nRp}, \tag{4b}$$

$$a_n(r)=\frac{a_{nR}R^n-a_{nRp}R_p^n}{R^{2n}-R_p^{2n}}r^n+\frac{a_{nR}R^{-n}-a_{nRp}R_p^{-n}}{R^{-2n}-R_p^{-2n}}r^{-n}, \tag{4c}$$

$$b_n(r)=\frac{b_{nR}R^n-b_{nRp}R_p^n}{R^{2n}-R_p^{2n}}r^n+\frac{b_{nR}R^{-n}-b_{nRp}R_p^{-n}}{R^{-2n}-R_p^{-2n}}r^{-n}, \tag{4d}$$

$$a_{nR}=\frac{1}{\pi}\int_0^{2\pi}\phi(R,\theta)\cos(n\theta)d\theta, \tag{4e}$$

$$a_{nRp}=\frac{1}{\pi}\int_0^{2\pi}\phi(R_p,\theta)\cos(n\theta)d\theta, \tag{4f}$$

$$b_{nR}=\frac{1}{\pi}\int_0^{2\pi}\phi(R,\theta)\sin(n\theta)d\theta \text{ and} \tag{4g}$$

$$b_{nRp}=\frac{1}{\pi}\int_0^{2\pi}\phi(R_p,\theta)\sin(n\theta)d\theta, \tag{4h}$$

where $\phi(R, \theta)$ and $\phi(R_p, \theta)$ are determined by the boundary conditions at $r = R$ and $R_p$ as discussed above. Note that the Fourier series in (4a) converges very rapidly; and in our calculation, 20 terms (i.e., $n = 20$) was found to give acceptable accuracy with relative errors less than 0.1%.

In the case of vertical venting, the pressure in the pipe will be lowered by the turbine and remains a constant for a given wind speed. The boundary condition at the pipe is therefore prescribed by a constant $\phi$. To derive the analytical solution of the airflow for this case, we introduce the following conformal mapping,

$$\eta = \frac{i\left(w^2 - 2w - 1\right)}{w^2 + 2w - 1}, \tag{5}$$

where $w = r(\cos\theta + i\sin\theta)$ is the complex variable for the physical plane, $\eta = \gamma(\cos\xi + i\sin\xi)$ is the corresponding variable for the mapped plane and $i = \sqrt{-1}$ is the imaginary unit. The solution in the $\eta$ plane is:

$$\Phi(\gamma, \xi) = \frac{R^2 - \gamma^2}{2\pi}\int_0^{2\pi} \frac{\Phi(R, \vartheta)}{R^2 + \gamma^2 - 2\gamma\cos(\xi - \vartheta)} d\vartheta \quad \text{with} \tag{6a}$$

$$\Phi(R, \vartheta) = p_{vp} \quad \text{for} \quad 0 \leqslant \vartheta \leqslant \pi \quad \text{and} \tag{6b}$$

$$\Phi(R, \vartheta) = \left\{1 - 4\sin^2[\theta(R, \vartheta)]\right\} p_{ref} \quad \text{for} \quad \pi \leqslant \vartheta \leqslant 2\pi, \tag{6c}$$

where $p_{vp}$ is the pressure in the vertical venting pipe. Eq. (6b) represents the boundary condition in the vertical pipe where (6c) defines the boundary condition around the pile. The solution in the physical plane can be obtained once $\gamma$ and $\xi$ are converted back to $r$ and $\theta$.

The results of airflow under natural conditions as predicted by (3) are shown in Fig. 3. The flow in the lower centre area is relatively weak. The horizontal venting pipe increased the flow rate in its vicinity but created a stagnant zone some distance above (Fig. 4). In contrast, the results from (6a–c) for the vertical venting condition show a strong and uniform flow pattern in the pile (Fig. 5). In the second and third simulations, we have assumed that the pressure in the venting pipe is the same as the surface pressure at $\theta = \pi/2$. In reality, the pressure in the vertical venting pipe is likely to be lower (in which case the flow rate in the pile would be higher), whereas the pressure in the horizontal venting pipe varies with the wind direction. In summary, the simulation results indicate that vertical venting is a better method for achieving an enhanced, uniform airflow in the pile.

## 3. Field experiments

### 3.1. Experimental setup

The purpose of the field trial was to compare the influence of the two types of aeration system on the biodegradation process. Two biopiles were constructed, one with a standard passive aeration and the other with

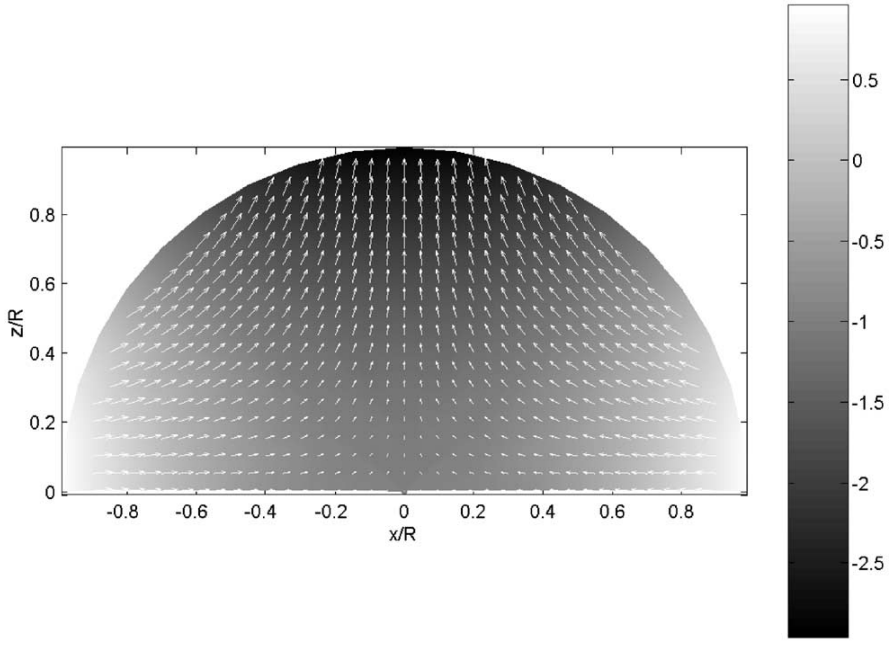


Fig. 3. Airflow pattern in a pile under natural conditions. The colour pattern represents the pressure variations in the pile and the arrows show the pressure gradient and hence the airflow velocity field.

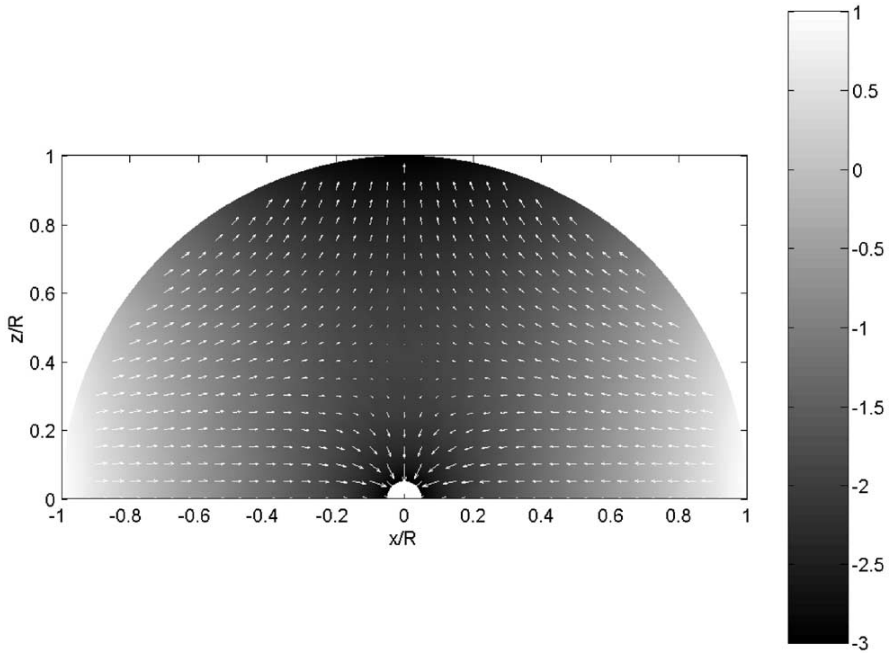


Fig. 4. Airflow pattern in a pile under horizontal venting conditions. The colour pattern represents the pressure variations in the pile and the arrows show the pressure gradient and hence the airflow velocity field.

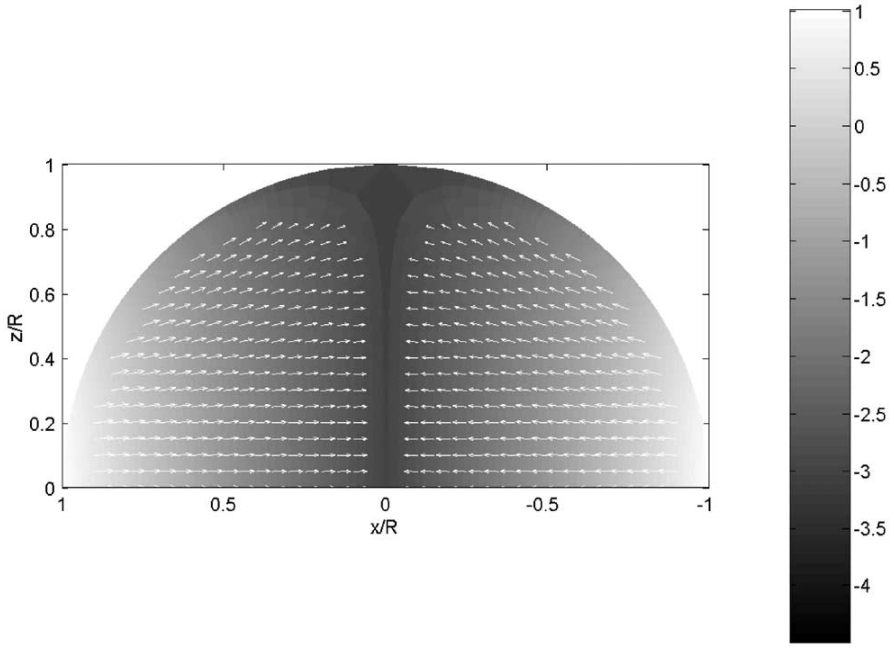


Fig. 5. Airflow pattern in a pile under vertical venting conditions. The colour pattern represents the pressure variations in the pile and the arrows show the pressure gradient and hence the airflow velocity field.

the vertically vented wind-driven system. Each biopile was constructed using 3 $m^3$ of diesel-contaminated soils collected from a railway siding (contaminated with diesel over several decades) and approximately 1.26 $m^3$ of soil amendments: woodchips 0.21 $m^3$ and compost 1.05 $m^3$. Soil hydrocarbon concentrations were between 18,000 and 25,000 ppm. The contaminated soil was piled approximately 1 m high, 2 m wide and 3 m long. Two perforated polyethylene pipes (land drain, 140 mm in diameter) were placed in the middle of one pile

("Normal" pile, N), in the manner shown in Fig. 1. The position of the land drain was chosen to maximise soil aeration. The other pile ("Aeration" pile, A) had the new aeration system. Two perforated flexible pipes (also 140 mm in diameter) were placed vertically in the centre of the pile at each end with turbines fitted to the top (Fig. 1).

Woodchips were used as a bulking agent to increase the porosity of the piles. A soil: woodchip volume ratio of 14:1 was used. In addition, 1.05 $m^3$ of garden compost was added to each pile. This compost was made with sphagnum peat mixed with essential plant nutrients including trace elements (multipurpose compost BALE, 150 l). This was incorporated to mitigate potential effects from heavy metals present in the soil and also served as a nutrient source for microorganisms (Von Fahnestock et al., 1996, 1998).

### 3.2. Soil sampling and analysis

Soil sampling was made on days 0 (initial conditions), 3, 15, 23 and 30. Each time, three soil samples per pile were taken from three different, representative locations in order to determine the overall behaviour/properties of the pile. The samples were preserved in plastic bags at 4 °C in a laboratory refrigerator. Analyses were carried out to determine the hydrocarbon content, pH and water content in each sample. For the determination of total hydrocarbon content, a total hydrocarbon gravimetric method was used. The oil contained in 10 g of contaminated soil was extracted using dichloromethane (DCM). The solvent was evaporated from the extract and the residue was weighed. Samples collected on days 0 and 30 were taken for further analysis by GC-FID (Hewlett Packard HP5890 gas chromatography with a flame ionisation detector). This was done to elaborate on the removal of specific hydrocarbon fractions. A 30-m, HP-5 column with 0.32 mm inside diameter and 0.25-μm film thickness was used to effect separation. Aliquots of 3 μl were injected using an auto-sampler and all analyses were carried out in splitless mode at a flow rate of 30 ml $min^{-1}$ and the purge time was set at 1.5 min. A linear temperature gradient was employed, the column temperature being held at 50 °C for 2 min following injection, ramped at 10 °C $min^{-1}$ to 320 °C, then held at this temperature for a further 10 min. Injector and detector temperatures were set at 285 and 315 °C, respectively. A helium carrier gas was used at a flow rate of 2.5 ml $min^{-1}$. A 10 component standard mixture of *n*-alkanes (Supelco, Poole, Dorset, UK) was used to quantify the total diesel range organic (DRO) content of the extracts. The compounds comprising this standard mixture were used to: (a) define and establish retention windows for the DRO in the extract, and (b) to determine calibration factors that in turn were used to calculate the collective concentration of diesel contained in the extract.

