



SID 5 Research Project Final Report

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2. Project title
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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Prior to July 2004, when a ban on co-disposal with domestic refuse was introduced under the Landfill Directive, contaminated soils accounted for about 32% of Hazardous Waste landfilled in the UK. Sustainable alternatives to "excavate and remove" (to landfill) to deal with contaminated soils utilise physical, chemical or biological processing which allow reuse of the treated material on site. Although various process-based remediation technologies are now available (e.g. thermal desorption, chemical treatments, bioremediation), these are not readily applicable to smaller urban brownfield sites due to the area, capital equipment and timescales required. The principal aim of this study was to investigate, using laboratory experiments, the combination of two existing processes (soil-washing and bioremediation), and also the use of natural soaps (biosurfactants), as a novel means of washing and recycling oily soils and similar waste products and a potential process suitable for a treatment centre.

Conventional soil-washing involves mixing soil and water as a slurry and then separating the particles into grain size fractions. During this process any fine-grained metallic contaminants and oils become associated with the clay fraction, reducing the volumes of this component requiring disposal as hazardous waste, whereas the sand and gravel fractions are left clean and may be reused as aggregate. Soil-washing is already widely used in Europe and in recent years has been successfully applied in the UK to remediate contaminated sites and to establish soil treatment centres.

Bioremediation of soils involves mechanical aeration, which allows naturally occurring soil organisms (bacteria) to breakdown hydrocarbon contaminants to less harmful by-products in a manner akin to composting, eventually producing carbon dioxide and water. It is usually achieved by turning soils in elongated piles (windrows) or by stacking above an aeration system (biopiles). Liquids or slurries can be aerated in a tank (bioreactor), which is advantageous in controlling temperature and other parameters.

Microbial biosurfactants are secreted by bacteria to enable them to digest hydrocarbons which are otherwise immiscible in water. Concentrated solutions of these and other plant-extracted biosurfactants are now widely available as commercial products for environmental applications. Washing trials with petroleum-based surfactants have shown that their addition enhances the removal of non-aqueous phase liquids (NAPLS) and can promote biodegradation (Mulligan et al. 2001). It was anticipated that the proposed use of biosurfactants during washing in this study should ensure that any surfactant residues in washed solids were biodegradable, and that the liquid eluates were likewise more amenable to bioremediation, after separation of useful oil (Mulligan, 2005). Potentially this could optimize any subsequent composting by reducing contaminant toxicity, or even eliminate the need to treat the bulk of the solids. The reduced volume of contaminated liquid produced could be treated intensively in controlled conditions in a bioreactor.

An additional aim of this study was to apply biosurfactant-assisted washing technologies to the reduction, reuse and recycling of other granular oily wastes than just soils. For example, iron ore feed stocks might be recovered from treatment of oily mill scale, a secondary product that is currently land filled. Following introduction of the European Landfill Directive this approach might also be used to reduce the hazardous properties of wastes destined for disposal and to fully meet the Waste Acceptance Criteria for organic

contaminants.

Our experimental approach was to select 10 wastes through our steering group and to sample these onsite for laboratory-scale experiments to determine the following:

- optimized washing conditions, based initially on British Standard 12457, but with analysis of solid residues and eluates
- the feasibility of separating oil during biosurfactant recovery for energy or reuse
- the effect on the speed or extent of secondary bioremediation treatment when biosurfactants are used
- the advantages of bioremediating the washed solids and the oily liquids separately
- the possibility of in-cycle production of biosurfactant during washing

Results

Experimentation began by characterising the effectiveness of available biosurfactant products. Surface tension measurements were made for different solution strengths to determine the minimum surfactant concentration to give the greatest reduction in surface tension (the critical micelle concentration). Comparisons were then made of the costs of preparing these solutions, which indicated that microbial biosurfactants (i.e. rhamnolipid) are economically and technically viable compared to other plant-based surfactants (e.g. orange oil).

Although literature reviews and our pilot studies using artificially oil “spiked” samples soils under a similar washing regime had indicated that oil would be released during washing it was soon apparent that this was not the case for weathered oil-contaminated wastes or soils, with virtually no oil released into solution by the washing. Moreover, many experiments suggested an apparent increase in soil hydrocarbon contamination levels after washing. Eventually it was demonstrated that this was partly an artefact of the smaller grain size fraction used for the standard analytical protocol (<2 mm), compared to that used in the standard washing protocol (<4 mm): The apparent increased contamination in the former resulted from the efficient transfer of oil contamination from the coarser particles (i.e. 2-4 mm) to the clay component during washing of soils.

In the absence of significant oil in the washings requiring separation, experimentation was focussed towards refurbishment of the washing solution and recovery of the biosurfactant for reuse. A simple process of foam fractionation was developed for the purpose: Aeration of the solution produced foam, which if allowed to overflow and collect contained a higher concentration of the surfactant.

Solid aerobic bioremediation microcosm trials were carried out on unwashed, water-washed, plant or microbial surfactant-washed portions of soil. Additions of hydrocarbon degrading bacterial consortia and seaweed-derived nutrient were also included (bio-augmentation and bio-stimulation). Again washing was found to increase the apparent concentration of contamination prior to bioremediation, especially with biosurfactant, and to reduce subsequent bio-degradation. The combined results meant that the unwashed soil showed optimum bio-degradation after 30 days, irrespective of other amendments. Our working hypothesis is that the washing process can hinder subsequent bioremediation by disrupting indigenous bacterial populations in the soils.

Additional washing trials were carried out on granular wastes with bentonite added as a fine-grained carrier. Some transfer of contamination to the fine-grained materials was demonstrated, although the addition of biosurfactant had no effect.

In conclusion, laboratory trials indicate that the envisaged combination of low intensity biosurfactant-assisted washing with bioremediation is unlikely to be applicable to recycle soils or other oily hazardous wastes due to the potent transfer of contaminants to the fine-grain fraction which is inherent in the soil-washing process. Biosurfactants appear potentially to offer technically and economically competitive alternatives to chemical surfactants derived from fossil fuels, and can be concentrated by foam fractionation. The availability of conventional on-site soil-washing from UK remediation companies has increased five-fold in the two years of this project, although regulatory issues surrounding the reuse of aggregates derived from waste soil are currently a limiting factor to widespread implementation of this potentially sustainable and low-carbon remediation method in fixed treatment centres.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other

journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

1. Scientific Objectives

The primary objective was to evaluate the use of biosurfactant-washing as a process-based treatment of particulate wastes, as a precursor to oil recovery or enhanced bioremediation, and as a mechanism of resource recovery or waste minimisation. The specific objectives required to achieve this were as follows:

- (1) To review the “state-of-the-art” of soil washing technology, identify commercially available biosurfactant products, candidate waste streams, and obstacles to implementation, using international scientific and technical publications.
- (2) To identify and sample 10 suitable oily waste streams of significant volume, economic or environmental significance.
- (3) To optimise experimental biosurfactant washing conditions, including surfactant: water:waste ratios, agitation, temperature, timing, reagents, and other physical, chemical or mechanical factors.
- (4) To develop effective physio-chemical separation of oil and aqueous phases, allowing recovery of oil and recycling of biosurfactant solution.
- (5) To investigate usage rate, lifetime and cost of biosurfactant solutions.
- (6) To assess the enhanced aerobic degradation of oil in eluates using liquid bioreactor experiments, and in washed solids using conventional composting techniques.
- (7) To evaluate the in-cycle biosurfactant enhancing potential of appropriate bacterial strains.

2. Extent to which objectives have been met

Objectives 1, 2, 3 and 5 were met in full. During the experimental washing trials (objective 3) it was found that the extent of oil removal into washing solutions from weathered samples of actual wastes (especially soils) was very limited compared to published results for spiked samples (Urum & Pekdemir, 2004). As a result it was not possible to complete as originally intended those aspects of objective 4 relating to oil separation, degradation of eluted oil under objective 6, or biosurfactant production using eluted oil under objective 7. The lack of eluted oil at this stage did not preclude the possibility of enhanced bioremediation, so there were no grounds to radically revise the remaining objectives and objective 4 was instead completed for separation of biosurfactant from solution for reuse. Objective 6 was completed for the washed solids (i.e. without eluted oil removal). Objective 7 was addressed using alternative sustainable growth media, including by-product glycerol from biodiesel production, and an alternative spiked-washing trial was completed to follow-up earlier results. The outcomes of these modified objectives were sufficient to meet the primary objective set although did not provide clear evidence of a significant advantage from biosurfactant addition during low intensity washing of hydrocarbon-contaminated hazardous wastes requiring disposal, especially in the case of soils.

The schematic diagram below illustrates the intended combination of stages in the hypothetical treatment process and provided the basis for sequencing the component technical objectives described above.

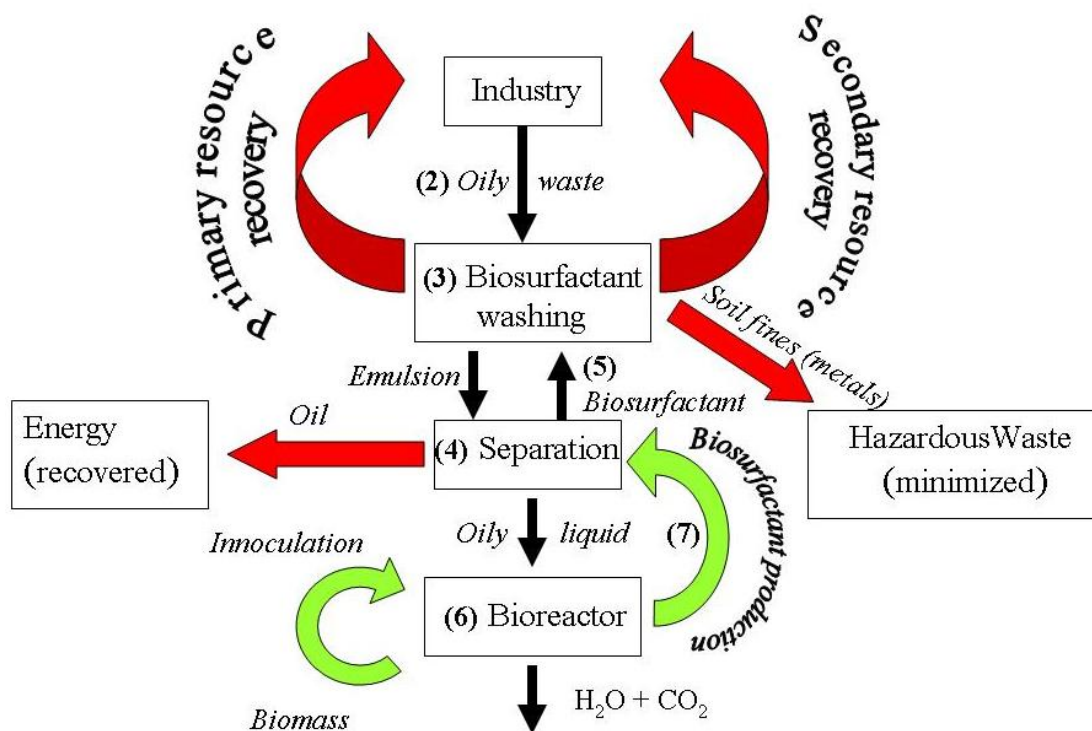


Figure 1. Schematic diagram of approach and project stages.

3. Research Methods & Results

The methods used and results of each objective will be discussed in turn.

3.1 Review of soil-washing, biosurfactants, and their combination

The Bio-recycling Project was initiated by organising an industrial steering group and then officially launched by convening a first steering group meeting (SGM) at the University of Teesside on 29/9/05. It was attended by representatives from eight relevant companies and also the Environment Agency. The steering-group members were selected on the basis of one or more of three criteria: firstly, they had first-hand experience of soil-washing practices as an indication of the “state-of-the-art” (e.g. Entec, DEC, FWS); secondly they were likely to generate or manage oily wastes which might be suitable for sampling (e.g. Oil Company 1, SNRH Landfill Operator A, Augean, Bradstone); thirdly, they were biotechnology providers who had experience of using biosurfactants (e.g. BioFuture, Cleveland Biotech, FWS).

