Journal of Environmental Management 92 (2011) 866–871

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

# Re-use of remediated soils for the bioremediation of waste oil sludge

Tanvi H. Makadia*, Eric M. Adetutu, Keryn L. Simons, Daniel Jardine, Petra J. Sheppard, Andrew S. Ball

*School of Biological Sciences, Flinders University of South Australia, Adelaide, GPO Box 2100, Adelaide SA 5001 Australia*

ARTICLE INFO

*Article history:*
Received 15 June 2010
Received in revised form
7 October 2010
Accepted 20 October 2010
Available online 27 November 2010

*Keywords:*
Bioremediation
Biodegradation potential
16S rDNA
ITS
DGGE

ABSTRACT

We investigated the possibility of re-using remediated soils for new bioremediation projects by spiking these soils with waste oil sludge in laboratory based microcosms. The level of Total Petroleum Hydrocarbon (TPH) reduction was high (>80%) in naturally attenuated microcosms and was not significantly improved by biostimulation, bioaugmentation and the combined treatment of bioaugmentation and biostimulation by week 12. This indicated that the observed TPH reduction might have been related to the soil's inherent hydrocarbon-degrading potential. Microbial community analysis (16S rDNA and ITS-based Denaturing Gradient Gel Electrophoresis fingerprints) confirmed the dominance of hydrocarbon degrading genera such as *Alcanivorax* and *Scedosporium*. Cluster and Shannon diversity analysis revealed similar but stable bacterial and fungal communities in naturally attenuated and amended microcosms indicating that rapid reduction in TPH may not always be accompanied by changes in soil microbial communities. This study has therefore shown that soils previously used for bioremediation can have an improved hydrocarbon degrading potential which was successfully re-harnessed for new projects. This ability to re-harness this potential is attractive because it substantially reduces operational costs as no additional bioremediation treatments are needed. It can also extend a landfill's lifespan as soils can be re-used again before landfill disposal.



## 1. Introduction

Oil sludges are classified as hazardous wastes and are composed of a mixture of petroleum hydrocarbons, mineral particles and water (Bartha, 1986). Petroleum hydrocarbons are complex mixtures of compounds which can be categorized into saturates, aromatics, resins and asphaltenes (Harayama et al., 1999). As a consequence of the toxic nature of oil sludges, there is a need to develop safe and environmentalally friendly methods for the treatment and disposal of sludge and sludge contaminated environments.

Disposal of hydrocarbon contaminated soil is usually carried out with reference to government legislations which vary in different countries. For example, in Australia, Total Petroleum Hydrocarbon (TPH) concentration below 10 000 mg $kg^{-1}$ is regarded as safe for disposal as Low Level Contaminant Waste in a landfill site by National Environmental Protection Council of Australia (National Environmental Protection Council, 1999). As the disposal of treated contaminated soils usually require the use of designated landfill sites, the logistics of waste transfer and maintenance of this disposal site represents an added cost. Therefore, in addition to legislative requirements, economic costs are also an important factor to be considered in the treatment and disposal of hydrocarbon contaminated soils.

Treatment of sludge contaminated soil can be carried out using a variety of technologies. These include excavation and containment of contaminated soils in secured landfills, stabilization and solidification, thermal desorption and incineration, with thermal desorption technologies now being more widely used than others (Varanasi et al., 2007). These treatments are not necessarily environmentally friendly and have substantial cost implications with remediation cost per cubic metre of polluted soil ranging from $880 for landfill disposal, $700 for incineration and $260 for thermal desorption (Cookson, 1995). Apart from this, recent legislative changes in Australia and other parts of the world have necessitated the development of environmentally friendly alternatives to some of these methods. For example, Australian legislation now limits the extent to which hydrocarbon contaminated environments can be excavated and disposed of in landfills or solidified.

Since hydrocarbons are susceptible to microbial attacks (Huesemann, 1995; Unterbrunner et al., 2007), biological treatment (bioremediation) is an environmentally friendly way of dealing with these contaminants. Exploitation of microbial hydrocarbon catabolic potential in treating contaminated environments via biostimulation and bioaugmentation processes (Mancera-Lopez et al., 2008) has proved very successful with comparatively lower

* Corresponding author. Flinders University, School of Biological Sciences, Bedford Park, Adelaide, SA 5042 Australia. Tel.: +61 8 8201 7961; fax: +61 8 8201 3015.
*E-mail address:* tanvihmakadia@gmail.com (T.H. Makadia).

0301-4797/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jenvman.2010.10.059

economic (Maila and Cloete, 2004) and environmental costs. Apart from this, dumping remediated soils into landfills can be a significant waste of microbial resources because the treated contaminated soil being disposed off may have a potentially increased catabolic capacity for hydrocarbons (Diplock et al., 2009).