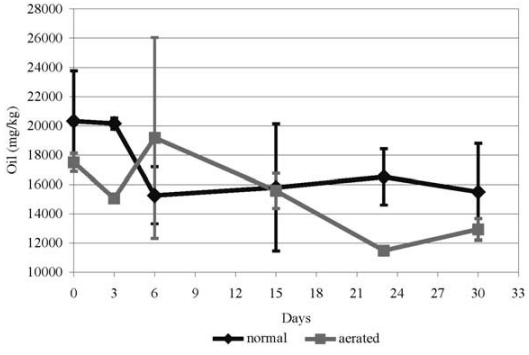


Fig. 6. Reduction of hydrocarbons in the piles during the experiment.

For the microbiological analysis, another three samples were taken per pile using a spatula sterilised with ethanol. These were preserved in sterile centrifuge tubes refrigerated at 4 °C prior to analysis. Microbiological analyses were carried out to indicate the total population of heterotrophic microorganisms. Soil samples (1 g) were added to 9 ml sterile 0.85% saline containing 0.2% tetra-sodium pyrophosphate (Klein, 1992), and treated in an ultrasonic cleaning bath for 60 s to break up clumps and desorb microorganisms. Subsequently, serial dilutions were made in sterile saline (0.85%), with samples plated out on nutrient agar. Colony counts were made after incubation at 27 °C for 7 days, and were expressed as colony forming units (cfu) $g^{-1}$ (Fig. 8).

Air pressure measurements were conducted on the site with a digital manometer (FCO 16 Bexhill, England, UK). Pressure sensors were not sensitive enough to provide data of the air pressure in the soil so measurements were made in the pipes. The measurements were made on the site on days 11 and 30.

## 4. Results and discussions

### 4.1. Hydrocarbon concentrations

The reduction of hydrocarbons in both piles is shown in Fig. 6 and Table 1. The overall trend of the hydrocarbon reduction in both piles seems to be exponential,

Table 1
Temporal variations of the oil content in the piles

| Day | Normal | | | Aerated | | |
|---|---|---|---|---|---|---|
| | Oil mg/kg | SEM | Variability | Oil mg/kg | SEM | Variability (%) |
| 0 | 20,318 | 3442 | 17% | 17,514 | 630 | 4 |
| 3 | 20,153 | 390 | 2% | 15,059 | 143 | 1 |
| 6 | 15,261 | 1948 | 13% | 19,180 | 6866 | 36 |
| 15 | 15,800 | 4342 | 27% | 15,567 | 1200 | 8 |
| 23 | 16,517 | 1917 | 12% | 11,480 | 185 | 2 |
| 30 | 15,497 | 3303 | 21% | 12,946 | 735 | 6 |

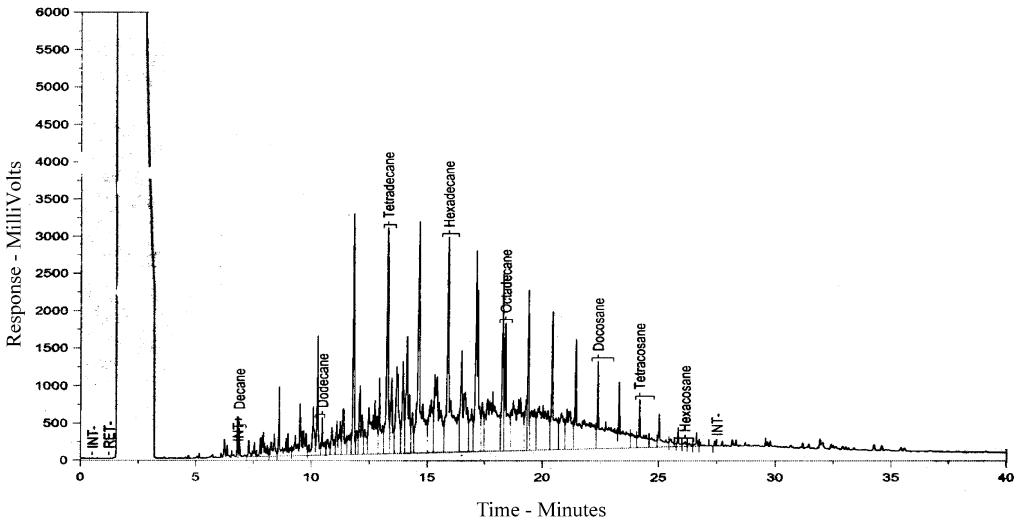


Fig. 7. Chromatogram for the sample collected from pile A on day 0.

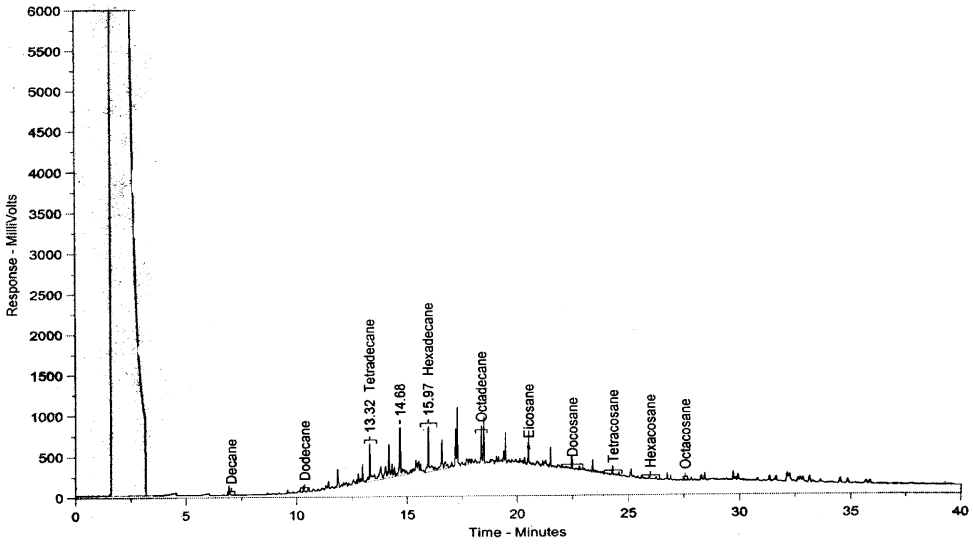


Fig. 8. Chromatogram for the sample collected from pile A on day 30.

i.e., first-order kinetics. Similar behaviour has been observed in other experiments (e.g., Cunningham and Philp, 2000). Although it is likely true that a zero-order model may give results that are statistically valid, the trend in the microbial population showed some higher order behaviour. After 30 days, the two piles had been decontaminated overall to 25% of the initial quantity of hydrocarbons.

It should be noted that the initial quantity of oil was not identical in the two piles. The normal biopile (N) was slightly more contaminated. To have a consistent comparison of the biodegradation between the two piles, the percentage of degradation was calculated (Table 2). Towards the end of the experiment the percentage of hydrocarbon degradation was higher in the aerated pile A than in the normal pile N, although the overall difference between the two piles was insignificant. Further analysis will be carried out in the following sections to examine the degradation rates.

Table 2
Percentage biodegradation

| Day | Normal | Aerated |
|---|---|---|
| 0 | 0 | 0 |
| 3 | 1 | 14 |
| 6 | 25 | −10 |
| 15 | 22 | 11 |
| 23 | 19 | 34 |
| 30 | 24 | 26 |

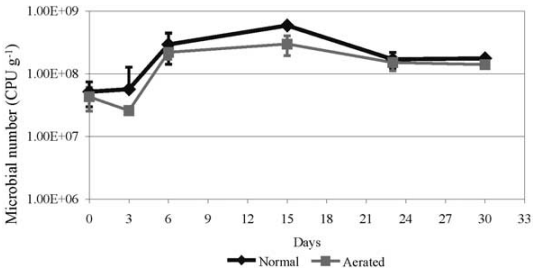


Fig. 9. Temporal variation of bacterial populations in the piles.

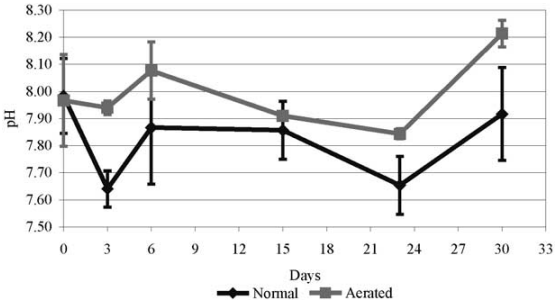


Fig. 10. Temporal variation of pH in the piles.

For both piles, we observed that some data points did not follow the general trend of decrease. For example, the mean oil content in pile A determined from the three samples collected on day 6 was higher than that of the first day. However, the variability in the sample was high (36%). Such variability reflects the physical and biochemical heterogeneity of the piles and should be taken into account when interpreting the results.

Example gas chromatographs are shown on Figs. 7 and 8. The graphs show the presence of many different molecules in the study. Low molecular weight, very volatile components appear between 5 and 10 min. Long, complex, difficult-to-degrade carbon chains appear above 20 min. Day 0 results showed a relatively high proportion of short chain, volatile components. On day 30, the results showed a significant decrease of most components, with the volatile molecules (between decane and dodecane) almost totally gone.

The results confirmed that the initial quantity of hydrocarbon was higher in pile N than in pile A, and that by the end of the experiment hydrocarbons in the two piles had undergone equivalent biodegradation.

### 4.2. Microbial population

The culturable heterotrophic microbial population in both piles is shown Fig. 9. Microbial numbers peaked at 15 days and, at this stage, had increased over the initial counts by approximately one order of magnitude. In the aerated pile, an initial decrease in the number of viable microorganisms was observed. This observation is

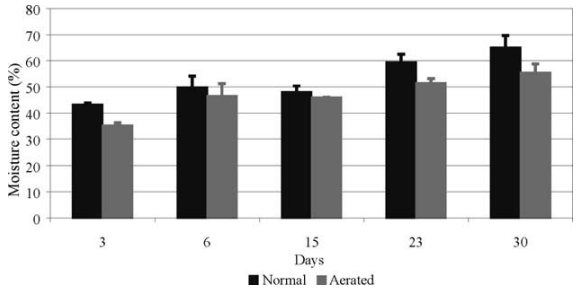


Fig. 11. Temporal variation of water content in the piles.

common, and may be due to biotic (stimulated grazing by protozoa), and abiotic factors such as UV irradiation (van Veen et al., 1997).

The pattern was very similar in both pile types, and enhanced aeration had no effect in the stimulation of microbial numbers. The initial population was slightly lower in pile A than in pile N. Initially, both populations increased rapidly. The rate of increase slowed until reaching a peak, consistent with the batch culture of bacterial populations. Subsequently the populations decreased in a manner consistent with the post-stationary phase of batch culture. All the data are moisture-corrected. The variability of the experimental analysis is low (typically $<6\%$). No datum point has been rejected [based on the Dixon ratio with a 95% confidence interval (Manly, 2001)].

### 4.3. Other measurements

The pH was monitored, with values between 7.5 and 8.5 in the piles as shown in Fig. 10. The optimal pH range for hydrocarbon degradation in soil has been commonly reported as being between 6.5 and 8.0 (Morgan and Watkinson, 1989). Throughout the experiment, the pH in both piles was within this range.

Adequate moisture is essential to the biodegradation process. The piles were watered following construction (day 0), and again on day 6. The results of moisture analyses are shown in Fig. 11. The water content in pile A was always lower than that in the pile N. This may be due to the enhanced aeration in the former pile.

The temperature of soils at various locations of the piles was recorded at the end of the experiment. The results are shown in Fig. 12. This measurement was to establish if there were any significant temperature differences between the two piles. The centre of the two piles had a higher temperature than the extremities, possibly due to local airflow rates and stimulated metabolism. However, these data were insufficient to make a link with the efficiency of airflow.

A digital manometer (FCO, 16 Bexhill England) was used to record the pressure (with respect to a reference, fixed atmospheric pressure) in the pipes at the centre of the piles. Some sample readings from the manometer

are given in Table 3 for pile A. In pile N, the measured pressure was higher (less suction). The new aeration system clearly stimulated suction in the pipe, leading to enhancement of airflow in the pipe. The new system also has the advantage of being sensitive and responsive to wind from all directions, which is not the case for pile N.

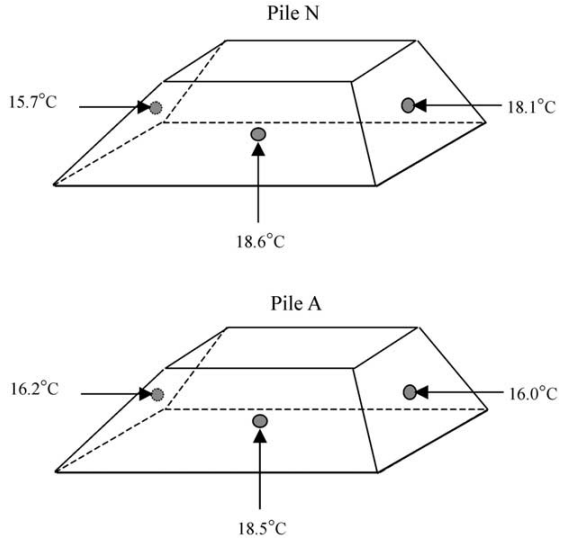


Fig. 12. Temperature profiles in the piles.