As a result of the first SGM a visit to an active soil-washing site in NW England was arranged with the soil-washing contracting company DEC (DEME Environmental Contractors) and the site owner (National Grid UK), following which samples of washed and unwashed soil were obtained.

Attendance at the CL:AIRE-First Faraday training course on soil-washing (26/10/05), which was partly delivered by DEC and other expert practitioners, gave further insight into the state-of-the-art and scientific basis of full scale deployment of this process-based brownfield remediation technology. DEC were subsequently invited to join the steering group. The knowledge gained from these activities indicates that conventional soil-washing is successful on sandy soils for two reasons; firstly, inorganic contaminants (such as heavy metals) are commonly fine-grained or adsorbed to clay particles; secondly, organic contaminants (such as coal tars) released by physical attrition during the washing process are also preferentially adsorbed by the high surface area of this finer fraction when it settles out of suspension. Thus separation and removal of the finer fraction of a washed soil can remove a greater proportion of both types of contamination. If this fraction is disposed of to landfill as hazardous waste then sandy soils (ideally 10% clay fraction, typically up to 20%) provide the optimum economic benefits of reduced disposal volume to justify the additional cost of the soil-washing, typically £15-25/T (Pomphrey, 2006). Use of this technology is well-established and reported in the literature (e.g. Beck et al., 2003; CIRIA 1995; Evans et al., 2001). A useful review has recently been published by CL:AIRE (Pearl, 2007).

This review of conventional soil-washing, although highly relevant, illustrated the fundamental differences of the proposed low-intensity laboratory washing trials with biosurfactants and oily wastes in this investigation. Such a fundamentally different approach was chosen mainly due to the likely behavioural differences between granular

wastes (investigated during this project) and waste soils (treated in conventional soil washing). Other reasons include the practicalities of laboratory replication, and the successful results achieved with pilot scale washing of oil-spiked material using this method, which were supported by precedents in the scientific literature. Moreover the ambition was to refurbish waste materials for reuse or to better meet the Waste Acceptance Criteria, which are actually defined by such a low intensity washing process test (i.e. BS12457/3), and so differs from the usual application of soil-washing to reduce the contaminant source term in a pollution linkage under the risk-based approach of the UK Contaminated Land Regime.

An internet-based search revealed that many biosurfactants are being commercially marketed for application in environmental technology. Six plant and microbial biosurfactants were purchased for use in the project, chosen from a range of sources. BioFuture Ltd. (Dublin) supplied a rhamnolipid microbial biosurfactant as an aqueous concentrated solution. Cleveland Biotech (Teesside) supplied Citrasolv, an orange peel extract as a concentrated solution. Ecochem Ltd (Canada) supplied two products recommended for environmental application, EC1800 and EC601, which were described as a rhamnolipid biosurfactant with and without bacterial amendment respectively. Petrosolv, a bacterial surfactant of unspecified origin, was supplied by Enzyme Technologies Inc (USA). Finally, Fisher Scientific supplied a preparation of saponin extracted from *Quillaja saponaria Molina*.

A review was conducted specifically to identify past research involving soil-washing and biosurfactant use. The study incorporated an investigation into both the academic and commercial activities taking place in Europe. During the industrial survey, UK remediation companies offering soil-washing services were identified from a table published in Brownfield Briefing (Remediation Solutions III, Oct '05). The companies were contacted and their opinion canvassed on the current and potential use of biosurfactants in the field of soil-washing. The survey uncovered the following information:

- In total, twenty companies advertised the capacity to carry out commercial soil-washing. However, only six of these companies could offer the service using in-house equipment. Of the six companies: three used water as the only solvent in the soil-washing process; one had considered using a biosurfactant and were planning to conduct trials within the next year; two had recently undertaken trials using "biodegradable surfactants" which had shown positive results
- All six companies expressed an interest in the technology, with two companies offering the future use of equipment to undertake field trials

A further review of the commercial availability of soil-washing in October 2007 is presented in section 6.

A review of the academic literature uncovered three research groups within Europe that had conducted lab trials using biosurfactants for washing soils. These groups were located at Herriot Watt University, Scotland (Urum & Pekdemir, 2004); Bologna University, Italy (Berselli, S *et al.*, 2004); and Lithuania Technical University, Lithuania. The studies revealed the following:

- Success was dependant on soil characteristics
- The bacterial rhamnolipid biosurfactant was more successful than the plant based biosurfactants aescin, saponin, lecithin and tannin at removing an oil phase from soils
- The studies were carried out on lab spiked soil, at masses as small as 5 g
- Only one study was reported that had investigated washing historically contaminated soil

The survey revealed growing interest in the use of biosurfactants for enhancing washing of soils, with most of the activity taking place in the last two years.

3.2. Waste Identification & sampling

Seventeen potential target waste streams were identified by participants of the brainstorming session during steering group meeting one. This list was consulted, along with the European Waste Catalogue, when sourcing the actual waste samples for the project. The ideal waste would have the following characteristics; granular, oily, potentially hazardous and would have a re-use value if cleaned. Single bulk samples of the waste materials shown in Table 1 were collected from appropriate sources or locations and used in the experiments shown.

The following potential waste streams were identified but were not sampled:

- Tertiary-butyl-tin contaminated sediments
- Hydraulic fluid contaminated sediments
- Rail ballast*
- Phenolic moulding sand
- Municipal road sweepings
- Smelter Zn-Cd contaminated soil
- Refinery tank sludge

Table 1. Waste materials cross-referenced to experimental use.

| | Waste material | Report sections |
|----|---|-----------------------------|
| 1 | Unwashed gas works soil from soil-washing remediation site (GWS) | 3.3.1, 3.3.4, 3.3.5 |
| 2 | Contemporaneous filter cake from soil-washing at the same former gas works as (1) | 3.3.1 |
| 3 | Oily mill scale*† (OMS) | 3.3.1, 3.3.4, 3.7 |
| 4 | Fresh diesel spill contaminated soil from construction site (FDS) | 3.3.1, 3.3.6, 3.4, 3.6, 3.7 |
| 5 | Historically hydrocarbon contaminated soil from brownfield site, including tars | |
| 6 | Metalworking swarf grindings* | |
| 7 | Oily rags/PPE | |
| 8 | Tank scale | |
| 9 | Diesel contaminated soil from underground storage tank leak remediation site | |
| 10 | Oily tank crust | |
| 11 | Oily used polypropylene sorbents* | |
| 12 | Oilfield drill cuttings | |
| 13 | Garden Soil (uncontaminated control) (CTR) | 3.3.1, 3.3.2, 3.3.4 |

* indicates a high reuse potential if decontaminated

† typically non-hazardous but oily

3.3 Optimisation of biosurfactant washing

3.3.1 Waste characterization and analytical development.

The following five waste samples: gas works soil (GWS), soil-washed gas works filter cake, oily mill scale (OMS), diesel spill contaminated soil (FDS) and garden soil (CTR), were selected as representing the range of arisings and likely candidate waste streams described. These were used extensively in the project washing trials, and consequently a full suite of chemical analysis was performed by a commercial laboratory (Severn Trent Laboratories) to determine the full extent of contamination in the samples, including metal and polycyclic aromatic hydrocarbon (PAH) content (Table 2). Due to the presence of total petroleum hydrocarbons (TPH) fractions, the soils could potentially be categorized as hazardous by the European Waste Catalogue definitions. Both the oily mill scale and the diesel spill soil could also fail Waste Acceptance Criteria if presented for landfill based on their Total Organic Carbon (TOC) levels above 6 % (unless LOI is <10 %). Both have a high TPH content, with the diesel spill soil containing mostly mid range hydrocarbons (C10 to 20) and the OMS containing longer chain hydrocarbons (C20 to 40), but no PAH. The soil-washed gas works filter cake has approximately twice the metal and TPH contaminants of the untreated gas works soil, and over three times the level of PAH. The analysis demonstrates the partitioning of contaminants into the finer fraction of soil during the washing process.

As TOC would be affected by content of coal, peat or carbonate rock fragments, washing success would be determined by removal of TPH as an indicator of oil content and the incumbent hazardous properties. The waste samples from the washing tests in sections 3.3.4 and 3.3.5 were also analysed for TPH content by a commercial laboratory. The waste samples in sections 3.3.6, 3.5 and 3.6 were subject to chemical analysis in-house using a method of analysis for TPH modified from British Standard ISO16703:2004. This method entails the extraction of aliphatic hydrocarbons from the fraction of soil smaller than 2 mm into a non-polar solvent for subsequent analysis using Gas Chromatography with Flame Ionization Detection.

The homogenized bulk samples were prepared by air-drying in a fume hood at room temperature for 24 to 48 hours, as significant volatile contamination was not expected in weathered soils or wastes from ambient conditions. A portion of waste was oven dried at 105 °C to determine the dry weight of the waste prior to calculation of TPH. Air-dried wastes were disaggregated then sieved and the portion < 2 mm was retained for solvent extraction into heptane: 10 g of waste (< 2 mm fraction) was weighed into a 100 ml glass flask with Teflon lined lid, and 20 ml acetone was added and mixed thoroughly; 10 ml retention time window solution (heptane with 30 µg/ml decane and tetracontane) was added and the flask mixed on a horizontal shaker at 120 rpm for 60 minutes; flasks were settled for 10 min, followed by removal of liquid to a fresh flask. 50 ml water was added and the flask mixed on horizontal shaker at 200 rpm for 5 min; flask contents were transferred to a 100 ml glass separating funnel and settled to two separate layers; the lower aqueous layer was removed and discarded, and 50 ml water added to the separating funnel; the separating funnel was shaken vigorously for 1 min and settled to two separate layers; the lower aqueous layer was removed and discarded, and the upper organic layer transferred to a fresh flask; the organic extract was filtered using a 0.45 µm Teflon filter and 10 ml syringe into a fresh flask; sodium sulphate was added to the flask until no clumping was observed; a portion of the extract was transferred to a 2 ml glass vial and sealed with crimp cap; the extract was filtered through a glass clean-up

column (2 g Florisil and 2 g sodium sulphate on a glass wool frit) until clear; a portion of the extract was transferred to a 2 ml glass vial and sealed with crimp cap.

Heptane extracts were analysed by gas chromatography with flame ionization detection (GC-FID) according to the following method: Injection split PTV (Programmable Temperature Vaporizing) at 340 °C; split ratio 20; injection volume 1 µl; column type WCOT (Wall Coated Open Tubular) fused silica; column length 10 m; column internal diameter 0.1 mm; liquid phase BPX-5; film thickness 0.1 µm; carrier gas Helium; detector temperature 350 °C; temperature programme 45 °C for 30 seconds; 90 °C/min to 220 °C, 55 °C/min to 340 °C, 340 °C for 6 min, total time 10.43 min.

Solvent extraction and GC-FID analysis were performed in triplicate on sub-samples of the fresh diesel spill soil and gas works contaminated soil to evaluate the heterogeneity of the bulk samples after mixing. Data indicated that the TPH content of three replicate (10g) samples of soil, removed from a 20kg homogenised sample, were significantly similar to suggest thorough mixing had taken place.