Molecular techniques such as PCR-DGGE, cloning sequencing which target universal eubacterial genes or functional genes coding for hydrocarbon degradation are now increasingly being used to assess soil hydrocarbon-degradation potential (Hamamura et al., 2008). These molecular tools have also been used to monitor microbial activity *in situ* and to carry out highly detailed analysis of the oil-degrading microbial community. Some studies have reported a substantially higher percentage of hydrocarbon degrading genes (potential) in contaminated soils than in pristine soils, indicating that these organisms had been enriched after soil contamination (Margesin et al., 2003). However, it is unclear whether this hydrocarbon degrading potential can be re-harnessed or exploited for new bioremediation projects. It is also unclear how many times this potential can be exploited (assuming that it was exploitable) and what would be the most appropriate bioremediation strategy to use in terms of efficiency and economic and environmental costs.

Therefore, in this study, the utilisation of a previously bioremediated soil's degradative capacity in treating a new contaminant was evaluated as an alternative to using pristine soil or dumping into landfill and compared to other bioremediation strategies. If successful (natural attenuation), then the metabolic capacity of this previously bioremediated soil would have been harnessed to treat a new contaminant. Re-use of this soil to treat a new contaminant would add a new incentive to the process of natural attenuation and ensure that the soil's hydrocarbon catabolic capacity is fully utilised prior to disposal while ensuring the longevity of the disposal site. Consequently, the primary objectives of this study were to (i) study the effectiveness of the use of a previously treated soil to treat a new contaminant and (ii) compare this monitored natural attenuation with other bioremediation treatments such as biostimulation, bioaugmentation and biostimulation–bioaugmentation, assessing their effects on the soil bacterial and fungal communities. This was carried out using PCR-DGGE-sequencing techniques in soil microcosms spiked with oil sludge from a crude-oil tank bottom of an oil refinery based in New South Wales, Australia.

## 2. Materials and methods

### 2.1. Initial analysis and pre-treatment of soil

The soil samples used in this study came from a biopile that had been previously used to bioremediate oil contaminated soil samples to below 10 000 mg $kg^{-1}$. This soil was going to be dumped as Low Level Contaminated Waste at a landfill site in South Australia. The soil was air dried and sieved to remove stones and other unwanted materials prior to any analysis. The pH, organic carbon, nitrogen, and phosphorus content of the soil was determined using standard methods while the soil's water holding capacity (WHC) was determined according to the method of Guerin (2000).

### 2.2. Inoculum preparation

*Scedosporium apiospermum*, a hydrocarbonoclastic fungus (Martin-Gil et al., 2008) was cultured on Potato Dextrose Agar plates containing 0.015 g $l^{-1}$ of tetracycline at 30 °C for up to 10 d to produce conidia for spore suspension preparation (Lestan and Lamar, 1996). Aliquots of this spore suspension were inoculated into sterile tryptone soya broth containing tetracycline and incubated at 37 °C for 3 d at 121 rpm to generate substantial quantities of mycelia. The mycelia were washed in phosphate-buffered saline, filtered with Whatman No. 1 filter paper (120 mm) and used to inoculate the soil microcosms.

### 2.3. Experimental design and treatments

Four treatment strategies were employed (biostimulation, bioaugmentation, natural attenuation and combination of biostimulation and bioaugmentation) to assess waste oil sludge degradation in soil. The microcosms were set up in triplicate in 1 l conical flasks containing 200 g of soil with 20% (w/w) sludge oil and treated with the desired amendments. Bioaugmented microcosms (BA) consisted of hydrocarbonoclastic fungus (0.1 g dry cell weight) and soil-sludge mixture while biostimulated microcosms (BS) were prepared by adding a modified nutrient formulation (8% *w*/*w*) based on Bushnell Haas medium (Eriksson et al., 2000) to the soil-sludge mixture. The combined treatment microcosms (A + S) contained the modified nutrient solution (8% *w*/*w*), fungus (0.1 g dry cell weight) and soil-sludge mixture while naturally attenuated microcosms (NA) consisted of only the soil-sludge mixture. Amendments and water were added in such a way that the microcosms were at 40% of the soil WHC. Weekly sampling was carried out for up to 12 weeks with samples being stored at −20 °C prior to any analysis.

### 2.4. Determination of soil TPH contents

Hydrocarbons in the replicate soil samples were extracted using a modified standard protocol of determining soil hydrocarbon content in soil according to International Standard Organisation (ISO/DIS GC-method, 2009). The TPH concentrations of the soil extracts were determined using Gas Chromatography, performed on a Varian 3800 gas chromatograph equipped with a Varian 8200 autosampler, Flame Ionisation Detector and splitless injector valve. The capillary column used was an Alltech EC-5 (30 m × 0.25 mm with 0.25 μm film thickness), with helium as a carrier gas.