Table 3
Measured pressure in the vertical pipe of pile A

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time (min) | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 |
| Suction (Pa) | −0.5 | −0.6 | −0.4 | −0.2 | −0.1 | 0 | −0.1 | −0.3 | −0.4 | −0.7 | −0.5 |

### 4.4. Biodegradation rates

The trend displayed in Fig. 6 suggests that the biodegradation reaction in the piles can be described by the first order kinetics, i.e.,

$$\frac{dH}{dt} = -bH, \tag{7}$$

where $H$ is the hydrocarbon concentration and $b$ is the degradation rate. The solution of $H$ is thus,

$$H = H_0 \exp(-bt), \tag{8}$$

where $H_0$ is the initial oil content. The logarithm of Eq. (8) is,

$$\ln(H) = \ln(H_0) - bt. \tag{9}$$

A weighted regression method was applied to fit the data to Eq. (9) in order to determine the degradation rates for both piles. This method took into account the variability of the data points. The fitting weighs more the data points with low variability. The results are shown in Figs. 13 and 14 for piles N and A, respectively.

The results of the weighted regression limit were used to calculate the confidence limits of the slopes: $b_N = 0.0111 \pm 0.0090$ day$^{-1}$ for pile N and $b_A = 0.0117 \pm 0.0090$ day$^{-1}$ for pile A (note that the fitted parameter values shown in the figures need to be multiplied by 2.3026 to give $b_N$ and $b_A$). Although the degradation rate in pile A was slightly higher than that of the standard pile, the difference between the two was small and statistically insignificant considering the variability of the data. The ratio of $b_A$ to $b_N$ was 1.05. On the other hand, the microbial population was lower

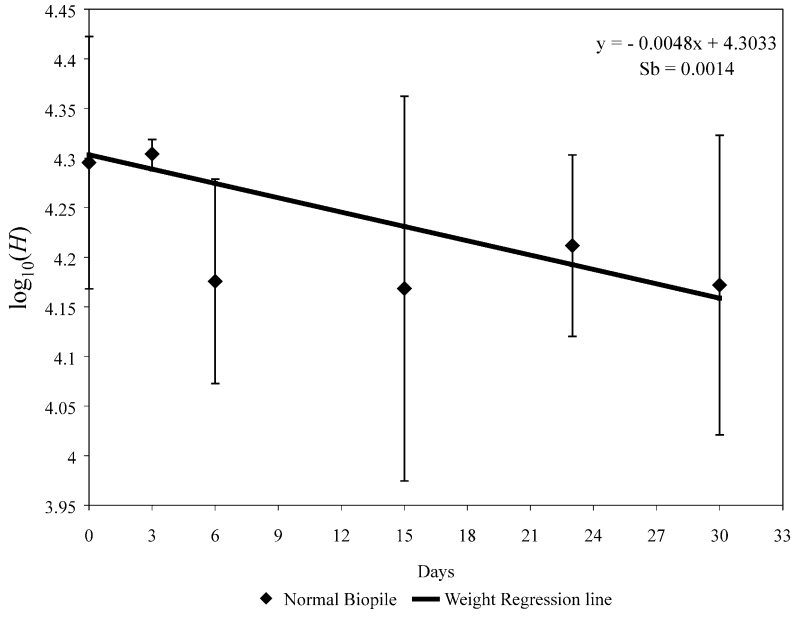


Fig. 13. Logarithm (base 10) of the hydrocarbon concentration in the pile and the regression results for pile N.

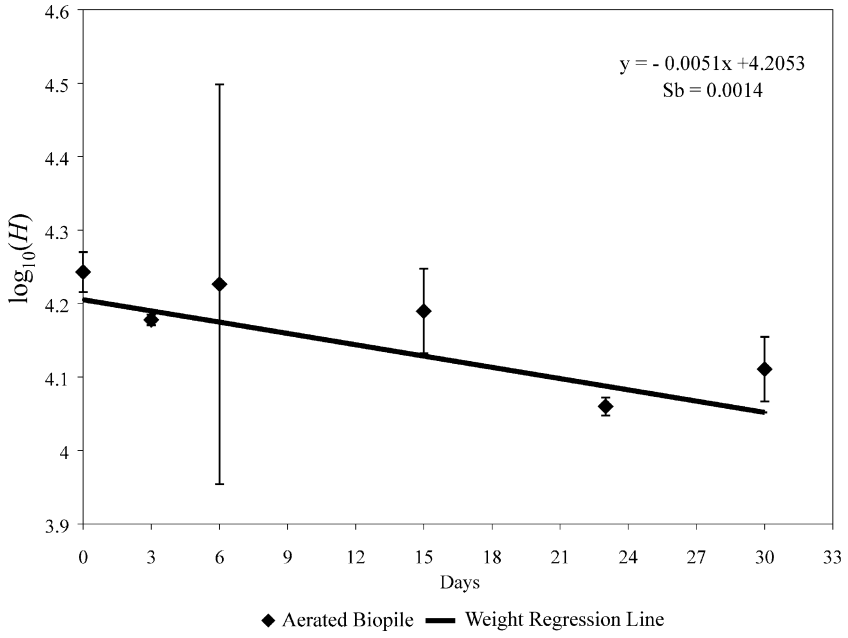


Fig. 14. Logarithm (base 10) of the hydrocarbon concentration in the pile and the regression results for pile A.

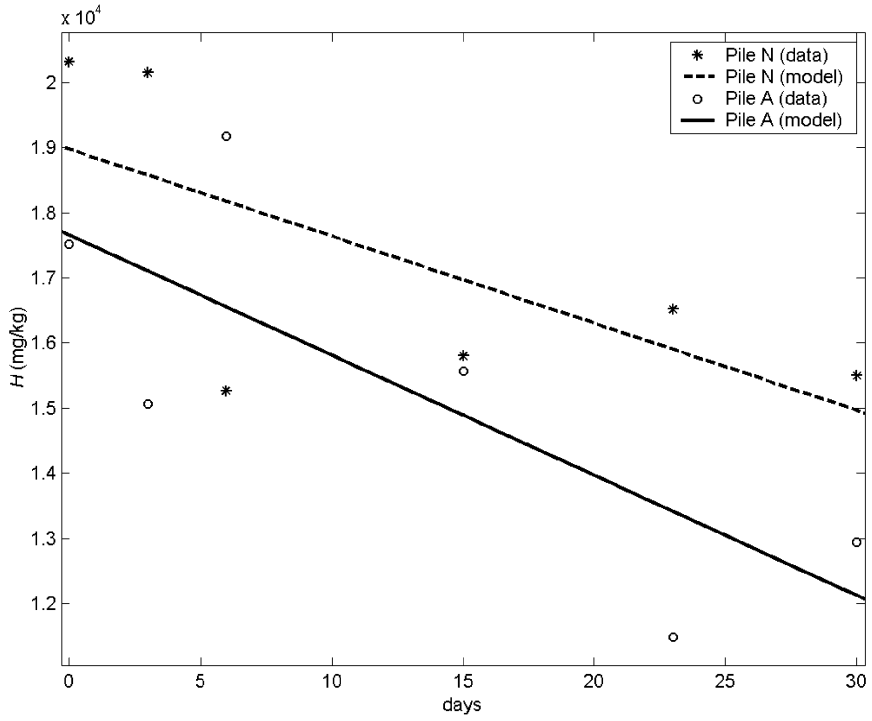


Fig. 15. Reduction of the hydrocarbon concentration with time-analysis based on the zero-order model.

in pile A than that in pile N. If the rates are normalised by the average biomass in the piles, the difference becomes larger. The ratio of normalised $b_A$ to normalised $b_N$ was 1.26.

A zero-order model was also applied to the data, i.e.,

$$\frac{dH}{dt} = -f, \tag{10}$$

where $f$ is the constant degradation rate coefficient. The results, displayed in Fig. 15, indicate that the degradation

process in the new aeration system ($f = 184.6$ mg/kg/day) was faster than that in the normal pile ($f = 133.7$ mg/kg/day), consistent with the analysis based on the first-order model. More sophisticated models that incorporate the growth of microbes can also be employed to analyze the data, e.g., Eqs. (27) and (28) of Barry et al. (2002). Due to the uncertainties about the background microbes, we did not proceed with this model. Nonetheless, the analyses based on the first and zero-order models suggest that the new aeration system might have improved the efficiency of biodegradation in the pile.

Other factors, including pH and water content, may also affect the degradation process. The pH values measured in both piles were in the range for a normal development of a degradative microbial population and thus were less influential in causing different behaviours of the piles. The measurements of the water content showed there was a difference in moisture content between the two piles. Pile A had less moisture than pile N, even taking into account the variability. This may be a result of enhanced airflow in pile A. As discussed previously, excessive loss of water can be counter-productive for the biodegradation process. The collected data are not adequate to determine whether the water loss in pile A was excessive and counter-productive. These results, however, reaffirm that aerating a biopile requires an optimal balance between enhanced airflow (hence oxygen supply) and many other factors.

## 5. Conclusions

The purpose of this study was to test a new aeration system for biopiles of diesel-contaminated soils by comparison with a standard system. The data show that the suction in the venting pipes was raised with the new aeration system, which likely led to enhancement of the airflow in the pile. The results of hydrocarbon degradation were not conclusive regarding the effectiveness of the new system in improving the performance of biopiles. The degradation rates obtained from the zero-order and first-order models indicate that the new system might have increased the efficiency of the bioreaction in the pile.

The results also confirmed that as a result of enhanced aeration, excessive water loss may occur, which will have a negative impact on the biodegradation process. Further studies need to be carried out to quantify how the airflow affects the moisture content in the pile. An ideal aeration system must reach an optimal balance between the enhancement of airflow (oxygen supply) and other factors including moisture in order to achieve the maximum biodegradation.

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## Appendix G. Kuyukina *et al*. (2003)

Kuyukina, M.S., Ivshina, I.B., Ritchova, M.I., **Cunningham, C.J.**, Philp, J.C., & Christofi, N. (2003). Bioremediation of crude oil contaminated soil using slurry-phase biological treatment and landfarming techniques. *Soil and Sediment Contamination*. 12 (1), 85-99.

### *G.1 Declaration of contribution*

My colleagues Philp and Christofi from the School of Life Sciences in Napier University had established a link in the early 1990s with the Russian institute (IEGM) where Kuyukina and Ivshina worked. The author first visited IEGM in 1999 and there was a great deal of knowledge exchanged and an evolving synergy in the field of bioremediation. The author was involved at all stages of the project including a period of time spent in Russia designing the project. I later returned to contribute to establishing the fieldwork and then again towards the end of the fieldwork when the author was involved in undertaking some of the laboratory analyses e.g. the plant biomass and length data. TLC-FID analyses were conducted in the UK on samples brought back by the author although these were undertaken at an external laboratory. The author closely involved in the interpretation of results and at all stages of writing the paper.

*Soil and Sediment Contamination*, 12(1):85-99 (2003)

# Bioremediation of Crude Oil-Contaminated Soil Using Slurry-Phase Biological Treatment and Land Farming Techniques

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*Field-scale experiments on bioremediation of soil heavily contaminated with crude oil were undertaken on the territory of the Kokuyskoye oil field (Perm region, West Urals, Russia) owned by the LUKOIL Company. The pollution consisted of the contents of a oil waste storage pit, which mostly received soils contaminated after accidental oil spills and also the solid n-alkane (paraffin) wastes removed from the surface of drilling equipment. Laboratory analyses of soil samples indicated contamination levels up to 200 g/kg of total recoverable petroleum hydrocarbons (TRPH). Average oil composition consisted of 64% aliphatics, 25% aromatics, 8% heterocyclics, and 3% of tars/asphaltenes. Ex situ bioremediation techniques involved the successive treatment of contaminated soil using a bioslurry reactor and land farming cells. An oleophilic biofertilizer based on Rhodococcus surfactant complexes was used in both treatment systems. An aerobic slurry bioreactor was designed, and the biofertilizer applied weekly. Slurry-phase biotreatment of the contaminated soil resulted in an 88% reduction in oil concentration after 2 months. The resulting reactor product, containing approximately 25 g/kg of TRPH, was then loaded into land farming cells for further decontamination. To enhance bioremediation, different treatments (e.g., soil tilling, bulking with wood-chips, watering, and biofertilizer addition) were used. The rates of oil biodegradation were 300 to 600 ppm/day. As a result, contamination levels dropped to 1.0 to 1.5 g/kg of TRPH after 5 to 7 weeks. Tertiary soil management involved phytoremediation where land farming cells were seeded with a mixture of three species of perennial grass. The effect of phytoremediation on the residual decontamination and rehabilitation of soil fertility is being evaluated.*

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***KEY WORDS:*** *bioremediation, oil-contaminated soil, oleophilic biofertilizer, slurry bioreactor, land farming cells.*

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## Introduction

Perm region has one of the largest oil-extracting areas in Russia where crude oil has been extracted using traditional drilling technology for several decades. This involved the preliminary settling of crude oil in settling pits to achieve separation of the hydrocarbon fraction from drilling fluids and cuttings. In later years, modern separation systems have reduced the need for so many settling pits. Some of the pits have begun to be used as waste storage reservoirs for the disposal of oily wastes from drilling wells and oil-contaminated soil. The content of these pits represents significant potential harm for the local environment due to the release of volatile hydrocarbon into the atmosphere and from the accidental penetration of oily material into soil and ground water. Therefore, there is an obvious requirement for technology to remediate the content of these pits (there are about 40 waste and settling pits in the Perm region). Bioremediation has been recognized as an acceptable, cost-effective alternative to physiochemical methods (e.g., incineration, solvent extraction, etc.) for the treatment of petroleum contamination (Atlas, 1981; Bartha, 1986; Radwan *et al*., 1995; Koronelli, 1996; Philp *et al*., 2000; U.S. EPA, 2001a, 2001b; Whyte *et al*., 2001).