Table 2. Full suite chemical analysis data for five different wastes.

| Analyte (ppm unless stated) | Waste Tested | | | | |
|-----------------------------|---------------------|----------------------|-----------------------|-------------------------|-------------------|
| | Gasworks soil (GWS) | Gasworks filter cake | Oily mill scale (OMS) | Diesel spill soil (FDS) | Garden soil (CTR) |
| Arsenic | 66 | 120 | 20 | 14 | 17 |
| Boron | 0.91 | 2.4 | 1.5 | 2.9 | 4.7 |
| Cadmium | <0.50 | 0.75 | <0.50 | <0.50 | 0.77 |
| Hexavalent Chromium | <0.10 | <0.10 | 1.6 | <0.10 | <0.10 |
| Chromium | 28 | 41 | 150 | 24 | 31 |
| Copper | 29 | 89 | 170 | 34 | 65 |
| Lead | 340 | 480 | 180 | 110 | 340 |
| Mercury | 0.9 | 3 | <0.20 | 0.29 | 0.25 |
| Nickel | 26 | 54 | 180 | 21 | 33 |
| Selenium | 1.7 | 2.6 | <0.30 | 0.72 | 0.81 |
| Zinc | 140 | 290 | 59 | 120 | 370 |
| Cyanide (Free) | <5.0 | <5.0 | <5.0 | <5.0 | <5.0 |
| Cyanide (Total) | 140 | 180 | <2.0 | 11 | 8.5 |
| Phenol | 2.6 | 3.3 | <0.50 | 0.85 | 0.86 |
| Sulphate (%) | 0.36 | 0.89 | <0.020 | 0.27 | 0.1 |
| Sulphide | <5.0 | <5.0 | <5.0 | 120 | 15 |
| Sulphur (Total %) | 0.8 | 1.5 | 0.093 | 0.7 | 0.22 |
| Thiocyanate | 0.62 | 2.3 | 0.64 | 1.1 | 0.7 |
| TOC (%) | 4.3 | 5.9 | 6.1 | 6.8 | 7.2 |
| pH | 8.8 | 8.1 | 9.5 | 10.3 | 7.7 |
| TPH (>C6-C10) | <50 | <50 | 580 | 1200 | <50 |
| TPH (>C10 - C20) | 430 | 610 | 2000 | 15000 | 140 |
| TPH (>C20-C40) | 1000 | 1900 | 29000 | 8700 | 380 |
| TPH Total (>C6 - C40) | 1400 | 2500 | 31000 | 24000 | 520 |
| naphthalene | 3 | 11 | <0.50 | 9.5 | 3.8 |
| acenaphthylene | <0.50 | 1.1 | <0.50 | <0.50 | <0.50 |
| acenaphthene | 0.97 | 3.1 | <0.50 | 3.8 | 2.3 |
| fluorene | 1.1 | 3.4 | <0.50 | 5.8 | 1.6 |
| phenanthrene | 6.9 | 14 | <0.50 | 38 | 14 |
| anthracene | 3.3 | 12 | <0.50 | 14 | 5.2 |
| fluoranthene | 16 | 40 | <0.50 | 44 | 20 |
| pyrene | 11 | 36 | <0.50 | 39 | 14 |
| benzo(a)anthracene | 5.3 | 22 | <0.50 | 21 | 7.7 |
| chrysene | 4.8 | 26 | <0.50 | 19 | 8.2 |
| benzo(b)fluoranthene | 5.5 | 28 | <0.50 | 13 | 5.1 |
| benzo(k)fluoranthene | 3.3 | 52 | <0.50 | 8.9 | 3.6 |
| benzo(a)pyrene | 8.1 | <0.50 | <0.50 | 19 | 6.6 |
| dibenzo(ah)anthracene | 0.55 | 3 | <0.50 | 1.4 | 0.66 |
| benzo(ghi)perylene | 5.9 | 36 | <0.50 | 14 | 6.9 |
| indeno(123cd)pyrene | 6.2 | 39 | <0.50 | 14 | 5.3 |
| PAH (Total) | 81 | 330 | <2.0 | 260 | 110 |

3.3.2 Biosurfactant characterization and behaviour

It has been postulated that pollutant removal from soil during washing trials may be dependant on biosurfactant characteristics and the formation of micelle structures by biosurfactant monomers in solution, at a concentration above the critical micelle concentration or CMC. The critical micelle concentration is the lowest concentration of surfactant that produces the minimum achievable surface tension. However, recent experimental findings have been contradictory (as discussed below) and a definitive explanation has not yet been offered (Urum & Pekdemir,

2004). In order to further investigate this theory, a range of biosurfactant concentrations ranging from above to below CMC were employed during the washing trials. Solutions of the six biosurfactants were prepared using de-ionised water and the CMC was determined using a method outlined by Urum et al. (2005). A two fold serial dilution was prepared from each surfactant in triplicate, and the surface tension (mN/m) repeatedly measured using a KRULL tensiometer with platinum ring. Concentration of biosurfactant was calculated as a volume to volume percentage solution, except saponin which was calculated as a weight to volume percentage solution. Surface tension (mN/m) data was plotted against biosurfactant concentration (log scale) and the CMC of each biosurfactant calculated from the resultant graph (Figure 2).

All six biosurfactant products were supplied without supplementary information regarding the purity and concentration of the material. The attributes of lowest achievable surface tension and CMC for each of the six commercial biosurfactants were measured and employed as a means to compare the performance of the material. This was the only feasible benchmark for such natural products, which are typically supplied as liquids of unknown dilution. The cost of surfactant in a washing solution at CMC was calculated, allowing comparison of the six biosurfactants at optimum dilution as shown in Table 3. A key finding here is that dilute solutions of microbial secretions (e.g. rhamnolipid) may be competitive with solutions of cheaper plant extracts (e.g. orange oil). The plant based surfactant saponin stands out as having the highest cost – however, the material was sourced as a highly pure product from a scientific laboratory supplier. Further suppliers of this material will be sought to determine if a cheaper and more sustainable source of saponin can be located.

CMC of Biofuture Biosurfactant

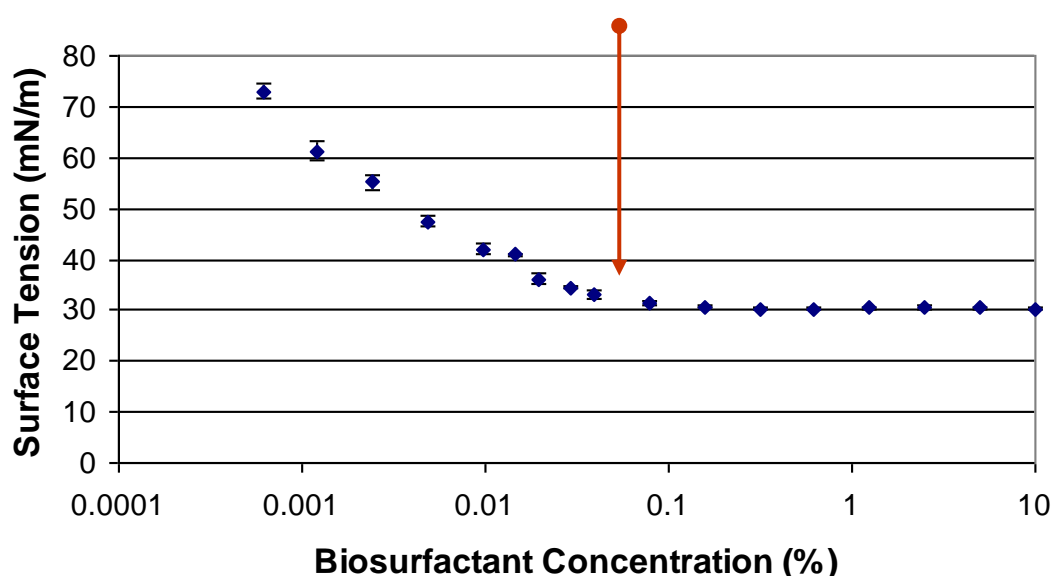


Figure 2. Graphical determination of CMC by serial dilution and surface tension measurement.

Table 3 Summary of biosurfactant characteristics: ST - Surface Tension; CMC - Critical Micelle Concentration (*v/v or † m/v); Cost of biosurfactant in 1 litre of solution at CMC.

| Biosurfactant | Origin | Supplier | ST mN/m | CMC (%*) | Cost (£/L) |
|---------------|-----------------------|------------------------------------|------------|-------------|---------------|
| BioFuture | Bacterial rhamnolipid | BioFuture Ltd Dublin | 28 | 0.08 | 0.02 |
| Citrasolv | Orange peel | Cleveland Biotech Ltd, Teesside | 30 | 0.9 | 0.01 |
| EC601 | Bacterial rhamnolipid | Ecochem Ltd, Canada | 29 | 0.2 | 0.23 |
| EC1800 | Bacterial consortium | Ecochem Ltd, Canada | 28 | 0.04 | 0.01 |
| Petrosolv | Bacterial unknown | Enzyme Technologies Inc, USA | 34 | 0.2 | 0.01 |
| Saponin | Plant bark | Sigma UK | 45 | 0.1 † | 0.50 |

The adsorption of biosurfactant molecules onto the surface of clay or silt particles will remove surfactant particles from solution and will therefore lead to a lower effective concentration in wastes with soil matrices, and an increased surfactant addition to achieve a CMC. The effect of soil adsorption was investigated by adapting the method of Urum & Pekdemir (2004). A 2-fold serial dilution was prepared of the BioFuture and Citrasolv biosurfactant, representing microbial and plant-based examples. Each biosurfactant dilution was mixed with garden soil (representative of soil matrices discussed above) in a 1:6 ratio of soil to solution and shaken for 24 hours at 150 rpm. The soil was removed by centrifugation and a CMC plot prepared of the separated biosurfactant solutions. The CMC plot for BioFuture biosurfactant with and without the addition of soil is shown in Figure 3. The coherent pattern of increased surface tension below CMC, trending towards a similar constant surface tension when in excess, strongly supports the theory of surfactant adsorption by soil and this model of surfactant behaviour. The difference between the CMC of the biosurfactant solution with and without soil mixing indicated that approximately 60 % of the biosurfactant was adsorbed onto a clay soil. Such loss of surfactant from solution needs to be considered when planning washing experiments and could affect the cost of the biosurfactant washing process if this observation is applied to all wastes and soils.

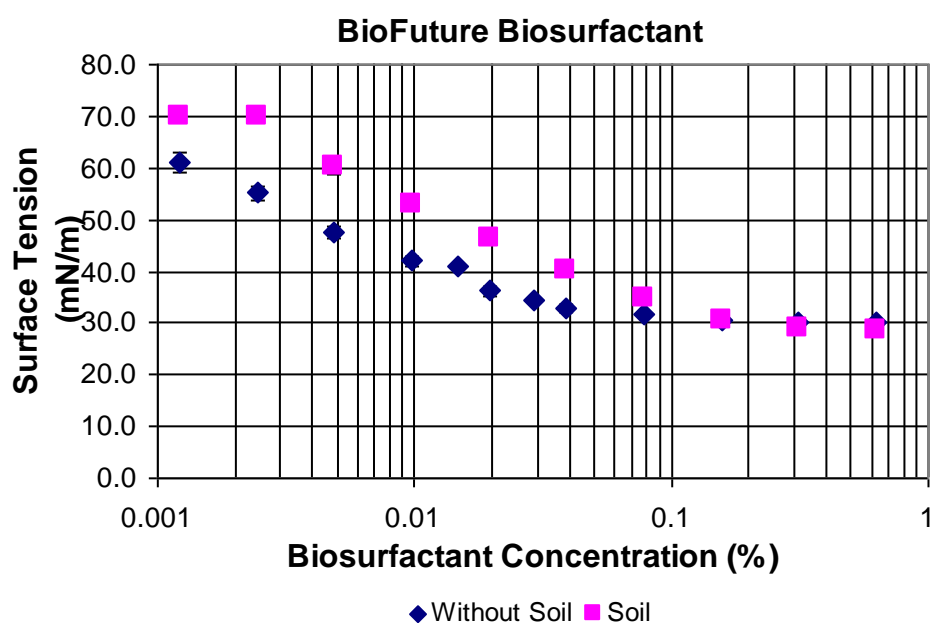


Figure 3. Plot of surface tension (mN/m) plotted against concentration both without (◆) and with (■) mixing with soil.