### 2.5. DNA extraction and Polymerase Chain Reaction

DNA was extracted from triplicate soil samples at day 0, weeks 4, 8 and 12 using a soil DNA isolation kit (MoBio PowerSoil, Carlsbad, CA USA) according to the manufacturer's instructions. The total soil bacterial community was evaluated by PCR using universal primers 314F GC and 518 R (Muyzer et al., 1993). The alkane degrading community (*alkB* genes) was evaluated with TS2S, Deg1RE and Deg1RE GC primers as described by Smits et al. (1999). *AlkB* genes were amplified using a semi-nested approach (Hendrickx et al., 2006) because of the reduced sensitivity of Deg1RE caused by the attached GC clamp. The first reaction was carried out with TS2S and Deg1RE primers while the second reaction was carried out with TS2S and Deg1RE GC primers using 2 μl of the product from the first reaction as template DNA. The fungal community in the samples were analysed via nested PCR reactions using (ITS) region based primers ITS 1, 1F GC, 2 and 4 (Anderson and Parkin, 2007). In both *alkB* and ITS reactions, the negative controls of the first reactions were used as template in the nested reactions to eliminate the possibility of carryover contamination.

### 2.6. DGGE, analysis of DGGE profiles, band excision and purification

PCR amplicons were analysed on a Universal Mutation Detection System D-code apparatus (Biorad, CA, USA). DGGE was carried out on 9% polyacrylamide gel using a 40–60% (bacteria) and 42–52% (fungi) denaturing gradient for 20 h at 60 °C and 60 V. Gels were silver

stained (Girvan et al., 2003) for community profile analysis (bacteria and fungi), scanned and saved as TIFF. files. DNA from bands of interest (aseptically excised) on both bacterial (silver stained) and fungal (SYBR Gold stained) DGGE gels were eluted by overnight incubation in nuclease free water at 70 °C. This DNA was then re-amplified with the appropriate primer pair and subject to further rounds of DGGE using a narrower gradient range, band excision and further PCR (as described before) in order to ascertain the purity of the excised bands. Only bands deemed to be pure were sequenced. The sequence data from bands of interest were analysed and their putative identities determined as described by Aleer et al. (2010).

### 2.7. Statistical analysis

The digitized image was analysed using Phoretix 1D advanced analysis package (Phoretix Ltd, UK) with the relatedness of the microbial communities being expressed as similarity clusters using the unweighted pair group method with mathematical averages (UPGMA). Shannon index (*H*') was calculated from DGGE profiles (Girvan et al., 2003) using the formula $H' = -\sum p_i \cdot LN \cdot p_i$. Statistical significance was determined in replicate samples comparison by either *t* test and or analysis of variance (ANOVA) and Tukey tests (Sigma Stat 2.03, Systat, London, UK).

## 3. Results

### 3.1. Biodegradation of TPH

Fig.1(a) shows the level of TPH biodegradation in the four laboratory based soil microcosms (sandy loam, pH 8.2, with 29.6% organic carbon, 0.42% total nitrogen and 1.64 mg $l^{-1}$ phosphate) over a 12 week period. Compared to the initial TPH soil content of over 20 000 mg $kg^{-1}$, BS samples had TPH levels below the safe landfill disposal limit of 10 000 mg $kg^{-1}$ (67% degradation rate, 7000 mg $kg^{-1}$) by week 2 while others including naturally attenuated samples fell below 10 000 mg $kg^{-1}$ by week 3. These results showed that both biostimulated and the bioaugmented–biostimulated samples initially had substantially higher rates of hydrocarbon reduction (≥67%) than bioaugmented and naturally attenuated samples (53–54%). However by week 12, the substantial reduction in the TPH content of the soil microcosms of NA samples (2915 mg $kg^{-1}$, 86% reduction) was not significantly different (ANOVA, $P > 0.05$) to the reductions observed in the amended soil microcosms; BS (1921 mg $kg^{-1}$, 91% reduction), BA (1747 mg $kg^{-1}$, 91% reduction) and A + S (1766 mg $kg^{-1}$,92% reduction) (Fig. 1a). Comparison of the week 12 chromatographic profiles in amended and non amended samples in Fig. 1(b) showed that the disappearance of the different hydrocarbon fractions in representative amended and naturally attenuated samples were identical.