Bioremediation technologies currently used in Russia are mostly directed to the remediation of oil spillages on land and include *in situ* biotreatment of contamination, for example, the addition of bacterial fertilizers, mineral, and organic nutrients to the oil-contaminated soil (Koronelli *et al*., 1997; Borzenkov *et al*., 1998; ISC-UNIDO, 2001). However, these technologies are not acceptable for the treatment of oil wastes, as the high concentration of toxic contaminants and anaerobic conditions in the pit content prevent the development of an active oil-oxidizing microbial consortium.

In previous field experiments, *Rhodococcus* biosurfactants have been used for the bioremediation of oil-contaminated agricultural soils after an accidental oil spill (Christofi *et al*., 1998; Ivshina *et al*., 1998). The application of composting systems enhanced by nutrient addition, bulking with straw and inoculation of *Rhodococcus*-biosurfactant complexes provided a 57% decrease in oil contamination during a 3-month treatment. In this study we attempted to develop an *ex situ* biotechnology employing a *Rhodococcus* biosurfactant-based biofertilizer (Ivshina *et al*., 2001) for the decontamination of heavily oil-polluted soil. The results from field-scale experiments using slurry-phase and land farming biotreatment of oil wastes are discussed in this article.

## Materials and Methods

### Site and Contamination Characterization

The experimental site was located on the territory of the Kokuyskoye oilfield (Perm region, West Urals, Russia) owned by the LUKOIL Company. The Kokuyskoye oilfield, with an annual oil production of 500,000 to 600,000 tonnes,

is situated to the southeast of Perm region, approximately 7 km west of Kungur (population of 100,000). Crude oil processing at the Kokuyskoye oilfield began in early 1970s. Two 900 $m^3$ concrete-lined waste storage pits were used for disposal of the oil wastes collected from the oilfield. These pits mostly received polluted soil from accidental oil-spill areas and also the solid *n*-alkane (paraffin) wastes removed from oil wells and the surface of drilling equipment. Laboratory analyses of samples taken from storage pits showed that oil contamination was not homogenous, ranging from 120 to 250 g/kg of TRPH with an average of 200 g/kg.

### Microbiological Analyses

Procedures for microbiological sampling, handling, and analyses were performed according to traditional methods. To achieve maximum desorption of microorganisms from the surface of soil particles, soil samples with a small amount of water added were subjected to ultrasonic (22 kHz, 0.3 A, 1 to 2 min) treatment (Ivshina and Kuyukina, 1997). The enumeration of heterotrophic bacteria was made routinely by inoculation of nutrient agar plates. Enumeration of hydrocarbon-degrading microorganisms was performed using mineral agar plates with a mixture of $C_{12}$-$C_{17}$ *n*-alkanes used as an organic carbon source. Cultures were incubated at 28°C for 1 week. All analyses were undertaken in triplicate.

### Analytical Methods

The oil content in soil and slurry samples was determined gravimetrically as the amount of total recoverable petroleum hydrocarbons (TRPH) extracted by chloroform (Christofi *et al.*, 1998; Capelli *et al.*, 2001). Oil fraction analyses were performed using an Iatroscan TLC-FID Analyzer MK-5 (Iatron Laboratories Inc., Japan). Soil and slurry samples were extracted in a 3:1 mixture of dichloromethane-pentane, the pentane-soluble fractions were applied onto chromarods (type S III), and consecutively eluted with *n*-hexane to separate saturated hydrocarbons, dichloromethane-pentane (55:45) to separate aromatics, and dichloromethane-methanol (98:2) to separate heterocyclics. The rods were scanned using an FID, the area for each peak calculated, and the composition (i.e., the percentage of saturated hydrocarbons, aromatic hydrocarbons, and heterocyclics) was determined (Cavanagh *et al.*, 1995; Bhullar *et al.*, 2000). Tars/asphaltenes content of samples (pentane-insoluble fractions) was determined gravimetrically (Uraizee *et al.*, 1998).

### Land Farming Cell Construction

The experimental site area was 80 $m^2$. Six land farming cells 2.0 m x 2.0 m in size were constructed, 0.5 m apart (Figure 1). To prevent the penetration of oil products

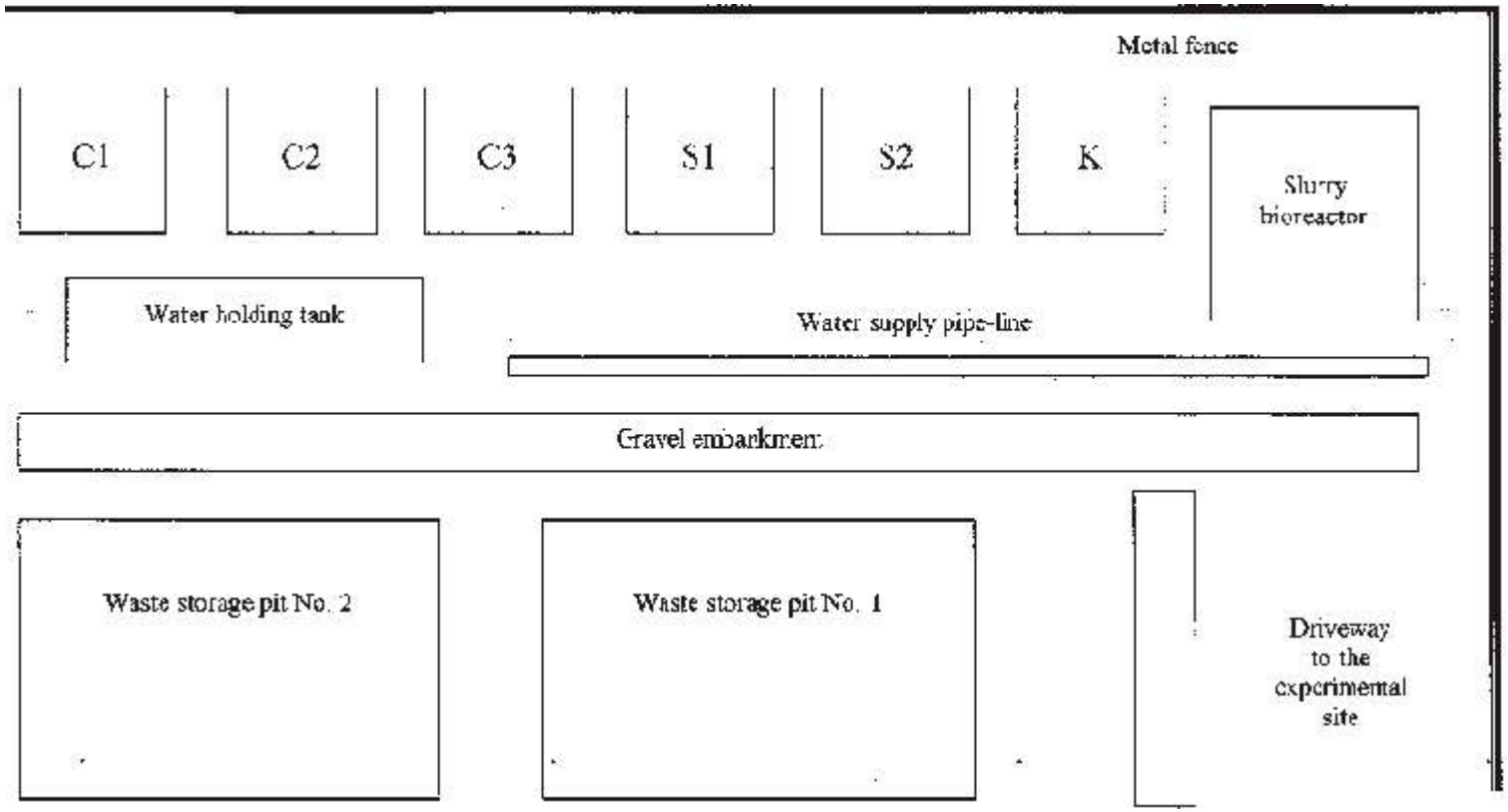


***FIGURE 1***

*Scheme of the experimental bioremediation site. C1 — control, untreated oil-contaminated soil; C2 and C3 — biofertilizer addition; S1 and S2 — initial biological treatment in a slurry bioreactor. K — noncontaminated agricultural soil.*

into ground waters, the base of each cell was lined with 10-cm clay layer. The ground between the cells was covered with gravel. Noncontaminated agricultural soil collected from a grain-growing field was loaded into the cells. The contaminated soil (0.5 $m^3$) collected from the first waste storage pit was loaded into and mixed with clean soil in the ratio of 1:3 (cells C1 and C2) and 1:10 (cell C3). Oil-contaminated soil had a heavy clay texture and low oxygen diffusivity, organic bulking material, particularly 0.1 $m^3$ of wood-chips was therefore added to increase aeration. The soil was tilled to a depth of 20 cm and large soil conglomerates destroyed using a rake. Oleophilic biofertilizer doses (2.0 and 1.0 kg/$m^2$ of soil) were applied to C2 and C3 cells weekly during the first month and monthly thereafter.

Over the course of the experiment, the land farming cells were tilled and watered weekly to maintain soil moisture levels of 20%. When the air temperature was below 14°C, the cells were covered with a nonwoven polymeric fabric covering. After 2 months of bioremediation, half of the area of C1, C2, C3, and K land farming cells was sown with a mixture of perennial grasses consisting of red clover (*Trifolium pratense*), brome (*Bromus exaristatus*), and timothy (*Phleum pratense*). The application rate was 3 g/$m^2$ in the ratio of 1:1:1.

### Slurry Bioreactor Design

The bioreactor was constructed using a 3-$m^3$ oil tank. Oil-contaminated soil (0.4 $m^3$) collected from waste storage pit was watered, homogenized, and added into the reactor along with 1200 l of tap water to give the final proportion of solid fraction an average of 30% (w/w). A compressor was used to supply air to the slurry at the pressure 5 kg/$cm^2$. The compressor was operated for 8 h per day. Mechanical mixing (20 rpm) was performed daily for 1 h before the compressor was switched on. Dissolved oxygen was maintained at the level of 6 to 7.5 mg/l during a day, and it dropped to 2 to 4 mg/l during the nighttime. The temperature of bioslurry ranged from 18 to 30°C. The biofertilizer (2 kg) was added to the slurry weekly.

After a 60-day treatment, the water fraction was removed from the bioreactor and placed into a water holding tank. The remaining solid fraction was loaded onto S1 and S2 land farming cells (see Figure 1). This material was mixed with clean soil in a ratio 1:1 (S1) and 1:4 (S2). Further treatment of these soil systems was performed as previously described for cells C1-C3. Contaminated water from bioreactor was used for the watering of S1 and S2 cells.

The temperature and pH of both systems and the dissolved oxygen (DO) in the slurry were monitored daily using pH Checker HI1270 (Hanna Instruments, UK) and portable DO meter ANKAT 7645 (Russia). Soil moisture content was monitored weekly using a standard soil analytical technique (Klute, 1986). Samples for microbiological and chemical analyses were taken weekly.

## Results and Discussion

### Oil-Contaminated Soil Bioremediation Using Land Farming Cells

Table 1 shows the counts of pysiological groups of microorganisms most important for bioremediation, that is, heterotrophic and hydrocarbon-oxidizing bacteria in the experimental land farming cells. These data indicate that all of the cells studied had the same numbers of heterotrophic ($10^7$ CFU/g soil) and hydrocarbon-oxidizing ($10^5$ CFU/g soil) bacteria prior to the bioremediation process. However, during bioremediation large variations in hydrocarbon oxidizers were detected in the control and in soil treated with biofertilizer. The addition of the biofertilizer resulted in a 100- to 1000-fold increase in the number of hydrocarbon-oxidizing bacteria in cells C2 and C3 compated with a 1--fold increase in the control cell (C1). The number of hydrocarbon-oxidizers was 3 to 15 times lower in heavily contaminated soil (C2) than that in soil with a lower initial contamination (C3) during the first month of bioremediation. High concentrations of toxic oil components in the initial contamination had an inhibitory effect on the soil hydrocarbon-oxidizing bacteriocenosis. However, as oil degradation proceeded, the numbers of hydrocarbon-oxidizing bacteria changed, and at the final stage of biodegradation their number in C2 land farming cell was nearly 20-fold that of the C3 cell.

Figure 2 shows the effect of biological treatment on oil degradation rates. During more than 2 months, similar initial oil concentrations (46 g/kg of TRPH) at C1 (control) and C2 cells decreased to 15.5 and 6.0 g/kg, respectively. Therefore, high numbers of hydrocarbon-oxidizing bacteria present in biofertilizer-treated land farming cells resulted in accelerated rates of biological oil degradation processes in soil. A significant decrease in oil concentration during the first week in all land farming cells was observed (Figure 2), due mostly to physiochemical processes, for example, volatilization and photooxidation of petroleum hydrocarbons. Thereafter, biodegradation of oil continued in C1, C2, and C3 cells at average rates of 320, 490, and 420 ppm/day, respectively.