The partitioning of oil into micelles has been proposed (Deshpande et al., 1999) as one of the potential mechanisms by which a surfactant removes oil from contaminated soil particles and is referred to as solubilisation. An alternative mechanism has also been proposed, often referred to as soil roll up or mobilisation (Deshpande et al., 1999; Rosen, 1989). The occurrence of both mechanisms during washing of soil was tested using a method described by Urum et al. (2005). Three concentrations of the biosurfactant BioFuture and Citrasolv were prepared at approximately 0.1, 1 and 10 times their CMC. The solutions were mixed at 200 rpm for 1 hour with a 1 % addition of crude oil, followed by settling for 1 hour in a separating funnel. A portion of each biosurfactant solution was removed and tested for TPH content. GC-FID chromatograms showed that no TPH had been extracted from the biosurfactant solution, indicating that the biosurfactants tested were not forming a stable emulsion or solubilising the crude oil for any length of time. This appears to support the proposal by Urum et al (2005) that mobilisation is the more likely mechanism for pollutant removal and that micelle formation (and CMC concentration) is not critical for removing oil particles from the soil surface. However, later experimental data indicates that micelles could play an important role in aiding the separation of finer soil particles from the soil matrix by solubilisation, and this process can enhance the removal of contamination.

3.3.3 Waste Washing Method

A method for laboratory scale waste washing was derived from British Standard BS 12457/3 (2002). Key factors predicted to affect washing were: biosurfactant concentration, wash time, waste: liquid ratio, agitation, and solution temperature. The following method was designed to allow testing of these parameters:

100g of waste was transferred to a reinforced glass 2 litre bottle with a Teflon lined lid; a pre-determined concentration and volume of biosurfactant solution was added to the soils and mixed thoroughly; bottles were placed on rollers and mixed at 9 rpm for a pre-determined time; bottles were removed and settled for 1 hour, after which solution was removed by peristaltic pump and up to 250 ml retained at 4°C for further analysis; wastes were rinsed with addition of 100 ml de-ionised water and horizontal rotary mixing at 100 rpm for 1 minute,

followed by settling for 30 minutes; rinse water was removed by pipetting and 30 ml was stored at 4°C for further analysis; wastes were transferred to glass evaporating dishes and dried in a fume extraction hood at room temperature for 24 – 48 hours; dried wastes were weighed and transferred to sealed plastic bags and stored at 4°C for further analysis.

Any modifications made to this method during individual experiments are described in that section.

3.3.4 Initial Washing Study

Initial testing of biosurfactant washing used two of the target wastes (oily mill scale & gas works soil) and three biosurfactants (BioFuture, Citrasolv & Saponin) at three concentrations (0.1, 1 and 10 factors of CMC). Garden soil and water were included as controls. TPH and TOC were determined on the washed soils by a commercial laboratory (Severn Trent Laboratories). The analytical results indicated that TPH concentration had apparently increased in many of the washed soils (Table 4). The data were therefore normalized to the apparent concentration reported in the water-washed control (Table 5) to eliminate this effect (discussed below).

Table 4. Final TPH concentration of wastes: oily mill scale (OMS), gas works soil (GWS) and garden soil (CTR), calculated as a percentage (%) of their initial (pre-wash) concentration. Data in bold show apparent increase, data in italic show decrease. Waste samples as Table 1.

| Solvent | Concentration | OMS | GWS | CTR |
|--|---------------|------------|------------|------------|
| BioFuture | 0.1 | 116 | 157 | 102 |
| | 1 | 106 | 179 | <i>79</i> |
| | 10 | 106 | 164 | 250 |
| Citrasolv | 0.1 | <i>84</i> | 150 | 102 |
| | 1 | 742 | 207 | 212 |
| | 10 | 126 | 464 | 137 |
| Saponin | 0.1 | 110 | 200 | <i>98</i> |
| | 1 | 148 | 164 | <i>69</i> |
| | 10 | 139 | 157 | 100 |
| Water | n/a | 129 | 164 | <i>73</i> |
| Unwashed soil TPH C ₆ -C ₄₀ (mg.kg ⁻¹) | | 31000 | 1400 | 520 |

Table 5. Results from table 4 normalised to the water-washed control. Data in bold show apparent increase relative to water washing, data in italic show decrease.

| Solvent | Concentration | OMS | GWS | CTR |
|-----------|---------------|------------|------------|------------|
| BioFuture | 0.1 | <i>90</i> | <i>96</i> | 139 |
| | 1 | <i>83</i> | 109 | 108 |
| | 10 | <i>83</i> | 100 | 342 |
| Citrasolv | 0.1 | <i>65</i> | <i>91</i> | 139 |
| | 1 | 575 | 126 | 289 |
| | 10 | <i>98</i> | 283 | 187 |
| Saponin | 0.1 | <i>85</i> | 122 | 134 |
| | 1 | 115 | 100 | <i>95</i> |
| | 10 | 108 | <i>96</i> | 137 |
| Water | n/a | 100 | 100 | 100 |

The TOC content of many of the wastes was reduced by the washing process, the extent of which can be seen in Table 6. However, the addition of biosurfactants did not appear to provide any benefit over washing with water alone, except in a few cases. Where the TOC level increased, this could indicate adsorption of the biosurfactant, an organic molecule, onto the soil surfaces or transfer during washing to the finer grain size fraction (<2mm) in the analytical sample. It was clear that some reduction of organic contaminant occurred during the washing process, albeit often a modest one. However, the identity and quantity of the removed contaminants was not clear from the data obtained, possibly due to sample heterogeneity.

Linear regression analysis was performed on the TPH and TOC data to determine any patterns. No relationship was observed between the TPH and TOC concentrations. Additional biosurfactant was not found to have a significant effect on the physico-chemical removal of oil during the washing process.

Table 6. Final TOC concentration of wastes: oily mill scale (OMS), gas works soil (GWS) and garden soil (CTR), calculated as a percentage (%) of their initial TOC. Data in italic show decrease relative to water washing, data in bold show apparent increase relative to water washing, data in bold italic show apparent increase relative to unwashed sample.

| Solvent | Concentration | OMS | GWS | CTR |
|------------------|---------------|------------|------------|------------|
| BioFuture | 0.1 | 92 | 84 | 100 |
| | 1 | 92 | 84 | 97 |
| | 10 | 93 | 88 | 104 |
| Citrasolv | 0.1 | 97 | 91 | 111 |
| | 1 | 102 | 109 | 110 |
| | 10 | 113 | 63 | 126 |
| Saponin | 0.1 | 98 | 84 | 99 |
| | 1 | 100 | 67 | 83 |
| | 10 | 79 | 81 | 100 |
| Water | n/a | 92 | 77 | 89 |

The counter-intuitive results of increased TPH in washed samples were presumed at this stage to indicate that the washing process had substantially increased the chemical availability of the contaminants to the organic solvent used in the analytical extraction. The un-normalised TPH data showed that the addition of biosurfactant increased this effect in nine washes out of ten for the control soil. However, the effect upon OMS and GWS is ambiguous, with some washed soils having elevated levels of hydrocarbons, and others lowered. Using this data set alone it was not possible to identify a pattern.

Further experiments were undertaken to investigate this phenomena, and the potential increase in the availability of hydrocarbons after washing. This data and an alternative explanation for these observations (via transfer between grain size fractions) are given in section 3.7.

3.3.5 Factors Affecting Washing – First main washing study

A thorough investigation of the washing process was undertaken to allow further examination of the earlier observations and factors affecting oil removal. The experiments were designed using a three factor, three level (3 x 3), factorial design to allow thorough statistical analysis of the results. The conditions tested are outlined below (Table 7). Each wash was performed in triplicate. Throughout the experiment the waste tested was gas works soil (GWS), the biosurfactant was BioFuture rhamnolipid, and the control was water washing. Due to the anticipated heterogeneous nature of the organic contaminants in the gas works soil, a pre-treatment waste preparation method was undertaken as follows:

- Soil was homogenized using a cement mixer and air dried for 24 hours in a fume hood
- Dried soil was sieved to <4mm and stored in an air tight polypropylene container prior to experimentation and analysis

Overall, 108 washed soils were analysed for TPH content by a commercial laboratory (Severn Trent Laboratories). In addition, the unwashed soil, water supply, final wash water, and biosurfactant wash solution (0.01, 0.1 & 1 %) were analysed for TPH content.

The results were analysed in Minitab (version 14) using a multi-factor ANOVA, to determine the effect of biosurfactant concentration, waste: liquid ratio and washing time on washed soil TPH concentration. The results indicated that biosurfactant concentration had a significant effect on soil TPH ($\alpha = 0.05$ level), with a higher biosurfactant concentration leading to a lower final TPH concentration. These findings contrast with the initial results for the unsieved undried and unhomogenized portion of the same soil (Table 4), particularly for the water wash. This indicates that biosurfactant micelles may play a role in removal of TPH from the gas works soil tested. Whether this is indeed due to the direct transfer of hydrocarbons from the soil surface to the micelle core, as in the postulated mechanism of solubilisation, cannot be determined from this data alone. Furthermore, the results indicated that the waste:liquid ratio and washing time did not have a significant effect on TPH removal. The effect graphs for the three factors are shown in Figure 4.

Table 7. Design outline of washing experiments showing factors and levels tested.

| Factors Tested | Levels Tested | | |
|---------------------------------|---------------|---------|---------|
| | Level 1 | Level 2 | Level 3 |
| Biosurfactant Concentration (%) | 0.01 | 0.1 | 1 |
| Waste:Liquid Ratio (g/ml) | 1:1 | 1:5 | 1:10 |
| Washing Time (hours) | 1 | 2 | 3 |

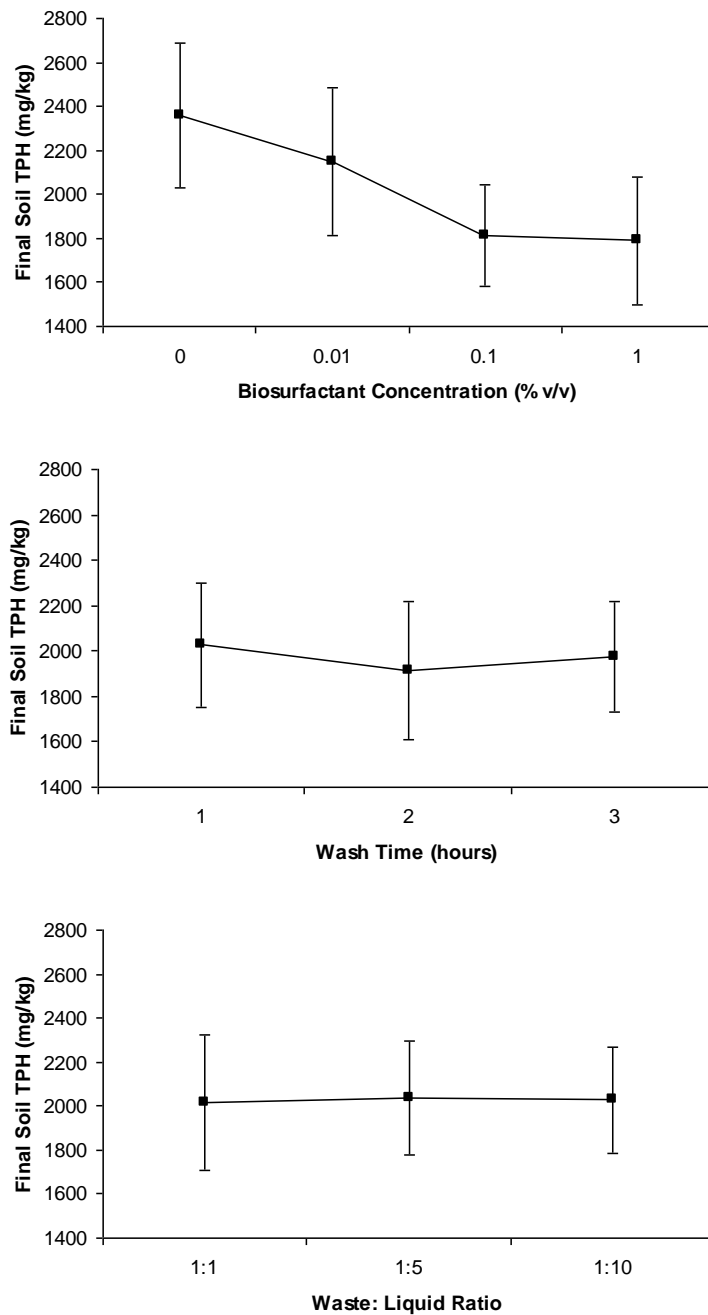


Figure 4. Effect of factors: biosurfactant concentration (%), wash time (hours) and waste:liquid ratio (g/ml) on soil TPH concentration after washing. Error bars represent two standard errors of the mean.