### 3.2. Effect of amendments on bacterial and fungal community profile and diversity values

UPGMA dendogram analysis showed that in both 16S rDNA genes based bacterial and ITS based fungal communities (data not shown), clusters were largely formed based on the sampling time rather than by treatment. This indicated that biotreatments had very little effect on these communities' banding patterns at these times (weeks 4, 8, 12). Assessment of the total *alkB* bacterial community over 12 weeks also showed time rather than treatment related clustering (Fig. 2a). The Shannon Diversity (*H*') values of the universal bacterial (data not shown), *alkB* (Fig. 2b) and fungal communities (data not shown) in naturally attenuated microcosms were not significantly different from the *H*' in most of the amended samples over the course of the experimental period (ANOVA, $P > 0.05$) (Fig. 2b).

### 3.3. Identification of microbial groups from DGGE community profiles

Sequence analysis of four dominant sets of bands (a–d) (Fig. 3a) which were detected at all sampling points in the bacterial community DGGE profile (independent of amendments) showed that they were closely related to either uncultured gammaproteobacteria or

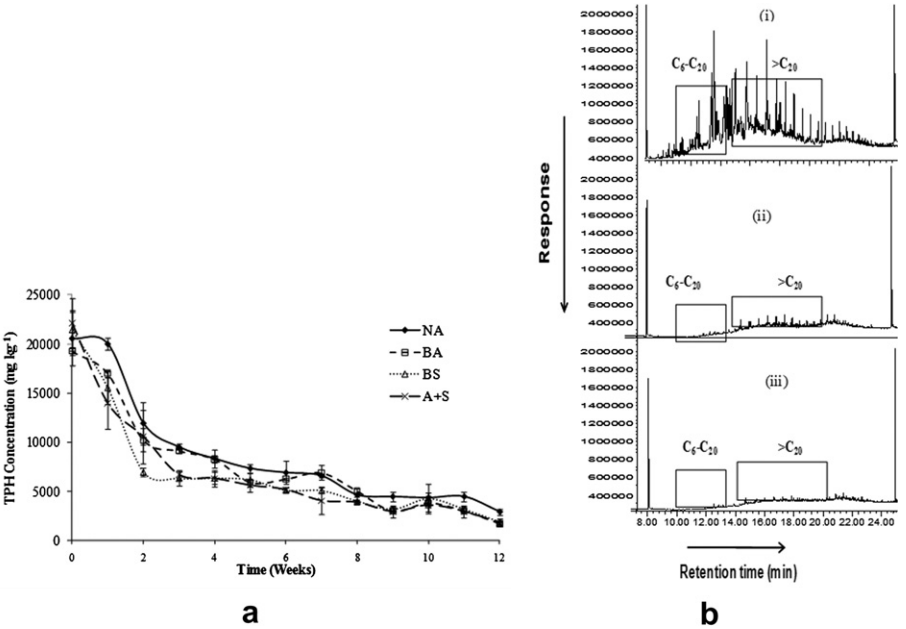


**Fig. 1.** TPH degradation levels of oil sludge (a) and representative chromatograms (b) from laboratory based soil microcosms incubated for 12 weeks. Note for (a), NA–natural attenuation, BA–bioaugmentation, BS–biostimulation and A + S–combined treatment of bioaugmentation and biostimulation. For (b) (i)–Chromatogram of sludge oil contaminated soil at time zero, (ii)–Chromatogram of samples natural attenuation microcosms at week 12 (day 84) and (iii)–Chromatogram of samples from Bioaugmentation–Biostimulation microcosms at week 12 (day 84).