These data provide evidence that oil-contaminated soil remediation occurred more efficiently in C2 and C3 cells treated with the biofertilizer. Total biodegradation effectiveness (calculated as percentage of oil degraded) at these cells was 80 to 90% after 5 to 8 weeks of bioremediation.

Table 2 compared the proportions of major hydrocarbon fractions in oil contamination of land farming cells. Rapid degradation of aliphatics and aromatics in biofertilizer-treated cells led to the relative increase of asphaltene-tar content in the residual contamination. The ratio of major oil fractions in the control cell (C1), however, changed insignificantly. Bioremediation in C3 cell characteristically lead to a relatively high degradation rate for aromatic and heterocyclic compounds.

Table 1. Microbiological data for samples taken from land farming cells (number of colony forming units/g soil)

| **Sample characterization** | Time, weeks | **Heterotrophic bacteria** | **Hydrocarbon–oxidizing bacteria** |
|---|---|---|---|
| Cell C1 – Control, no treatment (TRPH = 46.1 g/kg) | 0 | $(3.1 \pm 0.9) \times 10^7$ | $(4.4 \pm 1.3) \times 10^5$ |
| | 1 | $(5.5 \pm 1.7) \times 10^7$ | $(1.1 \pm 0.2) \times 10^6$ |
| | 2 | $(1.2 \pm 0.6) \times 10^7$ | $(2.0 \pm 0.5) \times 10^6$ |
| | 3 | $(8.3 \pm 3.0) \times 10^6$ | $(6.2 \pm 1.8) \times 10^6$ |
| | 4 | $(5.0 \pm 1.1) \times 10^6$ | $(5.7 \pm 1.3) \times 10^6$ |
| | 5 | $(2.4 \pm 0.5) \times 10^7$ | $(5.1 \pm 0.8) \times 10^5$ |
| | 6 | $(4.0 \pm 0.2) \times 10^6$ | $(6.0 \pm 1.7) \times 10^4$ |
| | 7 | $(7.9 \pm 1.7) \times 10^6$ | $(2.2 \pm 0.5) \times 10^5$ |
| | 8 | $(6.5 \pm 1.6) \times 10^7$ | $(9.2 \pm 3.4) \times 10^5$ |
| | 9 | $(4.3 \pm 1.4) \times 10^7$ | $(1.3 \pm 0.4) \times 10^6$ |
| | 10 | $(9.0 \pm 3.4) \times 10^6$ | $(1.5 \pm 0.5) \times 10^6$ |
| Cell C2 – Biofertilizer addition (TRPH = 46.0 g/kg) | 0 | $(4.6 \pm 1.2) \times 10^7$ | $(3.9 \pm 1.2) \times 10^5$ |
| | 1 | $(6.4 \pm 1.5) \times 10^7$ | $(2.0 \pm 0.5) \times 10^7$ |
| | 2 | $(3.5 \pm 1.0) \times 10^8$ | $(4.1 \pm 1.0) \times 10^7$ |
| | 3 | $(1.4 \pm 0.1) \times 10^9$ | $(1.0 \pm 0.1) \times 10^8$ |
| | 4 | $(2.2 \pm 0.6) \times 10^9$ | $(1.7 \pm 0.2) \times 10^8$ |
| | 5 | $(2.7 \pm 0.7) \times 10^8$ | $(4.3 \pm 0.8) \times 10^7$ |
| | 6 | $(2.9 \pm 0.6) \times 10^8$ | $(4.8 \pm 1.0) \times 10^7$ |
| | 7 | $(3.0 \pm 1.0) \times 10^9$ | $(4.5 \pm 1.5) \times 10^8$ |
| | 8 | $(5.2 \pm 1.1) \times 10^8$ | $(2.0 \pm 0.3) \times 10^8$ |
| | 9 | $(9.8 \pm 2.5) \times 10^8$ | $(6.5 \pm 2.2) \times 10^8$ |
| | 10 | $(9.3 \pm 2.1) \times 10^7$ | $(8.0 \pm 2.2) \times 10^7$ |
| Cell C3 – Biofertilizer addition (TRPH = 14.0 g/kg) | 0 | $(4.9 \pm 0.4) \times 10^7$ | $(3.7 \pm 0.6) \times 10^5$ |
| | 1 | $(5.2 \pm 1.6) \times 10^8$ | $(1.5 \pm 0.1) \times 10^8$ |
| | 2 | $(3.3 \pm 0.2) \times 10^9$ | $(6.2 \pm 2.0) \times 10^8$ |
| | 3 | $(5.8 \pm 1.2) \times 10^9$ | $(5.5 \pm 1.7) \times 10^8$ |
| | 4 | $(2.1 \pm 0.3) \times 10^9$ | $(5.6 \pm 1.0) \times 10^8$ |
| | 5 | $(3.0 \pm 1.5) \times 10^8$ | $(7.8 \pm 2.9) \times 10^7$ |
| | 6 | $(4.0 \pm 1.1) \times 10^7$ | $(1.5 \pm 0.2) \times 10^7$ |
| | 7 | $(1.5 \pm 0.7) \times 10^7$ | $(1.3 \pm 0.1) \times 10^7$ |
| | 8 | $(7.5 \pm 2.0) \times 10^7$ | $(2.1 \pm 0.8) \times 10^7$ |
| | 9 | $(3.8 \pm 0.5) \times 10^7$ | $(3.3 \pm 0.5) \times 10^7$ |
| | 10 | $(6.9 \pm 1.3) \times 10^7$ | $(1.4 \pm 0.1) \times 10^7$ |
| Cell S1 – Initial treatment in slurry bioreactor (TRPH = 24.0 g/kg) | 0 | $(1.8 \pm 0.3) \times 10^8$ | $(7.4 \pm 1.9) \times 10^7$ |
| | 1 | $(2.4 \pm 0.1) \times 10^8$ | $(3.5 \pm 0.1) \times 10^8$ |
| | 2 | $(8.6 \pm 2.0) \times 10^8$ | $(7.3 \pm 2.5) \times 10^8$ |
| | 3 | $(9.1 \pm 3.3) \times 10^8$ | $(6.4 \pm 1.3) \times 10^8$ |
| | 4 | $(8.3 \pm 1.5) \times 10^8$ | $(3.3 \pm 0.8) \times 10^8$ |
| | 5 | $(3.1 \pm 0.4) \times 10^8$ | $(1.8 \pm 0.6) \times 10^8$ |
| Cell S2 – Initial treatment in slurry bioreactor (TRPH = 9.1 g/kg) | 0 | $(5.3 \pm 0.1) \times 10^7$ | $(2.0 \pm 1.3) \times 10^7$ |
| | 1 | $(8.0 \pm 2.3) \times 10^7$ | $(3.7 \pm 0.4) \times 10^7$ |
| | 2 | $(4.3 \pm 0.3) \times 10^8$ | $(1.9 \pm 0.6) \times 10^8$ |
| | 3 | $(9.6 \pm 3.2) \times 10^8$ | $(6.3 \pm 0.4) \times 10^8$ |
| | 4 | $(8.5 \pm 2.0) \times 10^8$ | $(5.3 \pm 0.6) \times 10^8$ |
| | 5 | $(2.1 \pm 0.3) \times 10^8$ | $(9.5 \pm 2.1) \times 10^7$ |

Note. Mean values of three determinations ± standard deviations are given.

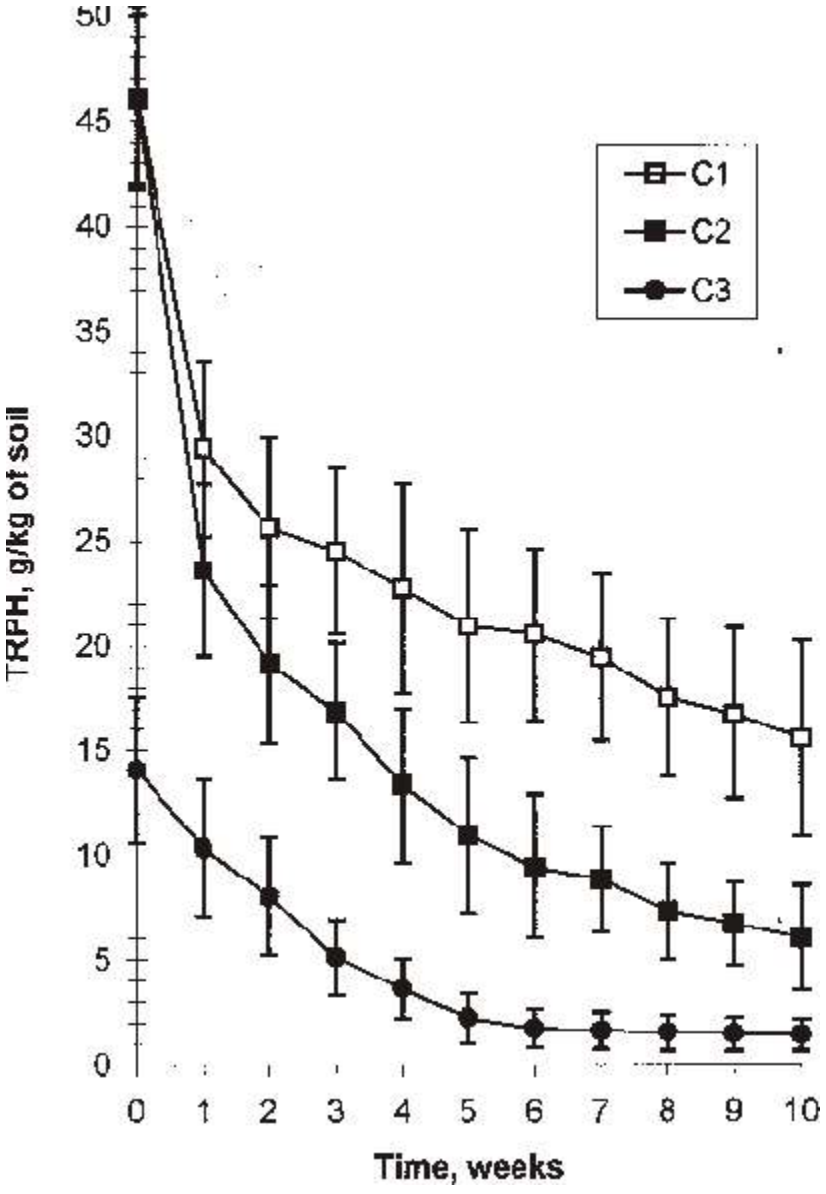


***FIGURE 2***

*Effects of biological treatment on residual oil content in C1, C2, and C3 land farming cells. Residual oil content is indicated as total recoverable petroleum hydrocarbons (TRPH) concentration in dry soil. The mean values of three determinations are given. Bars indicate standard deviations. C1 — control (no treatment); C2, C3 — oleophilic biofertilizer addition.*

Table 2. Fractional composition of residual oil in samples from land farming cells

| Land farming cell | Time, weeks | Major oil fractions, % | | | |
|---|---|---|---|---|---|
| | | Aliphatics | Aromatics | Heterocyclics | Asphalthenes and tars |
| Cell C1 | 0 | 65.9 | 26.2 | 6.0 | 1.9 |
| | 5 | 70.7 | 21.3 | 6.2 | 1.8 |
| | 10 | 71.2 | 18.7 | 7.2 | 2.9 |
| Cell C2 | 0 | 64.0 | 25.3 | 7.4 | 3.3 |
| | 5 | 63.5 | 26.7 | 7.8 | 2.0 |
| | 10 | 58.7 | 20.4 | 9.0 | 11.9 |
| Cell C3 | 0 | 57.9 | 23.8 | 14.4 | 3.9 |
| | 5 | 56.9 | 26.3 | 12.9 | 3.7 |
| | 10 | 50.5 | 10.0 | 10.7 | 28.7 |
| Cell S1 | 0 | 68.5 | 20.2 | 6.8 | 4.5 |
| | 3 | 64.6 | 17.5 | 10.3 | 7.6 |
| | 5 | 55.3 | 18.4 | 9.3 | 17.0 |
| Cell S2 | 0 | 62.1 | 27.4 | 6.0 | 4.5 |
| | 3 | 68.0 | 14.7 | 10.1 | 7.2 |
| | 5 | 54.2 | 16.6 | 17.2 | 12.0 |

## Slurry-Phase Biotreatment of Contaminated Soil

The use of a biofertilizer in a slurry reactor facilitated a high density of heterotrophic and hydrocarbon-oxidizing bacteria. Prior to the biofertilizer addition, heterotrophs and hydrocarbon-oxidizers were present at $7.9 \times 10^5$ and $5.1 \times 10^4$ CFU/ml, respectively. This increased to $1.5 \times 10^7$ and $9.2 \times 10^7$ CFU/ml, respectively, following the biofertilizer addition. These levels remained at around $10^7$ to $10^8$ CFU/ml through the bioslurry reactor treatment.

Figure 3 shows data for oil concentration changes in the samples from the slurry bioreactor. As evidenced from the data, oil concentration decreased from 4.9 to 0.6 g/l of TRPH in the liquid phase of the reactor after 8 weeks of bioremediation. However, a significant proportion of oil adsorbed onto clay particles and formed a film on the inner surface of the bioreactor not available for microbiological oxidation due to the lack of effective mixing of the reactor content.