An interaction plot was prepared, which allows any relationship between the factors tested to be identified (Figure 5). The interaction between biosurfactant concentration and wash time was found to be significant at $\alpha = 0.05$ level. It can be seen that in washed soil TPH is lower after a wash time of 1 hour, when washed with a biosurfactant solution above CMC (i.e. 0.1 and 1 %), whereas a concentration of biosurfactant below CMC (0.01 % and water) leaves a significantly higher concentration in the soil. This differential effect is shown to reduce and reverse as the wash time increases to 2 and 3 hours. Although this pattern suggests that extended washing could eventually have the same outcome irrespective of surfactant addition, such residence times were considered impractical. There were no other significant interactions observed between the factors.

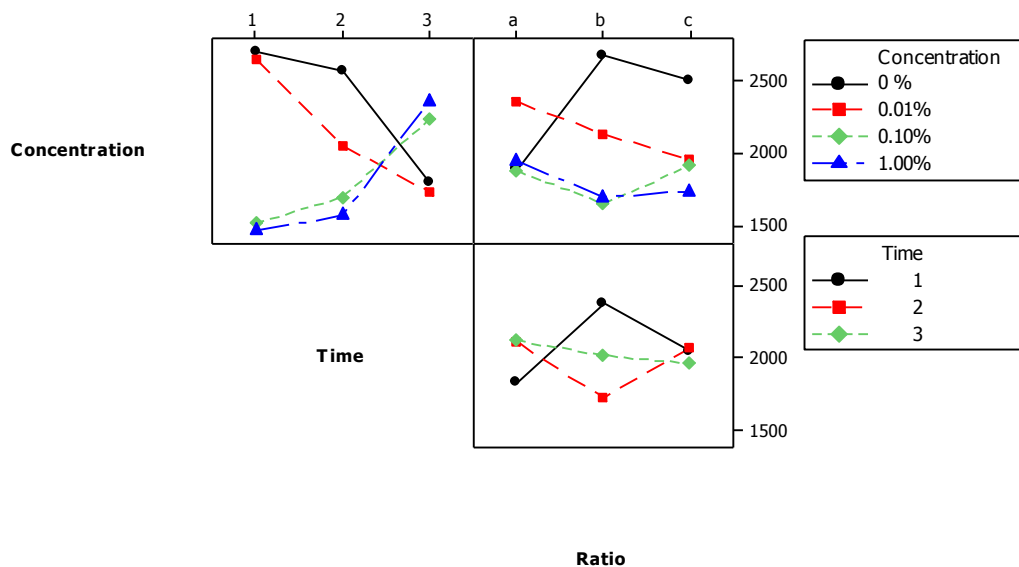


Figure 5. Interaction plot showing the relationship between biosurfactant concentration (0.01 %, 0.1 % and 1 % v/v), wash time (1, 2 and 3 hours) and waste:liquid ratio (a [1:1], b [1:5] and c [1:10]). Average final TPH concentration in solid is shown on vertical axis (1500-2500 mg.kg⁻¹).

3.3.6 Effect of Agitation – Second main washing study

Previous data suggested that increasing the concentration of biosurfactant above CMC achieved greater removal of TPH from soil. The effect on post-washed soil TPH content of increasing agitation from 9 rpm to 100 rpm and 200 rpm was also investigated. Again a factorial design was implemented, with agitation varied alongside biosurfactant concentration and waste:liquid ratio (Table 8). Throughout the experiment the waste tested was fresh diesel spill soil (FDS), as insufficient gas works soil remained, using BioFuture rhamnolipid and a water wash as a control. This sample was not sieved beforehand as it was sufficiently homogeneous and fine grained.

Table 8. Design outline of washing experiments showing factors and levels tested

| Factors Tested | Levels Tested | | |
|---------------------------------|---------------|---------|---------|
| | Level 1 | Level 2 | Level 3 |
| Biosurfactant Concentration (%) | 0.01 | 0.1 | 1 |
| Waste: Liquid Ratio (g/ml) | 1:1 | 1:5 | 1:10 |
| Agitation Speed (rpm) | 100 | 200 | |

The results were again analysed in Minitab (version 14) using multi-factor ANOVA, to determine the effect of agitation, biosurfactant concentration and waste: liquid ratio on final soil TPH (Figure 6).

On initial inspection of results it appears that agitation speed and biosurfactant concentration did not have a significant effect on total final soil TPH ($\alpha = 0.05$ level), although P values were very close to 0.05, which suggests that they were nevertheless important in the process. Further break down of the individual factors in the multifactor analysis allow a more specific consideration of individual effects. When the TPH concentration was split into molecular size fractions, the analysis showed that biosurfactant concentration did have a significant effect on the level of C₂₁₋₃₀ hydrocarbons in the soil, and that agitation speed had a significant effect on the levels of both C₂₁₋₃₀ and C₃₁₋₄₀ in the soil. A significant interaction was observed between agitation speed and biosurfactant concentration across all final levels of TPH in the soil (Figure 7). It was also observed that soil TPH increased slightly as the concentration of biosurfactant increased to 1 %, possibly due to an enhanced transfer of TPH to the finer particles during washing.

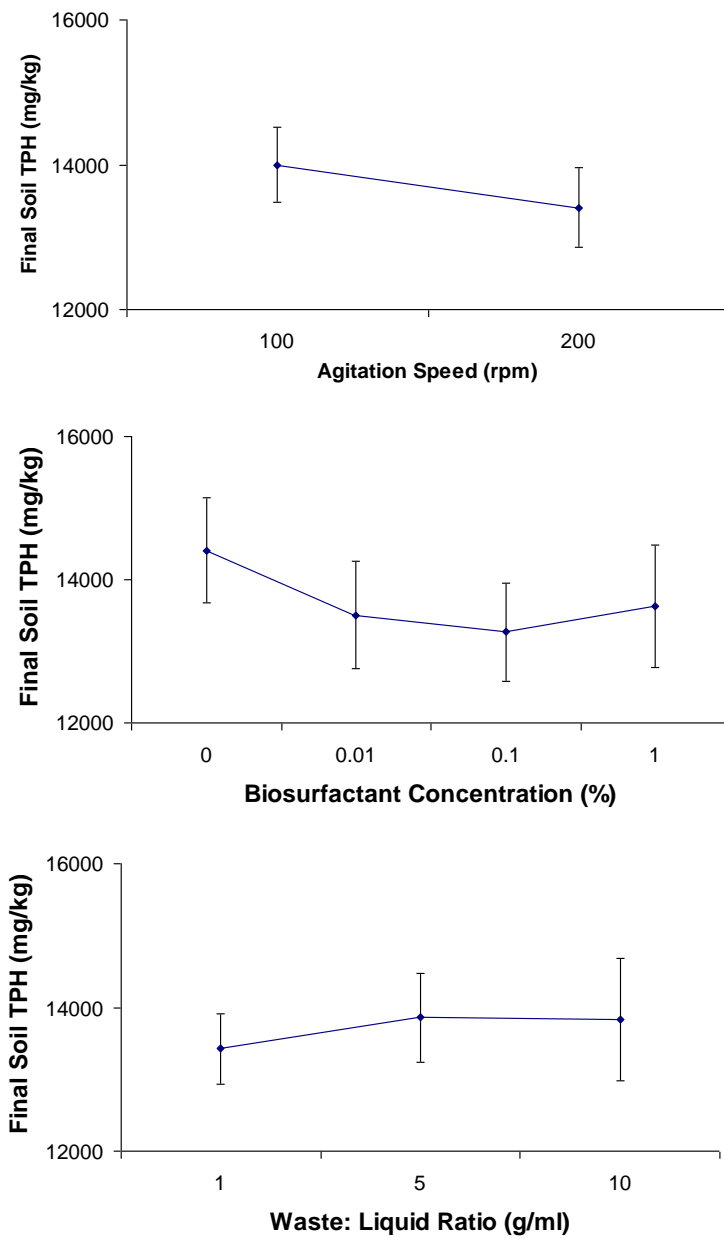


Figure 6. Effect of agitation speed (rpm), biosurfactant concentration (%) and waste:liquid ratio (g/ml) on washed soil TPH concentration (mg/kg). The points represent an average of 36, 24 and 24 determinations respectively. Error bars represent two standard errors of the mean.

Interaction Plot (fitted means) for TPH

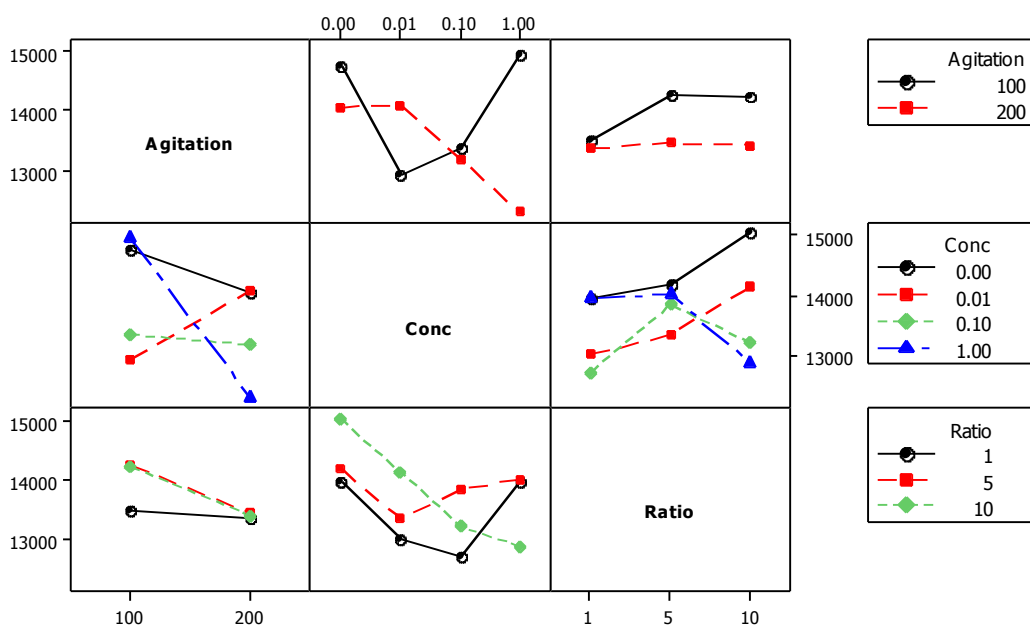


Figure 7. Interaction plot showing the interaction between agitation speed (100 and 200 rpm), biosurfactant concentration (0 %, 0.01 %, 0.1 % and 1 %) and waste: liquid ratio (1 [1:1], 5 [1:5] and 10 [1:10]). Average final TPH concentration in solid is shown on vertical axis (13,000-15,000 mg.kg⁻¹).