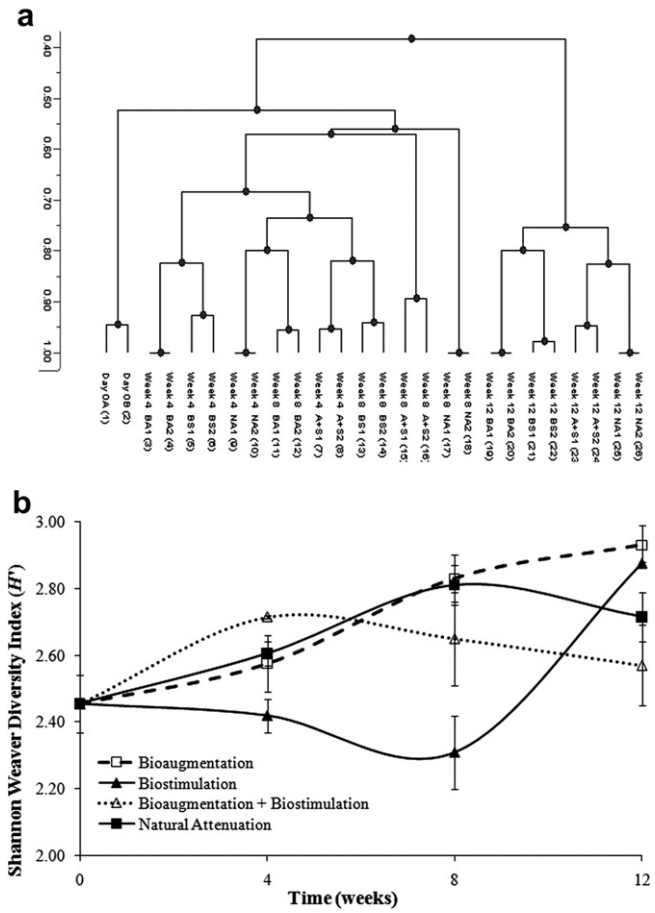


**Fig. 2.** UPGMA dendogram (a) and Shannon Weaver Diversity values (b) derived from *alkB* genes based DGGE profiles. Note: scale is representative of similarity levels between the samples.

*Alcanivorax* species. While a total of thirty two bands were excised from SYBR G stained ITS-based fungal DGGE, the identities of only eighteen of these bands could be resolved. Problems with resolving the identities of bands from fungal DGGE are known and reported in literature (Anderson et al., 2003; Kowalchuk et al., 2003). Sequence analysis of these eighteen bands revealed that they were closely related to six fungal genera. Four dominant bands which were found in all the lanes (in amended and non amended microcosms) were *Arthrobotrys oligospora*, *Scedosporium apiospermum*, *Motierella* sp and *Coprinus cordisoporus*. Other putatively identified bands include *Scytalidium lignicola* and *Chrysosporium pseudomerdarium* (Table 1 and Fig. 3b).

## 4. Discussion

In investigations carried out in this study, the safe landfill TPH disposal target of 10 000 mg $kg^{-1}$ (National Environmental Protection Council, 1999) was first attained in biostimulated samples by week 2 and in other samples by week 3. Nutrient addition is known to enhance the activities of indigenous microorganisms including hydrocarbon degrading organisms and the addition of nutrients to both BS and A + S might have contributed to the increased hydrocarbon-degradation seen in the first three weeks of incubation (Mancera-Lopez et al., 2008; Stallwood et al., 2005). However, this beneficial effect of nutrient addition was short term as both naturally attenuated and amended samples had similar rates of TPH reduction by week 12. Therefore, natural attenuation was as effective as the other bioremediation strategies as nutrient and fungal augmentation did not cause any significant improvement in TPH degradation over time. The significant TPH reduction observed in naturally attenuated and amended samples could have been related to the soil properties and soil hydrocarbon degrading potential (Bento et al., 2005; Vinas et al., 2005). The soil hydrocarbon degrading potential is related to the capacity of indigenous microorganisms to degrade different hydrocarbon fractions. Even though this potential exists in pristine environments, contacts with contaminating hydrocarbon can

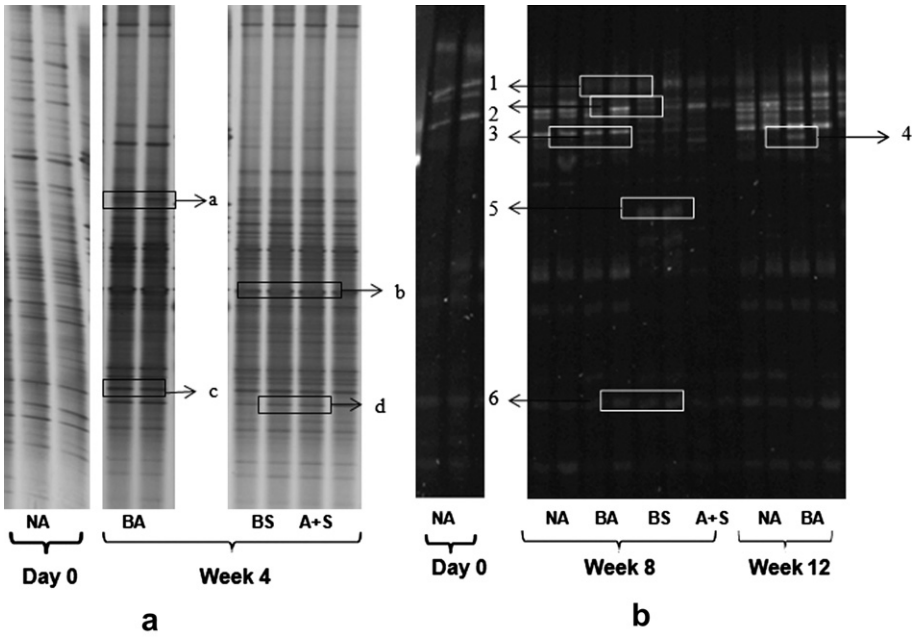


**Fig. 3.** Denaturant Gradient Gel Electrophoresis community profiles of bacterial (a) and fungal (b) groups in amended and naturally attenuated soil microcosms. Note: Since there was very little difference in the microbial community profile over the 12 week period, selected representative profiles are shown. Bands numbered and enclosed in boxes were excised and their putative sequence identities determined. NA–Natural Attenuation, BA–Bioaugmentation, BS–Biostimulation, A + S–Bioaugmentation + Biostimulation. In (a) bands a and b uncultured gammaproteobacteria (AJ640189) and bands c and d *Alcanivorax* sp (AB435642 and AB435644 respectively). In (b) identities of bands numbered 1 to 6 are shown in Table 1.