Microbial oxidation of aliphatic compounds occurred most intensively (Figure 3), and their relative proportion in the residual oil decreased from 68 to 63%. A notable characteristic of the bioreactor was a high degradation rate of aromatic hydrocarbons not readily degradable under normal soil conditions. The relative proportion of these compounds decreased from 20 to 11% within 2 months. Tar/asphaltene components degraded at a lower rate, and consequently the relative proportion in the residual contamination increased threefold.

The results of the slurry-phase biotreatment of heavily oil-contaminated soil indicated that a high biodegradation had occurred in the aqueous phase. However, partly due to limited physical mixing, a considerable proportion of the oil was not degraded. Further treatment of the bioreactor content therefore was performed using land farming cells. The microbiological data presented in Table 1 showed that the oil-oxidizing bacteriocenoses at S1 and S2 cells grew actively and exceeded the microbial communities of the C2 and C3 cells.

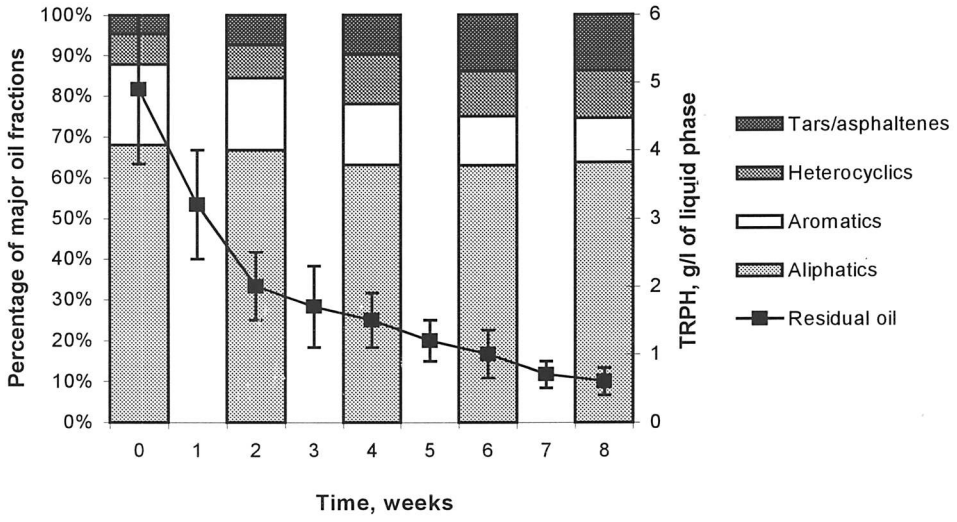


***FIGURE 3***

*Changes in concentration and fractional composition of oil contamination in liquid phase of slurry bioreactor. On residual oil curve the mean values of three determinations are given. Bars indicate standard deviations.*

Preliminary activation of the bioreactor's oil-degrading microflora provided a high degradation rate of residual oil in the land farming cells studied. Thus, the amount of oil in cells S1 and S2 decreased by 67 to 70% within 3 weeks of bioremediation (Figure 4). Total oil removal in these cells was 86 to 89% after 5 weeks.

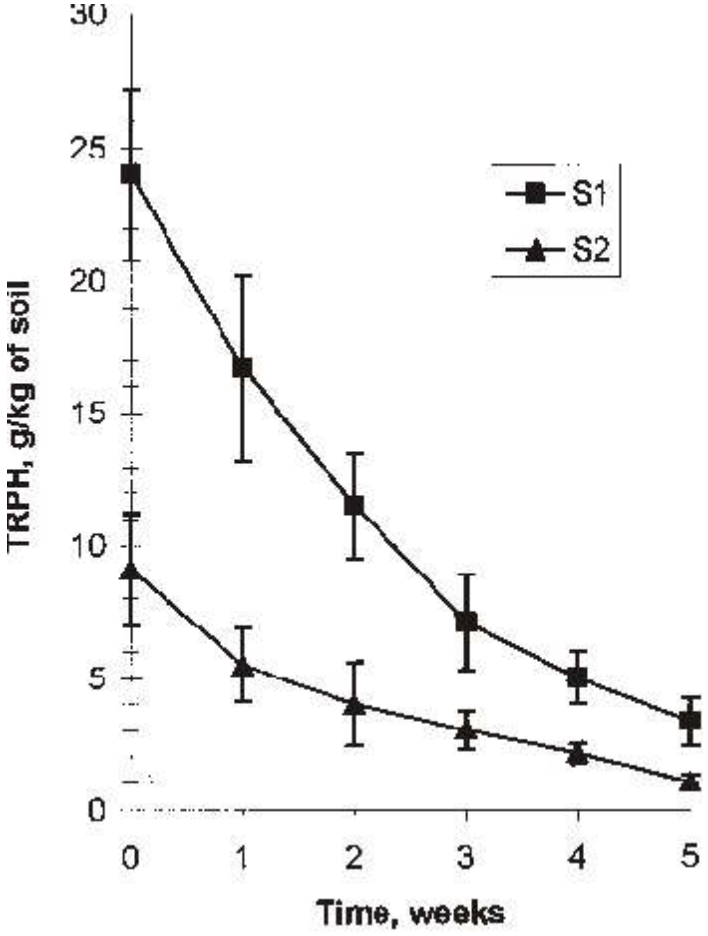


***FIGURE 4***

*Effects of biological treatment on residual oil content in S1 and S2 land farming cells. Residual oil content is indicated as total recoverable petroleum hydrocarbons (TRPH) concentration in dry soil. The mean values of three determinations are given. Bars indicate standard deviations. S1 and S2 — initial biological treatment in slurry bioreactor.*

The use of a soil slurry bioreactor to enhance the biodegradation process proved to be effective and, in combination with land farming cells, may be used to eliminate heavy oil contamination (up to 200 g/kg of TRPH). For lower levels of contamination, it was sufficient to construct land farming cells alone.

It is noteworthy that the use of a bioreactor allowed more precise control of operating parameters (temperature, pH, oxygen concentration, biofertilizer consumption, microbial biomass density) and also operation under cold conditions. An added advantage was the ability to reduce the application rate of the biofertilizer.

### Soil Phytoremediation

To recover soil quality for further agricultural use, phytoremediation of soil was performed using the mixture of perennial grasses described. Plate 1 shows C1 and C2 land farming cells after phytoremediation.

Comparative data on plant size and biomass (Table 3) shows a 1.8 to 6.2-fold inhibition of plant growth in untreated oil-contaminated soil (C1) compared with that of the clean agricultural soil (K). the greatest reduction on biomass of 96% was observed for *Bromus ezsaristatus.* The growth of introduced plants at C2 and C3 cells treated with biofertilizer were similar to those of non-contaminated agricultural soil (K). The increased growth of clover and timothy at these cells compared with the clean soil was probably due to the stimulating effect of the biofertilizer.

Due to its ability to fix atmospheric nitrogen and to produce considerable biomass, clover appeared to be the most effective species in recovering soil fertility. The other two cereal grasses used were reported to enhance the growth and biodegradative activity of rhizospheric microflora (Boyle and Shann, 1998; Siciliano and Germida, 1998). Biofertilizer addition had a stimulating effect on both bacterial and plant components of soil biocenosis.

The field-scale study involved bioremediation of oil-contaminated soil using the oleophilic biofertilizer. The scheme included the construction of land farming cells; treatment of oil-contaminated soil in a slurry bioreactor; phytoremediation of residual oil contamination by seeding a mixture of perennial grass. The work performed resulted in cleaning of soil heavily contaminated (up to 200 g/kg of TRPH) with crude oil wastes. Biodegradation effectiveness was 80 to 90% in biofertilizer-treated land farming cells after 5 to 7 weeks. Maximal biodegradation rates of petroleum hydrocarbons were achieved following preliminary stimulation of the degradation process in a slurry bioreactor. The concentration of residual oil contamination in remediated soil was 1.0 to 1.5 g/kg of TRPH and did not exceed the standard allowable level of the Russian Federation for further use of this soil for general economic purposes.

***PLATE 1***

*Experimental land farming cells after the phytoremediation was performed. In the photo above — biofertilizer-treated cell C2, below — control untreated cell C1.*

ACKNOWLEDGMENTS

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## Appendix H. Kuyukina *et al*. (2005)

Kuyukina, M.S., Ivshina, I.B., Makarov, S.O., Litvinenko, L.V., **Cunningham, C.J.**, Philp, J.C. (2005). Effect of biosurfactants on crude oil desorption and mobilisation in a soil system. *Environment International*. 31(2), 155-161.

### *H.1 Declaration of contribution*

The author was involved at all stages of the project including a period of time spent in Russia designing the project and returned to assist with laboratory experiments. Kuyukina and Ivshina brought the collaborators Makarov and Litvinenko from Perm State University to the project to lead the modelling work and the author had minimal involvement in this aspect. TLC-FID analyses were conducted in the UK on samples brought back by the author although these were undertaken at an external laboratory. The author closely involved in the interpretation of results and at all stages of writing the paper.

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# Effect of biosurfactants on crude oil desorption and mobilization in a soil system

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Available online 5 November 2004

**Abstract**

Microbially produced biosurfactants were studied to enhance crude oil desorption and mobilization in model soil column systems. The ability of biosurfactants from *Rhodococcus ruber* to remove the oil from the soil core was 1.4–2.3 times greater than that of a synthetic surfactant of suitable properties, Tween 60. Biosurfactant-enhanced oil mobilization was temperature-related, and it was slower at 15 °C than at 22–28 °C. Mathematical modelling using a one-dimensional filtration model was applied to simulate the process of oil penetration through a soil column in the presence of (bio)surfactants. A strong positive correlation ($R^2$=0.99) was found between surfactant penetration through oil-contaminated soil and oil removal activity. Biosurfactant was less adsorbed to soil components than synthetic surfactant, thus rapidly penetrating through the soil column and effectively removing 65–82% of crude oil. Chemical analysis showed that crude oil removed by biosurfactant contained a lower proportion of high-molecular-weight paraffins and asphaltenes, the most nonbiodegradable compounds, compared to initial oil composition. This result suggests that oil mobilized by biosurfactants could be easily biodegraded by soil bacteria. *Rhodococcus* biosurfactants can be used for *in situ* remediation of oil-contaminated soils.


*Keywords:* Biosurfactants; Bacteria; *Rhodococcus*; Crude oil; Soil; Desorption; Mobilization; Mathematical modelling

## 1. Introduction

In natural conditions, oil penetration through soil is an extremely complex process involving physical, chemical, and biological factors. Crude oil is a highly hydrophobic material with most of its components having low water solubility. These components bind to soil particles and become nonbioavailable to microorganisms. To increase the bioavailability of hydrocarbon pollutants, surface-active agents (surfactants) may be used, allowing desorption and solubilization of petroleum hydrocarbons and thus facilitating their assimilation by microbial cells (Deshpande et al., 1999; Doong and Lei, 2003; Mulligan et al., 2001).

There are two mechanisms of surfactant-enhanced soil washing. One occurs below the critical micelle concentration (CMC), when surfactant monomers increase the contact angle between the soil and hydrophobic contaminant, thereby promoting the separation of contaminant from soil particles and finally displacing the oil from the soil (soil roll-up mechanism). The other mechanism, solubilization, occurs above the CMC, when contaminants are partitioned from the

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soil into the hydrophobic core of surfactant micelles (Deshpande et al., 1999). Micellar phase bioavailability of hydrophobic organics means that contaminants partitioned into the micellar phase are biodegradable without having to transfer to the dissolved phase first (Deshpande et al., 1999). Solubilization using surfactants at concentrations above their CMC values is widely explored in *in situ* soil washing (Mulligan et al., 2001). Nonionic surfactants with high hydrophobicity (i.e., low HLB[4] value), such as Triton's, Tween's, and Brij's, are considered to be suitable for enhancing solubilization of hydrophobic organics in soil. It was shown though for nonionic surfactants that the increase of surfactant concentration from 2× to 10× CMC did not affect much the desorption efficiency of petroleum hydrocarbons (Doong and Lei, 2003). The inhibition of contaminant biodegradation in soil systems at surfactant concentrations above CMC has also been reported (Billingsley et al., 2002). Moreover, many commonly used synthetic surfactants are toxic and poorly biodegradable; their application may lead to the accumulation of ecologically harmful compounds in soil (Mulligan et al., 2001).

In recent years, microbially produced biosurfactants have found a new area of application in environment remediation processes. Biosurfactants possess distinct advantages over synthetic ones including biodegradability and biocompatibility, multifunctional characteristics, stable activity under extreme environmental conditions (e.g., high or low temperature and pH, high pressure, and salinity), and thus can be more effective in remediation of contaminated soil. Bacteria of various genera such as *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Arthrobacter*, and *Rhodococcus* are able to produce biosurfactants during hydrocarbon oxidation (Lang and Philp, 1998; Mulligan et al., 2001). *Rhodococcus* bacteria, when grown on liquid *n*-alkanes, produce glycolipid surfactants with low CMC and toxicity (Ivshina et al., 1998; Philp et al., 2002).

Biosurfactants from *Rhodococcus ruber* were found to partition high amounts (up to 93 %) of crude oil from sand slurries into the aqueous phase under shaking conditions (Ivshina et al., 1998). Here we have examined the oil removal activity of *R. ruber* biosurfactants using model soil-packed columns heavily contaminated with crude oil. Column systems were chosen for the study as they closely mimic natural soil conditions and thus they can be used for laboratory simulation of *in situ* soil washing process. The ability of biosurfactants to remove the oil from soil cores was compared with that of the synthetic surfactant, Tween 60. Tween 60 [polysorbate 60; polyoxyethylene (20) sorbitan monostearate] was chosen for its relatively low toxicity; this surfactant is widely used as an emulsifier, dispersant, or stabilizer in foods, cosmetics, and pharmaceutical preparations (FAO/WHO, 1974; Ema et al., 1988; RTECS, 1997; Arechabala et al., 1999).