3.3.7 Derivation of waste-washing Method Statement

The main observations were:

- Washing did not produce the anticipated movement of TPH into the liquid phase or allow recovery as NAPLS – A key finding for the project.
- Addition of wash water above a waste to liquid ratio of 1:1 (g:ml), has no significant effect on TPH removal from the soil. Again, this is consistent with the TPH not being removed into the waste water phase, as additional water does not lead to enhanced removal.
- Increasing the time of washing to above 1 hour did not have a significant overall effect on TPH removal from soil irrespective of surfactant concentration. This data suggests that any advantageous transfer of TPH due to surfactant addition above CMC would be achieved with the first 60 minutes of treatment.
- Addition of biosurfactant to the wash water during washing was found to have an effect on TPH removal from the soil matrix in two specific cases:
 - Firstly, at a low agitation speed of 9 rpm the final soil TPH was seen to decrease as biosurfactant concentration increased (see Figure 4).
 - Secondly, at a higher agitation speed (100-200 rpm) the final soil TPH decreased as biosurfactant concentration increased (see Figure 6), but the returned concentration was still higher than in the unwashed soil.

The apparent increase in TPH can be explained by considering the preparation of soils prior to chemical analysis. During this and all previously reported chemical analysis (according to BS ISO 16703), the fraction of soil < 2 mm is subject to solvent extraction and is taken as representative of the whole soil sample, whereas all washing experiments used the fraction < 4 mm (as BS12457/3). Therefore, all of the data – graphs and tables - showing 'Final Soil TPH' strictly refer to the results of analysis on the <2mm soil fraction from experiments performed using a <4mm soil fraction. Movement of TPH between soil size fractions is often observed in soil-washing (Pearl, 2007) so an additional batch of experiments was designed specifically to investigate this phenomenon (section 3.7).

Several other complicating factors were also surmised:

- At low agitation rates and surfactant concentrations mixing is inadequate.
- Decanting wash-water can transfer suspended fines with adsorbed TPH unless allowed to settle.
- Below CMC biosurfactant present as monomers (i.e. not micelles) is likely to bind to fines, reducing potential adsorption of TPH.

- Above CMC the dispersion of suspended fines is enhanced.

The following method statement was devised for waste-washing trials with biosurfactant following our protocols:

- The concentration of a rhamnolipid biosurfactant (e.g. BioFuture) should be above its CMC, which will lead to enhanced transfer of TPH to fine particles and enhanced recovery after settling of these fines from the wash water.
- The agitation speed should be sufficient to allow thorough mixing of the liquid and solid phases (above 100 rpm if at lab scale).
- The ratio of solid waste to liquid should be approximately 1 to 2, but an increase in liquid has no effect.
- A washing time of 1 hour is sufficient for batch washing to be completed.

3.4 Recovery of Oil from Wash Water

It was anticipated that any TPH removed from the soil during washing would be transferred to the biosurfactant solution (as free phase and/ or emulsion), presenting an opportunity for recovery of the surfactant (or product). One potential mechanism is solubilisation (section 3.3.2). As the hydrophobic ends of the biosurfactant molecules align themselves towards the inside of the micelle in an aqueous solution, so the TPH molecules could partition into the hydrophobic core. Another potential mechanism would be for the TPH molecules to align and aggregate on the surface of the aqueous solution forming a free phase. Both mechanisms were investigated by analysing wash-waters from previous experiments using BioFuture (0.1 % v/v) or Citrasolv (1 % v/v) biosurfactant and water. TPH analysis was performed on replicate wash waters from a washing experiment performed in triplicate with both the surface and sub-surface layers harvested for analysis using the following method: Wash water (approx 600 ml) was placed in a glass 1 litre separating funnel and settled for 10 min; the lower 590 ml was removed and homogenised, and 10 ml removed to a fresh tube (subsurface fraction); the upper 10 ml was removed from the separating funnel to a fresh tube (surface fraction); both fractions were centrifuged at 14000 rpm for 5 min to remove fine soil particles, transferred to fresh glass flasks and stored at 4 °C prior to solvent extraction. The stored wash water samples were analysed for TPH content by heptane solvent extraction followed by GC-FID analysis as described in section 3.3.1. Although the GC-FID experimental set-up is sufficiently sensitive to detect TPH down to 50 mg/l, none of the samples of surface or subsurface wash water layers contained detectable concentrations. It can be concluded that washing did not lead to any significant transfer of oil from the soil matrix to wash water that could be recovered for re-use.

Although recovery of eluted hydrocarbon was an anticipated outcome at the project planning stage, the low value of waste oil was unlikely to add value to the envisaged treatment process. Furthermore, if washing created an additional liquid waste from an original solid waste this could be disadvantageous on both environmental and economic grounds.

3.5 Biosurfactant Recovery and Re-use

The re-use of biosurfactant solution after washing would enable a more cost effective process, as it would require less biosurfactant and also reduce the waste water yield from the process requiring treatment. Some loss was expected, as it had been shown in section 3.3.2 that biosurfactant adsorbed onto the soil surface led to a reduction in the concentration in solution. If the biosurfactant could be recovered from waste wash solution in a more concentrated form, then optimised washing solutions could be regenerated.

Experiments were conducted using a foam fractionation method and fresh BioFuture rhamnolipid solution at a concentration of 0.1 and 0.01 % (v/v), corresponding to the CMC and a tenfold dilution respectively. The solution was placed in a 1 litre Buchner flask with side arm and connected to the collection vessel with PTFE tubing (Figure 8). Air was supplied by an air pump, through a sealed rubber bung, and dispersed into the fluid via an air sparger. The resulting foam was transferred via the arm along tubing into a collection vessel, where it subsequently settled into a concentrated liquid.

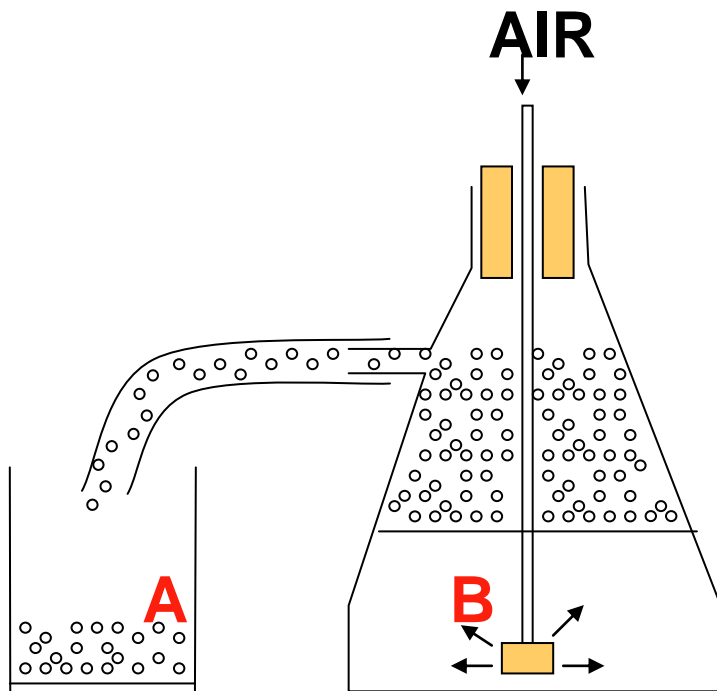


Figure 8. Foam fractionation apparatus, showing the recovered concentrate (A) and the residual wastewater (B).

The measurements indicated that the surface tension in residual solution B increased from 43.4 to 58 mN/m and from 29.4 to 48.6 mN/m for the 0.01 and 0.1 % (v/v) biosurfactant solutions respectively, indicating a decrease in the biosurfactant concentration. Solution A resulting from foam settling contained a more concentrated biosurfactant solution with a low surface tension of below 30 mN/m for both tests. The relationship between surface tension and concentration (Figure 9) can be used as a standard graph for solutions below CMC, providing an estimate of the amount of biosurfactant remaining in the residual solution (B). The concentration of biosurfactant in solution A was then calculated from mass balance (Table 9). The biosurfactant solution was concentrated by a factor up to 100 using foam fractionation.

In conclusion, air sparging foam fractionation offers a simple but highly effective method of recovering or re-concentrating BioFuture rhamnolipid biosurfactant from spent washing solutions, or to reduce foaming of waste washing water. Volume of foam will be a significant factor when designing such a process.

CMC of Biofuture Biosurfactant

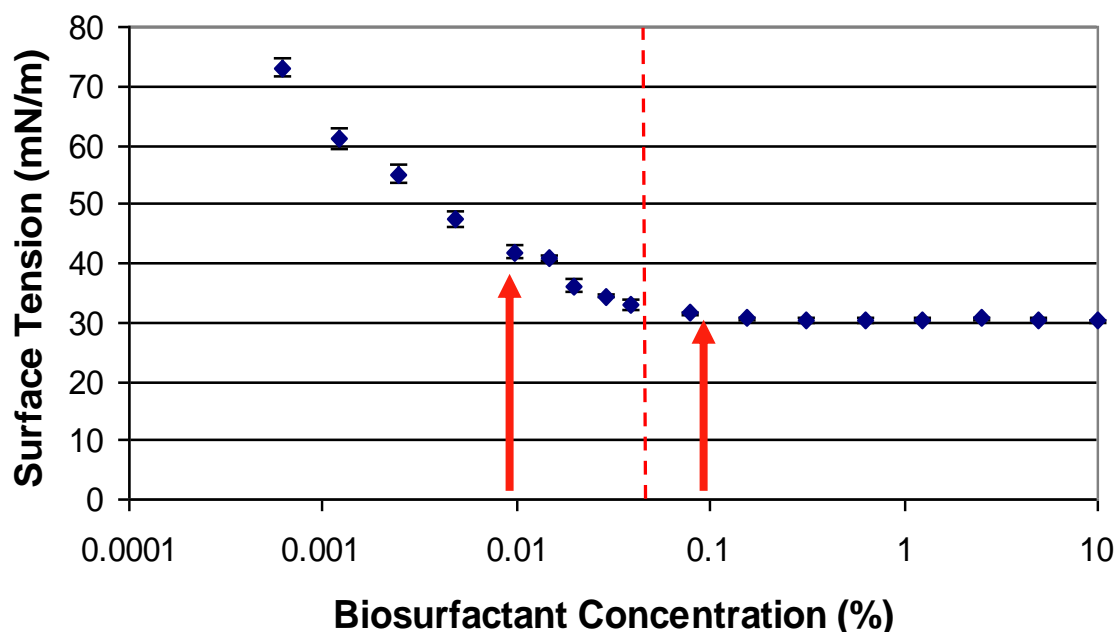


Figure 9. Concentration of biosurfactant (% v/v) plotted against surface tension (mN/m) for the BioFuture Ltd Rhamnolipid biosurfactant. The dotted line indicates the estimation of the CMC of the biosurfactant. The two red arrows indicate the surface tension at 0.01 and 0.1 % solutions which were used to test foam fractionation.