**Table 1**
Summary of sequence identification of bands excised from the fungal DGGE gel.

| DGGE band label | Closest Match | Accession Number | Sequence similatiry (%) | Reference |
|---|---|---|---|---|
| 1 | *Arthrobotrys oligospora* | EF445989.1 | 100 | van Elsas et al., 2000 |
| 2 | *Scedosporium apiospermum* | AB489090.1 | 91 | Martin-Gil et al., 2008 |
| 3 | *Coprinus cordisporus* | AY461814.1 | 98 | Pletsch et al., 1999 |
| 4 | *Scytalidium lignicola* | FJ903317.1 | 91 | Gramss et al., 1999 |
| 5 | *Chrysosporium pseudomerdarium* | AJ390386.1 | 94 | Nagai et al., 1998 |
| 6 | *Motierella* sp. | AJ541798.1 | 100 | Weber et al., 2002 |

enhance this potential by causing a sharp increase in hydrocarbonoclastic microbial population (as shown in a review by Joo et al. 2008). Since the soil samples used in this study came from a finished hydrocarbon contaminated biopile, the samples would be expected to have an increased population of hydrocarbonoclastic microorganisms and enhanced hydrocarbon degrading potential (Johnsen et al., 2007).

Evidence of the presence of adapted hydrocarbon degrading communities in these soil samples was provided by molecular analysis of the soils' microbial communities. The detection of *alkβ* genes in all the microcosms irrespective of treatment is indicative of the availability of alkane degrading potential in the soil's bacterial community. Although cluster and diversity ($H'$) analysis of soil microbial communities in naturally attenuated and amended soil samples showed very little changes, the selected dominant bands identified from DGGE profile were closely related to hydrocarbon degrading genera (Fig. 3 and Table 1) The dominant bacterial species identified from the bacterial profiles were uncultured gammaproteobacteria-like and *Alcanivorax* sequences which belong to a group of known hydrocarbon degraders (Fig. 3a) (Harayama et al., 2004; Kyung-Hwa et al., 2007; Vinas et al., 2005). Some of the selected dominant DGGE fungal bands were closely related to *Scedosporium* sp, *Scytalidium*, and *Coprinus* species (Fig. 3b and Table 1) which are known hydrocarbon degrading genera or have been isolated from hydrocarbon contaminated environments. Their presence could be evidence of fungal roles in hydrocarbon catabolism. This high dominance by sequences similar to those of known hydrocarbonoclastic microorganisms might be reflective of an environment that has an enhanced hydrocarbon degrading potential because of the source of the soil. The detection of hydrocarbonoclastic mycoagent (*Scedosporium* sp) in amended and non amended samples suggested that this mycoagent was already present in the community and probably explained why bioaugmentation was of little benefit during bioremediation.

Changes in bacterial communities (DGGE) have been reported in some studies (Kyung-Hwa et al., 2007; Vinas et al., 2005). These changes were due to the loss of microbial groups incapable of tolerating (little or no available microbial potential) certain hydrocarbon fractions (contaminant type) and dominance by groups capable of utilising these fractions. The lack of amendment induced shifts in microbial communities observed in this study could have been because the communities in these re-used remediated soils had the potential to degrade the alkanes in the oil sludge spiked soil. This could also explain why there was no long term benefit of amendments on TPH reduction in the oil sludge spiked soils. The absence of significant difference in the TPH reductions between naturally attenuated and amended soils therefore probably reflected the enhanced soil hydrocarbon degrading potential possessed by these soil samples. However, care must be taken in extrapolating the results of this study for field based studies where environmental conditions cannot be adequately controlled as was the case in laboratory based studies. In addition the success of any bioremediation strategy can be affected by a variety of edaphic and environmental factors such as contaminant and soil type, temperature and pH, (Hamamura et al., 2008; Stallwood et al., 2005; Vinas et al., 2005).