Mathematical modelling using a one-dimensional filtration equation based on Darcy's law was used to simulate the process of crude oil penetration through a soil column in the presence of (bio)surfactants. Values of main process parameters such as penetration rates and permeability coefficients were obtained by fitting the experimental data to the model.

[4] Hydrophilic–lipophilic balance.

## 2. Materials and methods

### 2.1. Biosurfactant production and surface-active properties

Bacterial strain *R. ruber* IEGM 231 from the Regional Specialized Alkanotrophic Microorganism Collection of the Institute of Ecology and Genetics of Microorganisms (IEGM; www.iegm.ru/iegmcol) was used for biosurfactant production. Growth conditions for *R. ruber* strain and crude biosurfactant extraction procedure have been described elsewhere (Ivshina et al., 1998; Kuyukina et al., 2001). Surface-active properties were determined as described previously (Philp et al., 2002). Emulsifying ability was determined by the method of Gerson and Zajic (1978). Five milliliters of *n*-hexadecane, 5 ml of distilled water, and 0.2 ml of biosurfactant were mixed in a graduated tube. The type of emulsion formed was noted (oil-in-water; water-in-oil) and percentage volumes of emulsion recorded after 0.1, 1, and 24 h.

### 2.2. Model soil column experiments

The model soil used contained (w/w) 50% sand, 30% clay, and 20% peat. Soil components were dried overnight at 105 °C and screened through a 2-mm sieve before mixing. Glass columns (length, 57 cm; diameter, 3.0 cm) with a glass filter at the bottom were dry-packed with the model soil under vibration, watered from the top to adjust soil moisture level to 20%, and then loaded from the top with crude oil taken from Chashkinskoe oilfield (well no. 179), Perm region. The amount of oil applied to individual columns was calculated to achieve the final oil concentration in soil of 10% (w/w). Physical parameters of soil (e.g., dry weight, bulk density, porosity, moisture content, and water-holding capacity) were determined by standard methods (Klute, 1986). Soil column properties are shown in Table 1. The levels (fronts) of water, oil, and surfactant penetration through the soil columns were measured at 15- and 30-min intervals and were represented as percentages of soil core height.

Solutions used for mobilization of crude oil entrapped in the soil were as follows: distilled water used as a control; *R. ruber* IEGM 231 crude surfactants used as biosurfactants; and Tween 60 (Sigma) used as synthetic surfactant. Aqueous surfactant solutions were prepared in distilled water. Biosurfactant solutions were sonicated (44 kHz, 0.7 A) for 10 min before usage. Surfactants were used at concentrations twofold of their CMC values. The CMC of *R. ruber* biosurfactant in distilled water at 25 °C was determined to be 0.72 g/l. The CMC of Tween 60 is 33 mg/l. Surfactant solution (300 ml) was applied to the top of each column. Three replicates of each variant (distilled water,

Table 1
Physical properties of model soil columns

| | | | |
|---|---|---|---|
| Soil composition (wt.%) | Sand—50; clay—30; peat—20 | Porosity coefficient (void ratio) | 0.51±0.04 |
| Soil dry weight (g) | 489.2±4.6 | Soil moisture content (%) | 20.3±0.53 |
| Soil core height (cm) | 50.3±0.8 | Water-holding capacity (ml/g) | 0.36±0.03 |
| Soil core diameter (cm) | 2.9±0.1 | Crude oil concentration (g/kg) | 100.0±0.9 |
| Soil bulk density (g/cm$^3$) | 1.41±0.08 | Crude oil specific density (g/cm$^3$) | 0.86±0.07 |
| Soil skeleton density (g/cm$^3$) | 0.94±0.03 | Crude oil kinematical viscosity at 20 °C (cm$^2$/s) | 0.0894 |
| Total (average) porosity (%) | 33.7±1.2 | Crude oil kinematical viscosity at 50 °C (cm$^2$/s) | 0.0427 |

Here and in Table 2, the mean values of three determinations ±S.D. are given.

biosurfactants, and Tween 60) were prepared. The effluents from the columns were collected and placed into glass separation funnels. The oil phase was discharged from the funnels and used for fractional analysis.

After oil washing, the experimental soil columns were extracted with 300 ml of chloroform to remove remaining oil. The solvent was rotary-evaporated at 50 °C, and the amount of extracted oil was determined gravimetrically. The recovery efficiency of the extraction procedure examined by the control chloroform extraction was 99.6%.

### 2.3. Chemical analyses

Oil fraction analysis was performed using an Iatroscan TLC-FID Analyzer MK-5 (Iatron Laboratories, Japan). Oil samples were dissolved in a 3:1 mixture of dichloromethane–pentane; the pentane-soluble fractions were applied onto chromarods (type S III) and consecutively eluted with varying solvent systems. Details of microextraction–microTLC can be found elsewhere (Kuyukina et al., 2003). After separation, the rods were scanned using an FID, the area for each peak was calculated, and the composition (i.e., the percentage of saturated hydrocarbons, aromatic hydrocarbons, and heterocyclics) was determined. Tar/asphaltene content of samples (pentane-insoluble fractions) was determined gravimetrically (Uraizee et al., 1998).

### 2.4. Model simulation

In our study, a one-dimensional model of unsteady filtration motion based on the Polubarinova-Kochina (1962) equation and Darcy's law was used to simulate the penetration of water and crude oil through a soil column.

The model describes a filtration process for Newtonian fluids (water, crude oil) through a porous medium (soil), assuming that the fluids studied are incompressible and isothermal, and the porous medium is homogenous. The simplified geometrical model used is a straight vertical cylinder having infinitely large radius and height, filled with porous medium and limited at the top by horizontal surface $z$=0. Fluid filtration through the porous medium occurs in the direction of the gravitational force $\vec{g} = g\,\vec{e_z}$. At initial time $t$=0, the fluid layer of height $h_0$ is applied to the surface of the porous medium $z$=0. At the time $t$, the fluid penetrates through porous medium to the depth $z$=$H(t)$.

The fluid filtration process is determined by the gravity force $g$ and by negative capillary pressure $-q(\Delta\sigma)$ of the porous medium, which is dependent upon the contact angle of liquid–solid interface and upon the difference in interfacial tensions $\Delta\sigma$ of dry and wetted porous matrix particles (or hydrophobic liquid and wetted particles, in case of crude oil penetration through wet soil) (Moseley and Dhir, 1996).

Assuming that external pressure is zero, the pressure at porous medium surface $z$=0 is defined as:

$$p(z=0,t) = \rho g h(t);$$

and the pressure at liquid–solid interface within a porous matrix $z$=$H(t)$ is defined as:

$$p(z=H,t) = -q(\Delta\sigma).$$

The porous medium force of resistance to the fluid flow is determined by Darcy's law:

$$\vec{f} = -\frac{\eta}{K}\vec{u},$$

where $u$ [cm/s] is filtration rate; $\eta$ [g/cm×s] is fluid dynamic viscosity; and $K$ [cm$^2$] is soil permeability coefficient. Soil permeability coefficient can be expressed as:

$$K = \frac{k_{\mathrm{hydr}} v}{g},$$

where $k_{\mathrm{hydr}}$ [cm/s] is hydraulic conductivity; $v$ [cm$^2$/s] is kinematical viscosity; and $g$ [cm/s$^2$] is gravity acceleration.

The effective seepage (or filtration) velocity is $\vec{u} = \varepsilon\vec{v}$, where $\vec{v}$ is average pore fluid velocity and $\varepsilon$ is soil porosity. Assuming that filtration velocity is equal to the fluid front velocity: $u(t)$=$\dot{H}(t)$.

The summarized equation (Eq. (1)) of the proposed one-dimensional unsteady filtration model is:

$$\frac{\rho}{\varepsilon}\frac{\partial\vec{u}}{\partial t} = -\nabla p + \rho\vec{g} - \frac{\eta}{K}\vec{u}; \quad \mathrm{div}\,\vec{u} = 0 \tag{1}$$

$$t=0: \quad h(0)=h_0; \quad H(0)=0; \quad u(0)=0 \tag{2}$$

$$z=0: \quad p(z=0,t)=\rho g h(t) \tag{3}$$

$$z=H: \quad p(z=H,t) = -q(\Delta\sigma)$$

where $u$ [cm/s] is filtration rate; $t$ [s] is time; ρ [g/cm$^3$] is water/oil density; $p$ [dyn/cm] is pressure; $g$ [cm/s$^2$] is gravity acceleration; $\eta$ [g/cm×s] is water/oil dynamic viscosity; $K$ [cm/s] is soil permeability coefficient; $q\Delta\sigma$ is

capillary pressure; $H$ [cm] is water/oil penetration level; and $h$ [cm] is water/oil column above soil core.

Eq. (2) describes the initial conditions when $t$=0, $H$=0, and there is no oil/water in the soil column. Eq. (3) describes the boundary conditions, when $z$=0, and the oil/water is applied to the top of the soil column; when $z$=$H$, and the oil/water is penetrating through the soil column.

## 3. Results and discussion

### 3.1. Biosurfactant properties

As was shown in our previous studies, the *Rhodococcus* actinobacteria grown on liquid *n*-alkanes ($C_{10}$–$C_{17}$) produce surface-active glycolipids (Ivshina et al., 1998; Philp et al., 2002). Table 2 summarizes the emulsifying properties of biosurfactants produced by *R. ruber* IEGM 231 grown on individual *n*-alkanes. Biosurfactants produced by *R. ruber* bacterium have formed stable emulsions of the oil-in-water type when added to a *n*-hexadecane–water system. Higher biosurfactant yield was recorded for bacterial cells grown on *n*-hexadecane, although greater emulsion indices were obtained for the biosurfactant produced on *n*-dodecane (Table 2). Biosurfactants obtained from dodecane- and hexadecane-grown *R. ruber* cultures were further examined for the ability to remove crude oil from a soil in the model column test.

### 3.2. Temperature effect on water, crude oil, and surfactant penetration through model soil column

Prior to crude oil mobilization experiments using (bio)surfactants, we have studied the process of penetration of hydrophilic (distilled water), hydrophobic (crude oil), and amphiphilic (surfactants) liquids through the model soil at different temperatures.

Fig. 1 shows the effect of temperature on the penetration of water and crude oil through the soil column. The penetrations of both water and oil through the soil core are nonlinear processes, and the filtration rates were maximal during the first 30 min of penetration. Water saturation of dry soil in a column has occurred more rapidly at higher temperature. Thus, at 28 °C, the soil column was completely saturated with a given amount of water after 3 h. However, at 15 °C, the water saturation of the soil column took 4.5 h. The average water penetration rates at 15 and 22 °C were similar (Fig. 1).

Table 2
Surface-active properties of *R. ruber* IEGM 231

| Carbon source | Biosurfactant yield (g/l) | Emulsion type | Emulsion index (%) | | |
|---|---|---|---|---|---|
| | | | $E_{0.1}$ | $E_1$ | $E_{24}$ |
| *n*-Dodecane | 6.5 | o/w[a] | 89.4±2.9 | 59.9±1.3 | 44.8±1.1 |
| *n*-Hexadecane | 9.9 | o/w | 79.7±2.3 | 46.5±3.5 | 25.3±3.1 |

[a] Oil-in-water emulsion.

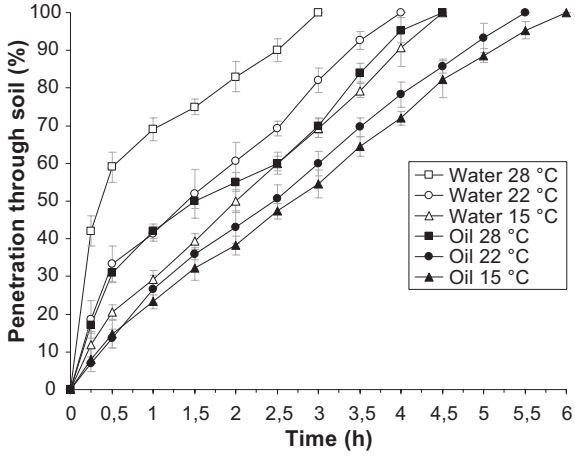


Fig. 1. Effect of temperature on water (open symbols) and oil (filled symbols) penetration through the model soil. Temperature: (□,■) −28 °C; (○,●) −22 °C; (△,▲) −15 °C. Here and in Figs. 2 and 3, the mean values of three determinations are given. Bars indicate standard deviations.

After the saturation of soil in columns with water at a relative moisture level of 20%, we have studied the penetration of crude oil through the soil core. This process was also found to be temperature-dependent and was slower than water penetration. Although there was no statistically significant difference in oil penetration rate at 22 and 15 °C, at 28 °C, it was twofold the rate at 15 °C.

Fig. 2 shows the penetration of surfactant solutions through oil-contaminated soil at different temperatures. *Rhodococcus* biosurfactant and Tween 60 penetration through contaminated soil has occurred at higher speed at higher temperature. The penetration rates of *Rhodococcus* biosurfactant were maximal and these exceeded the corresponding values for synthetic surfactant by 1.2–2.8 times. At 22 °C (average summer temperature for the Perm region), the biosurfactant solution completely penetrated through the column after 4.5 h; however, for the Tween 60 solution, this process took 6 h.