Table 9. Results of the foam fractionation experiments, showing the change in surface tension and concentration of each solution; A – the concentrated biosurfactant, and B – the residual wastewater.

| Biosurfactant Solution (% v/v) | 0.01 | 0.1 |
|--|----------|-------------|
| Initial Surface Tension (mN/m) | 43.4 | 29.4 |
| Concentrated Biosurfactant (A) Surface Tension (mN/m) | 28.2 | 29.3 |
| Residual Wastewater (B) Surface Tension (mN/m) | 58 | 48.6 |
| | | |
| Concentrated Biosurfactant (A) Final Concentration (% v/v) | 1.0 | 1.5 |
| Residual Wastewater (B) Final Concentration (% v/v) | 0.003 | 0.006 |
| | | |
| Air Flow | Low | High |
| Time | 10 min | 20 min |
| Volume Biosurfactant Residue | 5 ml | 50 ml |
| Volume Foam | ~ 100 ml | ~ 20 litres |

3.6 Bioremediation of Washed Soils

As transfer of TPH to wash water was insignificant, bioremediation trials focused on the washed soils using an aerobic composting approach. Fresh diesel spill soil (FDS) was washed at the optimal washing conditions for concentration of biosurfactant, waste:liquid ratio and agitation rate (0.1 %, 1:2 g/ml and 200 rpm respectively). The washed soils were then composted for 30 days in microcosms after undergoing both biostimulation and bioaugmentation. The experiment was planned in two stages:

- Stage 1. 350 g of contaminated soil was washed in BioFuture rhamnolipid or Citrasolv biosurfactant, with water washed and unwashed soil as controls (performed in triplicate with stage 2 performed on each 350 g replicate)
- Stage 2. 350 g of washed soil was split into 7 x 50 g for chemical analysis for TPH by GC/FID and TOC or bioremediation microcosm treatment for 30 days at room temperature

Microcosm treatments were:-

- 1) Water amendment & water inoculum

- 2) Water amendment & consortium bacteria inoculum PR (*Stenotrophomonas rhizophila* PR 292A
Achromobacter clevelandea PR292BT)
- 3) Water amendment & consortium bacteria inoculum CB (assortment of TPH degrading bacteria supplied by Cleveland Biotech)
- 4) Seaweed nutrient amendment (High Nitrogen Extra, Grotech, UK) & water inoculum
- 5) Seaweed nutrient amendment & consortium bacteria inoculum PR (*Stenotrophomonas rhizophila* PR 292A
Achromobacter clevelandea PR292BT)
- 6) Seaweed nutrient amendment & consortium bacteria inoculum CB (assortment of TPH degrading bacteria supplied by Cleveland Biotech)

All soils were analysed for TPH content by GC-FID after washing and at the end of composting. Initial average TPH levels were higher in the biosurfactant washed soils, than the unwashed and water washed soils (Figure 10), consistent with the working hypothesis that during washing TPH is transferred to the fine fraction of soil in the analytical portion < 2mm from the coarser portion of the feedstock (< 4 mm).

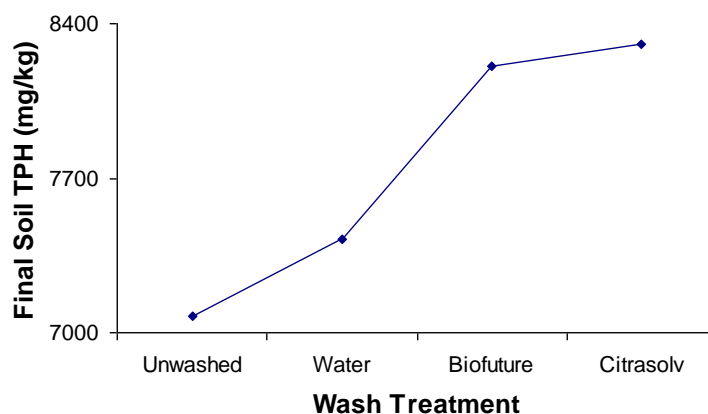


Figure 10. Soil TPH level before and after washing with water, BioFuture and Citrasolv biosurfactants.

Figure 11 shows the TPH level of the soils after bioremediation for each treatment. The results were analysed in Minitab (version 14) using a multi-factor ANOVA, to determine the effect of nutrient amendment and inoculum addition on final soil TPH. The results indicated that the addition of seaweed high nitrogen nutrient significantly enhanced ($\alpha = 0.05$ level) bioremediation and led to a reduction of final soil TPH for all treatments. The greatest amount of biodegradation was observed in the unwashed and Citrasolv washed soils. Treatment with BioFuture biosurfactant appeared to increase the soil TPH levels during bioremediation. This potentially could be due to breakdown products from bacterial degradation of the biosurfactant.

The findings indicate that the success of post-washing biodegradation of residual TPH was dependant on the type of biosurfactant. It was observed that the unwashed soil had the greatest level of biodegradation, followed by the soil washed with Citrasolv. The soils washed with water and BioFuture were observed to have the lowest level of biodegradation. The chemical analysis data alone can not indicate the nature of these differences in degradation rates. Many reasons can be attributed to the slower biodegradation rates observed in the BioFuture washed soil, such as: the inability of the bacterial inoculum to survive in the washed soil; a lack of the inoculum ability to degrade the contaminants. Future research should include 16S Polymerase Chain Reaction and Denaturing Gradient Gel Electrophoresis on the soil bacterial DNA to analyse bacterial community structure before and after bioremediation, which will help determine the fate of the bacterial inoculum introduced into the soils. It can also be speculated that washing disrupted the indigenous bacterial populations, since unwashed soils gave lower final TPH concentrations. Another possibility is that the BioFuture bacterial rhamnolipid was being preferentially degraded by the soil bacteria, thus diverting bacterial activity from TPH degradation. However, neither theory can be determined without further investigation.

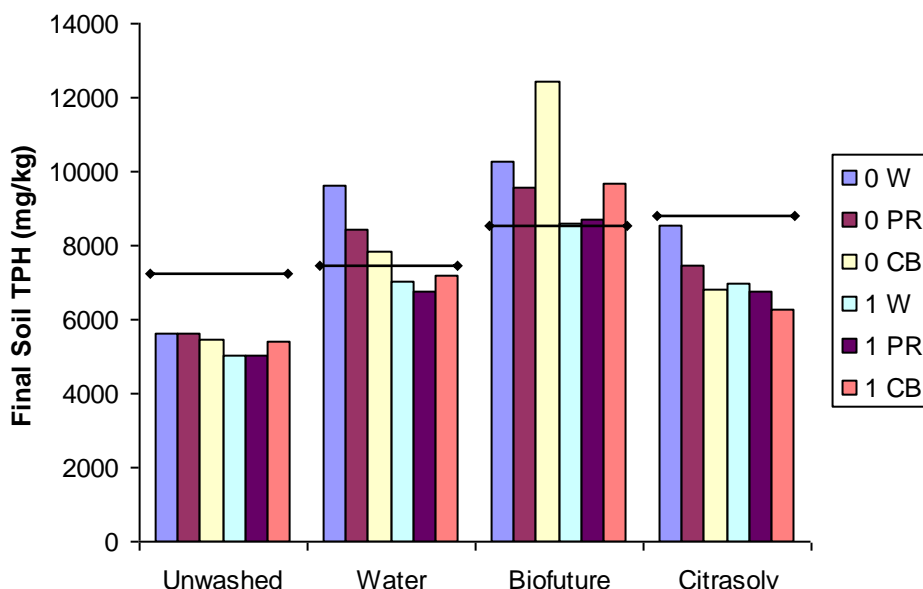


Figure 11. Final soil TPH level after bioremediation for unwashed soil and soil washed with water, BioFuture and Citrasolv biosurfactant. Soils were treated with water [control] (0) and seaweed nutrient (1) and inoculated with water [control] (W), consortium PR (PR) and consortium CB (CB). The black bars represent the pre-bioremediation level of TPH in the soil after washing and before bioremediation.

3.7 Sieved or spiked washing trials

During previous experiments, the TPH solvent extraction was performed on a specific size fraction of soil (< 2 mm), as required by the British Standard ISO16703:2004 to ensure a homogenous and representative sample of soil was subjected to chemical analysis. All washing experiments used soil fraction < 4 mm, with sieving if required, as per BS12457/3. Thus the observed increase in TPH after washing could be explained by redistribution and binding of TPH to the finer grain sizes included in the < 2 mm fraction. An equivalent decrease in TPH in the remaining fraction (> 2mm, < 4mm) was not originally tested for. A series of additional experiments were performed to investigate the effect of surfactant on this transfer of TPH contaminants between size fractions of soil using scaled-up washing experiments. Following these experiments, a trial was conducted to investigate the addition of a suitable fine grain carrier material as an adsorbent during the washing process.

A 1kg portion of diesel-contaminated soil (FDS) was washed with 2 litre solution (water, 0.01 %, 0.1 % and 1 % BioFuture biosurfactant) for 1 hour at 200 rpm. The soil was settled for 1 hour, following this the solution and any unsettled suspension were transferred to a separate container and allowed to evaporate (and designated 'fines'). The settled soil was air-dried and sieved into two size fractions: 'coarse' (> 2mm) and 'medium' (< 2mm). This gave three designated size portions for each soil which were each subject to solvent extraction and GC-FID analysis for TPH content. A 1 kg portion of soil was air-dried and sieved into coarse and medium fractions as a control (Table 10).

Analysis of TPH content in the washing experiments according to British Standard ISO16703:2004 was undertaken on the fractions defined as 'coarse', 'medium' and 'fines' in Table 10. However, no further preparation of the fractions was undertaken and the protocol was only applied at the stage of solvent extraction using 10 g of each fraction. It is worth noting here that the 'medium' fraction of soil here would equal the portion of soil < 2 mm tested using the British Standard ISO16703:2004 method of earlier experiments, since 1 hour settling of fines was standard throughout for the washing method.

The data indicated reductions in TPH content of the 'coarse' and 'medium' size fractions and hence the calculated total TPH content of the soil after washing with water and biosurfactant. The substantial amount of TPH that was unaccounted for means that these results must be interpreted with caution. Some movement of TPH to the 'fines' fraction was observed when washing with 1 % BioFuture, but the amount is not substantial enough to account for the observations in earlier experiments or support the working hypothesis that the apparent increases in TPH levels were due to movement to the <2mm fraction analysed under the BS methodology.

Table 10. The distribution of TPH in ‘fines’ (settled suspension), ‘medium’ (< 2mm) and ‘coarse’ (> 2 mm, <4 mm) soil fractions after washing with water, 0.01 %, 0.1 % and 1 % BioFuture. Unwashed soil was analysed as a control.

| | Unwashed (control) | Water washed | 0.01 % | 0.1 % | 1% |
|-------------------------------|--------------------|--------------|-------------|-------------|-------------|
| TPH Medium (mg/kg) | 12317 | 6748 | 4565 | 4204 | 5315 |
| TPH Medium (mg/g) | 12 | 7 | 5 | 4 | 5 |
| Mass Medium (g) | 321 | 480 | 372 | 423 | 416 |
| Total TPH Medium (mg) | 3959 | 3239 | 1697 | 1778 | 2213 |
| TPH Coarse (mg/kg) | 3852 | 3818 | 2688 | 2191 | 3795 |
| TPH Coarse (mg/g) | 4 | 4 | 3 | 2 | 4 |
| Mass Coarse (g) | 591 | 429 | 541 | 470 | 475 |
| Total TPH Coarse (mg) | 2275 | 1637 | 1455 | 1029 | 1801 |
| TPH Fines (mg/kg) | 0 | 7942 | 4356 | 4113 | 11329 |
| TPH Fines (mg/g) | 0 | 8 | 4 | 4 | 11 |
| Mass Fines (g) | 0 | 4 | 5 | 4 | 26 |
| Total TPH Fines(mg) | 0 | 34 | 21 | 16 | 297 |
| TPH Total in 1 kg (mg) | 6234 | 4910 | 3174 | 2824 | 4311 |
| Total Soil Dry Mass (g) | 912 | 913 | 918 | 897 | 917 |
| <i>TPH Missing (mg)</i> | 0 | 1324 | 3060 | 3410 | 1923 |

A further series of experiments were designed to test the ability of a fine-grained carrier material (bentonite) to remove TPH from a granular oily waste (oily mill scale) in a 1:10 ratio with the addition of biosurfactant to aid dispersion. Initial settling tests were performed on the bentonite and OMS to determine their settling times in both water and 1 % BioFuture biosurfactant:

- Water – OMS 1 minute; bentonite 30 minutes
- 1 % BioFuture – OMS 6 minutes, bentonite ~1 hour

A 20 g portion of dried bentonite was added to 200 g OMS and washed with 400 ml solution (water, 1 % BioFuture biosurfactant) at 200 rpm for 1 hour. The mixtures washed in water and 1 % BioFuture were settled for 1 minute and 6 minutes respectively, followed by transfer of the liquid and unsettled bentonite to a fresh vessel for settlement. All washes were performed in triplicate. Decants were settled for 1 hour and all liquid removed. All solids were air dried and stored at 4°C prior to chemical analysis. A 20 g portion of all washed solids was analysed for TPH content, alongside a 20 g portion of unwashed bentonite and OMS as a control.