Despite these limitations, the findings of this study are crucial for managing hydrocarbon contaminated soils. Apart from showing the benefits of natural attenuation, this study has also showed that molecular tools such as PCR-DGGE-sequencing techniques can be used to assess the hydrocarbon degrading capacity or potential of soils. The data obtained from these kinds of analyses can also be used as part of the model for determining appropriate bioremediation strategies for contaminated soils. For example, when such hydrocarbon catabolic capacities are high, monitored natural attenuation may be all that would be needed. This bioremediation strategy of monitored natural attenuation is also comparatively cheaper, improves the management of waste materials and enhances land management (US EPA, 2010). In addition, the potential to re-use soils is attractive because it can extend the landfill's lifespan as fewer soils are dumped because the soils can be re-used again for new projects before subsequent disposal.

## 5. Conclusion

This study has shown that soils previously used for successful bioremediation possess sufficient microbial hydrocarbon degrading potential which can be re-harnessed for new bioremediation projects. It has also demonstrated that depending on the history of the soil, monitored natural attenuation might be all that would be needed for subsequent bioremediation projects which could result in substantial cost savings. In addition, molecular tools can give important information on soil hydrocarbon degrading potential.

## References

Aleer, S., Adetutu, E.M., Makadia, T.H., Patil, S., Ball, A.S., 2010. Harnessing the hydrocarbon-degrading potential of contaminated soils for the bioremediation of waste engine oil. Water Air Soil Pollution. doi:10.1007/s11270-010-0628-1.
Anderson, I.C., Parkin, P.I., 2007. Detection of active soil fungi by RT-PCR amplification of precursor rRNA molecules. Journal of Microbiological Methods 68, 248–253.
Anderson, I.C., Campbell, C.D., Prosser, J.I., 2003. Potential bias of fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. Environmental Microbiology 5, 36–47.
Bartha, R., 1986. Biotechnology of petroleum pollutant biodegradation. Microbial Ecology 12, 155–172.
Bento, F.M., Camargo, F.A.O., Okeke, B.C., Frankenberger, W.T., 2005. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmetation. Bioresource Technology 96, 1049–1055.
Cookson, J.T., 1995. Bioremediation Engineering: Design and Application. McGraw-Hill, New York.
Diplock, E.E., Mardlin, D.P., Killham, K.S., Paton, G.I., 2009. Predicting bioremediation of hydrocarbons, laboratory to field scale. Environmental Pollution 157, 1831–1840.
Eriksson, M., Dalhammar, G., Borg-Karlson, A.K., 2000. Biological degradation of selected hydrocarbons in an old PAH/creosote contaminated soil from a gas work site. Applied Microbiology and Biotechnology 53, 619–626.
Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soil. Applied and Environmental Microbiology 69, 1800–1809.
Gramss, G., Kirsche, B., Voigt, K.D., Gunther, T.H., Fritsche, W., 1999. Conversion rates of five polycyclic aromatic hydrocarbons in liquid cultures of fifty-eight fungi and the concomitant production of oxidative enzymes. Mycological Research 103, 1009–1018.
Guerin, T.F., 2000. The differential removal of aged polycyclic aromatic hydrocarbons from soil during bioremediation. Environmental Science and Pollution Research International 7, 19–26.
Hamamura, N., Fukui, M., Ward, D., Inskeep, W., 2008. Assessing soil microbial populations responding to crude-oil amendment at different temperatures