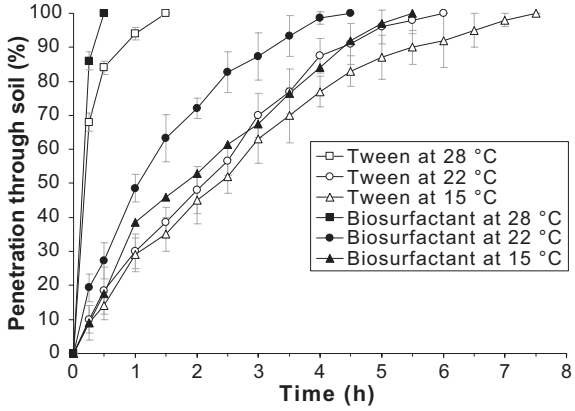


Fig. 2. Effect of temperature on Tween 60 (open symbols) and biosurfactant (filled symbols) penetration through oil-contaminated soil. Temperature: (□,■) −28 °C; (○,●) −22 °C; (△,▲) −15 °C.

### 3.3. Model simulation

Eq. (1) (see Materials and Methods section) has the exact solution for pressure ($p$):

$$p = \rho g h(t) - \{\rho g h(t) + q\} z / H(t) \quad (4)$$

Substituting solution (4) in Eq. (1) gives an ordinary nonlinear second-order differential equation for the $H(t)$ in dimensionless form:

$$\ddot{H} + \dot{H} = 1 + \frac{b}{H}; \qquad b = \frac{\varepsilon \nu^2 (q + \rho g h_0)}{(1-\varepsilon)^2 g^2 \rho K^2} \quad (5)$$

with the boundary conditions $t$=0: $H$=$\dot{H}$=0, where the unit of length is $\frac{gK^2(1-\varepsilon)}{\varepsilon\nu^2}$; the unit of time is $K/\nu\varepsilon$.

Eq. (5) has only a numerical solution for the integrated parameter $b$. Fig. 3 shows the theoretical curve $H(t)$ calculated for the arbitrary value of parameter $b$. It can be seen from the curve in Fig. 3 that fluid penetration through the porous medium increases rapidly at the initial time, after which it tends to the linear asymptotic limit.

It should be noticed that in real experimental conditions, fluid penetration through the soil column (glass cylinder with finite radius) was considerably affected by a wall wetting process resulting in boundary fluid layer build-up at the column wall. This wall wetting effect was especially significant during the initial stage of fluid filtration, impeding precise measurement of fluid front level during the first 10–15 min, and resulting in discrepancy between theoretical and observed dynamics of fluid filtration during this time. Furthermore, at small $H$ values, the error of measurement is comparable with the value of $H$. Taking these facts into account, we have performed the fitting of theoretical curves to the experimental data for water/oil filtration through the soil, recorded after the first 30 min (Fig. 4). When filtration time is large enough, then the parameter $H(t)$ is large, and therefore in Eq. (5), the ratio $b/H$ is much less than 1. Parameter $b/H$ can be vanished to obtain asymptotic relationship between $H$ and $t$. Eq. (5) with the vanished parameter $b/H$ has an analytical solution:

$$H = t - 1 + e^{-t}$$

Fig. 4 shows theoretical curves calculated for fluid penetration through the porous medium at different temperatures, fitted to the experimental data. It can be resumed from these curves and from the theoretical curve in Fig. 3 that the proposed model simulation provides a good fit to the experimental data within the whole time period, except during the first 10–15 min. Optimized model parameters, characterizing the process of water/oil penetration through the soil, such as soil permeability coefficient, hydraulic conductivities, and crude oil filtration coefficients at three different temperatures (15, 22, and 28 °C), are shown in Table 3. We are currently developing a complex filtration model allowing us to estimate the influence of (bio)surfactants on a process of crude oil filtration through the porous medium.

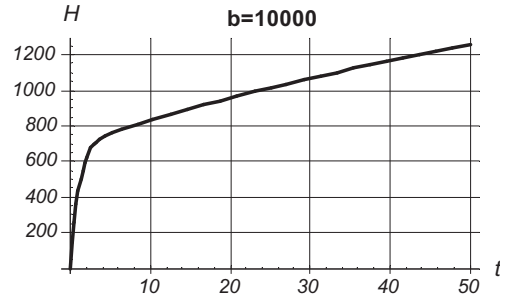


Fig. 3. Theoretical curve $H(t)$ calculated from the proposed filtration model for the arbitrary value of parameter $b$=10000.

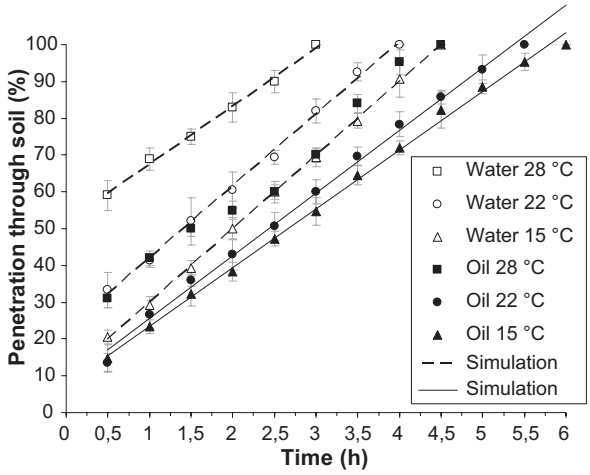


Fig. 4. Model simulations of the water/oil filtration through the soil at different temperatures, fitted to the experimental data.

### 3.4. Crude oil mobilization using (bio)surfactants

Fig. 5 shows the removal of oil contamination from the soil core using chemical (Tween 60) surfactant and biosurfactants. The ability of biosurfactants to remove crude oil entrapped within the soil matrix was 1.9–2.3 times greater than that of a synthetic surfactant. Oil removal rate was found to be temperature-related. At 28 °C, the maximal oil recovery (82%) was recorded for the *Rhodococcus* biosurfactant produced on *n*-hexadecane. Biosurfactant-enhanced oil removal decreased by 1.3 times at 22 °C compared to 28 °C. Biosurfactant produced by *R. ruber* grown on *n*-dodecane was most effective for oil removal from contaminated soil in colder conditions (at 15 °C).

However, *n*-hexadecane-produced biosurfactant was not effective in cold conditions as it froze at temperatures below

Table 3
Optimized model parameters

| Temperature (°C) | Permeability coefficient ($cm^2$) | Hydraulic conductivity (cm/s) | Crude oil filtration coefficient (cm/s) |
|---|---|---|---|
| 15 | $5.36\times10^{-8}$ | $4.60\times10^{-3}$ | $5.36\times10^{-4}$ |
| 22 | | $5.52\times10^{-3}$ | $6.10\times10^{-4}$ |
| 28 | | $6.18\times10^{-3}$ | $6.82\times10^{-4}$ |

16 °C, apparently due to a high proportion of residual hexadecane in biosurfactant content (Kuyukina et al., 2001).

Strong positive correlation was found between the penetration rate of surfactants through the oil-contaminated soil and their oil removal abilities at different temperatures ($R^2$=0.94, $P$=0.16 at 15 °C; $R^2$=0.99, $P$=0.08 at 22 °C; $R^2$=0.97, $P$=0.11 at 28 °C). Apparently, the *Rhodococcus* biosurfactant displayed lower sorption to soil particles than the synthetic surfactant, thus penetrating through the soil core at higher speed and effectively removing oil from the soil matrix.

It should be noticed that hydrocarbon release from soil depends on soil texture and mineralogy. Particularly, high clay content in soil can significantly influence surfactant-mediated removal of hydrophobic organic compounds. In the present study, we have used the model soil with high (30%) clay proportion, which is characteristic for heavy clay texture soils of Perm region (Kuyukina et al., 2003). It has been frequently observed that low permeability clayey soils with hydraulic conductivity less than $10^{-3}$ cm/s may significantly increase the time of surfactant penetration through the contaminated subsoil zone (Rajput et al., 1994; Roy et al., 1995; Lee et al., 2002).

Surfactants bind to clay particles, thereby decreasing the concentration of micelles and thus the extent of contaminant removal. *Rhodococcus* biosurfactant was less sorbed to the soil matrix than Tween 60, and therefore less influenced by soil hydraulic conductivity and more efficient in oil removal from clay-rich soil.

### 3.5. Composition of crude oil removed from contaminated soil by biosurfactants

Fig. 6 compares the composition of initial oil and that washed from the soil core using (bio)surfactants. Significant sorption of aliphatics (presumably high-molecular-weight paraffins) and asphaltenes by soil components led to a relative decrease of these fractions in the recovered oil.

The composition of oil washed by synthetic and bacterial surfactants, however, was similar. Oil washing

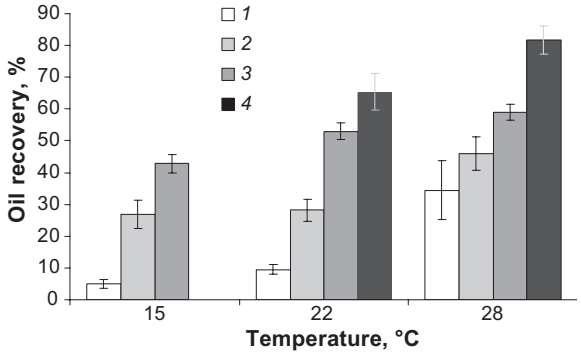


Fig. 5. (Bio)surfactant-enhanced oil recovery from the model soil at different temperatures. Surfactants used: (1) water (Control); (2) Tween 60 (synthetic surfactant); (3) *Rhodococcus* biosurfactant produced on *n*-dodecane; (4) *Rhodococcus* biosurfactant produced on *n*-hexadecane.

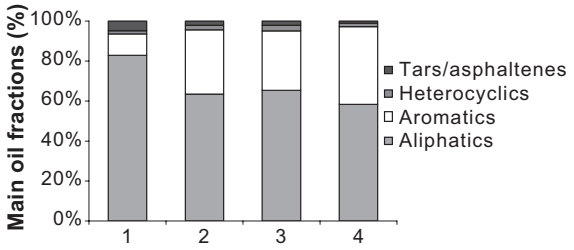


Fig. 6. Changes in oil composition before (1) and after (2–4) recovery from model soil using (bio)surfactant. Surfactants used: (2) water (control); (3) Tween 60 (synthetic surfactant); (4) *Rhodococcus* biosurfactant.

of soil by *Rhodococcus* biosurfactant characteristically led to a relatively high recovery of aromatic compounds; the proportion of aromatics increased by 3.6 times. Moreover, crude oil recovered from soil by *Rhodococcus* biosurfactant contained only 1.0 wt.% of asphaltene fraction, which is almost five times lower when compared to initial oil composition. These results suggests that biosurfactant from *Rhodococcus* bacteria is capable of recovery of crude oil with favorable characteristics (e.g., low asphaltene and high aromatics content) from porous media.

## 4. Conclusion

It was found that crude oil recovery from soil by water washing at different temperatures is relatively small (5%, 10%, and 34% at 15, 22, and 28 °C), indicating low effectiveness of water washing for remediation of crude oil-contaminated soil. In our experiments, the synthetic surfactant Tween 60 had increased the extraction efficiency by 1.5–5.0 times compared to water.

The effectiveness of (bio)surfactant-based mobilization of crude oil in contaminated soil can be limited by adsorption of surfactants to the soil, particularly clay minerals and organic soil matter (Billingsley et al., 2002; Deshpande et al., 1999; Mulligan et al., 2001). Clay and humus chemosorption reduces surfactant effectiveness for *in situ* remediation of subsoil and groundwater (Lee et al., 2002). Cationic and nonionic surfactants can be sorbed by negatively charged clay minerals, thereby decreasing micelle concentration and thus the extent of contaminant solubilization. Anionic surfactants can be precipitated by $Ca^{2+}$ and $Mg^{2+}$ from soil minerals. Nonionic surfactants are less likely to be adsorbed to the soil and thus are most suitable for soil remediation (Doong and Lei, 2003; Mulligan et al., 2001). In our experiments, nonionic *Rhodococcus* biosurfactant was more mobile and effective than the nonionic synthetic surfactant.

Crude oil mobilized by *Rhodococcus* biosurfactant contained relatively lower amounts of aliphatics (presumably high-molecular-weight paraffins) and asphaltenes, the most nondegradable compounds. Although asphaltenes have been reported as not significantly affecting growth

of soil oil degraders, at high concentration in soil, they can clog soil pores and reduce water and oxygen penetration. Asphaltenes can also decrease crude oil degradation rate by inhibiting the diffusion of biodegradable fractions into the oil–water interface and therefore decreasing their bioavailability for microorganisms (Uraizee et al., 1998). Laboratory reactor experiments (Uraizee et al., 1998) showed that artificially deasphaltened crude oil was degraded much more rapidly than this oil supplemented with certain amounts of the asphaltene fraction. These results suggest that crude oil recovered from soil by *Rhodococcus* biosurfactant and containing lower asphaltene concentration could be more bioavailable to soil microorganisms and easily biodegradable. Overall, our results suggest that *Rhodococcus* biosurfactants have potential applications in *in situ* remediation of oil-contaminated subsoil and ground waters.

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