Although the blank analysis of the bentonite was perhaps rather high, the increase in TPH concentration in the clay fraction (Table 11) indicates that some transfer of TPH from OMS to bentonite occurred during washing but there was no significant difference between the amounts transferred using water or 1 % biosurfactant. At the 1:10 mass ratio of clay to OMS this has no obvious effect on the TPH concentration in the remaining solid. However, there was a statistically significant difference (at the 95% level) between the amounts of clay recovered from the wash solutions, with 3.2 g recovered after washing with water compared to 10.7 g with biosurfactant. This indicated that suspension of fine particles was enhanced in biosurfactant solution above CMC, which could be advantageous in separation for disposal.

Table 11. The distribution of TPH (mg/kg) in OMS (oily mill scale) and bentonite clay fractions before (initial) and after washing with water and biosurfactant. Values are average of three replicates, with the numbers in brackets represent two standard errors of the mean (95 % confidence intervals).

| Material | TPH Concentration (mg/kg) | | |
|-----------|---------------------------|---------------------|-----------------------------|
| | Initial | After Water Washing | After Biosurfactant Washing |
| OMS | 32999 | 31294 (1295) | 32854 (310) |
| Clay | 4140 | 7858 (912) | 7257 (1508) |
| Total TPH | 37139 | 39152 | 40111 |

4. Reliability of Results

As the project set out to use samples of real waste streams, one possible source of error in the experimental methods was the extent of sample heterogeneity prior to treatment and chemical extraction. The soil samples in particular have a range of grain size fractions with an unknown mineralogical association and speciation of contaminants. It was expected that the contamination would not be evenly distributed through the soils. Care was taken to develop a rigorous method to ensure soil samples were thoroughly homogenised prior to sub-sampling for washing experiments with multiple sampling and chemical extractions performed to assess

homogenisation. A mechanical method was selected which ensured sub-samples of soil did not have a significantly different TPH content (significant at $\alpha=0.05$ level).

It should be stressed that the standardized leaching test used in the laboratory to compare generic waste-washing differs significantly from conventional commercial scale soil-washing, particularly in the intensity of washing and optimization, so any extrapolation of findings from one to the other is with this caveat.

The effects on washing soil and waste samples with biosurfactant as opposed to water of a range of variables has been tested statistically at different significant levels. A factorial design approach was used throughout to allowing the effect and interaction of many factors to be tested at once. This method allowed a more rigorous analysis of data using statistical methods and allowed interactions between factors to be identified. All statistical analysis was performed using ANOVA (analysis of variance) in Minitab Software Version 14. Experiments were all performed in triplicate as true replicates from the point of sub-sampling the laboratory waste stocks.

The principal measure of success of the washing trials involved determining TPH content. Initially this was carried out by a commercial laboratory with suitable external accreditation (UKAS, MCerts ISO9001 etc.). Internal analytical protocols were developed based on methods described in relevant British Standards. As described above the trials were performed in duplicate or triplicate, thus reducing the significance of any analytical imprecision.

Finally, a number of sequential trials with similar designs were performed iteratively to confirm key outcomes by producing consistent results.

5. Summary of Main Findings and Implications

(1) Our review of current remediation practice and stakeholder consultation indicated that conventional soil-washing involves the physical separation of a fraction of the soil which contains a disproportionately high percentage of the contamination, usually the finer particles. It is a cost effective remediation process for sandy soils where, for example, the bulk of the heavy metals are associated with a clay fraction typically non exceeding 10% which can therefore be disposed of to landfill, with the reduced disposal costs balancing those of treatment. Organic contaminants can also be removed - by virtue of the higher surface area of the fine fraction, onto which they are adsorbed.

(2) Factors which may affect the potential use of commercially available biosurfactants in washing wastes include:

- the achievable reduction in surface tension
- the CMC - the concentration of surfactant in solution at which the surfactant molecules aggregate together to form spherical structures (micelles), which provides a means of comparing the efficacy of different products, particularly rhamnolipids
- biosurfactant depletion by adsorption on the surface of clay particles in soils

The combination of variable CMC, ST and cost as supplied combine to determine the economics of a particular washing solution. If it assumed that CMC provides a valid basis for comparison of use in washing, then more dilute solutions of microbial secretions (e.g. rhamnolipid) may still be economically competitive with stronger solutions of cheaper plant extracts (e.g. orange oil).

(3) In our laboratory waste-washing studies on TPH-contaminated soil the extent of contaminant removal was variable, waste-specific and dependent on a number of factors. Enhanced transfer to finer (clay) size fractions occurs faster with rhamnolipid biosurfactant concentrations above CMC, which infers that removal of organic contaminants from sand and coarser fractions is enhanced. This indicates that addition of biosurfactant could potentially improve the performance of conventional soil-washing by enhancing the liberation of fines, leading to lower residual contamination in reusable grain size fractions. Initial trials to introduce a clay fraction to granular oily wastes as a carrier to exploit this phenomenon were inconclusive.

(4) Transfer of TPH to biosurfactant washing solution through solution, solubilisation in micelles, or formation of floating product was minimal in the weathered wastes studied, which contradicted earlier studies of spiked samples. This indicates that washing is unlikely to generate an additional liquid waste requiring (or amenable to) bioreactor treatment. This also means that contaminants cannot be adequately removed through movement to wash water, e.g. to avoid subsequent disposal of the finer fraction generated by soil-washing.

(5) Foam fractionation is a highly efficient method of concentrating and recovering high value rhamnolipid biosurfactant from the used wash solution for recirculation, achieving up to 90% recovery and concentration by a factor of 10-100. A key finding is that this could also be utilized to minimize undesirable foaming in any wastewater.

(6) Washing reduces the potential for aerobic biodegradation through biostimulation of indigenous bacteria. A key finding is that bacterial biosurfactant apparently inhibited bioremediation to a greater extent than orange oil.

6. Future work and replication potential

Initial studies have already begun to address the main area for additional work evidenced by the laboratory study. The addition of a fine-grained material to contaminated wastes, as a carrier material for removing remobilised contaminants, is a potential route for development. In particular, such carrier materials could be designed with favourable separation techniques (such as magnetic properties) or coatings to attract target contaminants. Studies should also focus on the reliable determination of true TPH contamination levels in washed and unwashed soil samples, as the extent of contaminant removal could not be reliably determined this way or mass-balance demonstrated after mechanical treatment. In addition, the engineering challenges posed by large-scale use of biosurfactant solution, such as foaming, would also need to be tackled prior to implementation at large scale.

The Bio-recycling Project was conceived in late 2004 in response to legislative drivers, in particular the introduction to the UK of the European Landfill Directive. The anticipated introduction of Waste Acceptance Criteria (July 05) was seen as a major driver for process-based remediation of soils classified as hazardous waste, many of which could potentially exceed 6% TOC (e.g. as contaminated soil with organic matter, fine coal, coke, clinker, peat etc.). Uptake of alternative treatment technologies has been slow, in part due to widespread 3 x WAC derogations on TOC, or by classification of some oily waste streams as non-hazardous (e.g. due to a low benzo[a]pyrene content in oily material). In June 2008 the Environment Agency published a regulatory position statement indicating that from 1/7/08 it expects landfilled hazardous waste to meet the WAC of 6% TOC.

A number of new or emerging regulatory drivers can be identified as potentially increasing demand for washing technologies including soil-washing: Landfill tax increases and the likely end of landfill tax remission for soils from contaminated sites should encourage further reuse of material onsite; from 30 October 2007 pre-treatment is required for any non-hazardous waste going to landfill.

The definition of waste from the Waste Framework Directive (based on an “intention to discard”) without a consensus definition of “recovery” has been a major hurdle for reuse of the cleaned secondary aggregates generated by soil-washing. Guidance from the UK Environment Agency (2006) on the definition of waste, recovery and reuse in brownfield sites has clarified the position for treated soils. This document allows reuse of processed material on site. The hub and cluster principle is also agreed for reuse on site of soil treated at an offsite soil treatment centre. However, reuse on a third site of treated material remains impossible at the time of writing, partly due to the conflict between the absolute definitions of hazard used in waste management, compared to the risk-based and site-specific assessments of “suitability for use” inherent in the UK Contaminated Land Regime. It is hoped by the authors that, as applied in the hub and cluster model, establishing “certainty of use” will avoid an “intention to discard”, providing an alternative to an accepted definition of “recovery” following the draft Waste Framework Directive revision. Further regulatory clarification and demonstration projects are required to evidence soil treatment as an example of acceptable best-practice.

The EU Soil Thematic Strategy and draft Soil Framework Directive could have required widespread strategic soil remediation. However, it is understood by the authors that following extensive revisions it is now unlikely to supersede the approach or progress of remediation achieved in the UK under Part IIA and the planning regime.

The recent focus on sustainability and carbon-footprint of remediation methods could also favour soil-washing versus transportation to landfill, thermal desorption or cement stabilization. Drivers include the house builders who have declared that they are now working towards zero-carbon construction for housing. A CL:AIRE project (SuRF UK) is currently assessing the sustainability of remediation methods using experience from the USA.

Table 12. UK soil-washing technology providers (source Brownfield Briefing Remediation Solutions October 2007).

| Number (%) | With MTL issued | No MTL | Total providers |
|--|-----------------|---------|-----------------|
| Soil/jet washing offered in house | 20 (23) | 7 (8) | 27 (32) |
| Soil/jet washing offered via subcontractor | 5 (6) | 14 (17) | 19 (22) |
| Soil/jet washing not offered | 10 (12) | 29 (34) | 39 (46) |
| Totals | 35 (41) | 50 (59) | 85 (100) |

Although the addition of biosurfactant appears not to be advantageous, conventional soil-washing remains a promising technology for pre-treatment of waste or soil treatment centres (e.g. Terramundo), and is finding widespread use in landmark remediation projects (e.g. London Olympics site). An equivalent survey of the availability of soil-washing in the UK (Table 12) to that completed at the beginning of the project (Section 3.1) indicates a four- to five-fold increase of technology providers in 2 years with over half of UK remediation consultants now offering soil-washing in-house or via a contractor. Two thirds of all remediation companies listed now either offer soil-washing or have obtained a Mobile Treatment Licence (MTL) to use it.

7. Action resulting from research.

The steering-group established by the Bio-recycling Project has met quarterly at the University of Teesside over a two year period and has become an established knowledge transfer network. We thank the representatives of the member organizations (listed below) for giving their time to attend steering-group meetings, for supplying materials, waste samples or know-how, and for ensuring that the research was fully focused towards up to date commercial and regulatory pressures.

Bio-recycling steering group members:

| | |
|--|----------------------------|
| • Oil Company 1 | • SNRH Landfill Operator A |
| • Entec | • FWS Consultants |
| • Augean | • Bradstone |
| • Cleveland Biotech | • DEC |
| • Environment Agency | • Corus |
| • National Industrial Symbiosis Programme (NE) | • National Grid Property |
| • BioFuture | |

Richard Lord & Helen Connolly, December 2007.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Project Publications

Bio-recycling Project Brief

<http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=14697#Description>

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