using phylogenetic, functional gene (*alkb*) and physiological analyses. Environmental Science and Technology 42, 7580–7586.
Harayama, S., Kasai, Y., Hara, A., 2004. Microbial communities in oil contaminated sea water. Current Opinion in Biotechnology 15, 205–214.
Harayama, S., Kishura, H., Kasai, Y., Shutsubo, K., 1999. Petroleum degradation in marine environments. Journal of Molecular Microbiology and Biotechnology 1, 63–70.
Hendrickx, B., Dejonghe, W., Faber, F., Boenne, W., Bastiaens, L., Verstraete, W., Top, E.M., Springael, D., 2006. PCR-DGGE method to assess the diversity of BTEX mono-oxygenase genes at contaminated sites. FEMS Microbial Ecology 55, 262–273.
Huesemann, M.H., 1995. Predictive model for estimating the extent of petroleum degradation in contaminated soils. Environmental Science and Technology 29, 7–18.
ISO/DIS 16703 GC-method, 2009. http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=39937.
Johnsen, A.R., Schmidt, S., Hybholt, T.K., Henriksen, S., Jacobsen, C.S., Andersen, O., 2007. Strong impact of polycyclic aromatic hydrocarbon (PAH)-degrading community of a PAH-polluted soil but marginal effect on PAH degradation when priming with bioremediated soil dominated by mycybacteria. Applied and Environmental Microbiology 73, 1474–1480.
Joo, H., Ndegwa, P.M., Shoda, M., Phae, C., 2008. Bioremediation of oil-contaminated soil using *Candida catenulata* and food waste. Environmental Pollution 156, 891–896.
Kowalchuk, G.A., van Os, G.J., van Aartrijk, J., van Veen, J.A., 2003. Microbial community responses to disease management soil treatments used in flower bulb cultivation. Biology and Fertility of Soils 37, 55–63.
Kyung-Hwa, B., Yoon, B., Kim, B., Cho, D., Lee, I., Oh, H., Kim, H., 2007. Monitoring of microbial diversity and activity during bioremediation of crude oil-contaminated soil with different treatments. Journal of Microbiology and Biotechnology 17, 67–73.
Lestan, D., Lamar, R., 1996. Development of fungal inocula for bioaugmentation of contaminated soils. Applied and Environmental Microbiology 62, 2045–2052.
Mancera-Lopez, M.E., Esparza-Garcia, F., Chavez-Gomez, B., Rodriguez-Vazquez, R., Saucedo-Casteneda, G., Barrera-Cortes, J., 2008. Bioremediation of an aged hydrocarbon-contaminated soil by a combined system of biostimulation–bioaugmentation with filamentous fungi. International Biodeterioration and Biodegradation 61, 151–160.
Maila, M.P., Cloete, T.E., 2004. Bioremediation of petroleum hydrocarbons through landfarming: are simplicity and cost-effectiveness the only advantages? Reviews in Environmental Science and Bio-Technology 3, 349–360.
Margesin, R., Labbe, D., Schinner, F., Greer, C.W., Whyte, L.G., 2003. Characterizaton of hydrocarbon-degrading microbial populations in contaminated and Pristine Alpine soils. Applied and Environmental Microbiology 69, 3085–3092.
Martin-Gil, J., Navas-Gracias, L.M., Gomez-Sobrino, E., Correa-Guimaraes, A., Hernandez-Navarro, S., Sanchez-Bascones, M., Ramos-Sanchez, M., 2008. Composting and vermicomposting experiences in the treatment and bioconversion of asphaltens from the prestige oil spill. Bioresource Technology 99, 1821–1829.
Muyzer, G., Waal, E., Uitterlinden, A., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of Polymerase Chain Reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59, 695–700.
Nagai, K., Suzuki, K., Okada, G., 1998. Studies on the distribution of alkalophilic and alkali-tolerant soil fungi II: fungal flora in two limestone caves in Japan. Mycoscience 39, 293–298.
National Environmental Protection Council, 1999. Assessment of site contamination measure. <http//soer.justice.tas.gov.au/2003/source/739/index.php>.
Pletsch, M., Araujo, B.S., Charlwood, B.V., 1999. Novel biotechnological approaches in environmental remediation research. Biotechnological Advances 17, 679–687.
Stallwood, B., Shears, J., Williams, P.A., Hughes, K.A., 2005. Low temperature bioremediation of oil contaminated soil using biostimulation and bioaugmetation with a *Pseudomonas* sp from maritime Antarctica. Journal of Applied Microbiology 99, 794–802.
Smits, T.H.M., Rothlisberger, M., Witholt, B., van Beile, J.B., 1999. Molecular screening for alkane hydroxylase genes in Gram-negative and Gram-positive strains. Environmental Microbiology 1, 307–317.
United States Environment Protection Agency, 2010. <http://www.clu-in.org/greenremediation/docs/GR_factsheet_biorem_32410.pdf> (accessed 07.10.10).
Unterbrunner, R., Wieshammer, G., Hollender, U., Felderer, B., Wieshammer-Zivkovic, M., Puschenreiter, M., Wenzel, W.W., 2007. Plant and fertiliser effects on rhizodegradation of crude oil in two soils with different nutrient status. Plant Soil 300, 117–126.
van Elsas, J.D., Duarte, G.F., Wolters, A.K., Smit, E., 2000. Analysis of the dynamics of fungal communities in soil in fungal-specific PCR of soil DNA followed by denaturing gradient gel electrophoresis. Journal of Microbiological Methods 43, 133–151.
Varanasi, P., Fullana, A., Sidhu, S., 2007. Remediation of PCB contaminated soils using iron nano-particles. Chemosphere 66, 1031–1038.
Vinas, M., Sabate, J., Espuny, M.J., Solanas, A.M., 2005. Bacterial community dynamics and polycyclic aromatic hydrocarbon degradation during bioremediation of heavily creseote-contaminated soil. Applied and Environmental Microbiology 71, 7008–7018.
Weber, R., Ridderbusch, D., Anke, H., 2002. 2,4,6–Trinitrotoluene (TNT) tolerance and biotransformation potential of microfungi isolated from TNT-contaminated soil. Mycological Research 106, 336–344.