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A polyphasic approach for assessing the suitability of bioremediation for the treatment of hydrocarbon-impacted soil

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HIGHLIGHTS

- Molecular/chemical tools were used to predict hydrocarbon bioremediation potential.
- Pyrosequencing showed microbial capacity for soil hydrocarbon degradation.
- Bioaccessibility assays predicted that total hydrocarbons would be reduced by <50%.
- Predictions were validated at pilot scale illustrating the success of this strategy.

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ABSTRACT

Bioremediation strategies, though widely used for treating hydrocarbon-contaminated soil, suffer from lack of biodegradation endpoint accountability. To address this limitation, molecular approaches of *alkB* gene analysis and pyrosequencing were combined with chemical approaches of bioaccessibility and nutrient assays to assess contaminant degrading capacity and develop a strategy for endpoint biodegradation predictions. In long-term hydrocarbon-contaminated soil containing 10.3 g C_{10}–C_{36} hydrocarbons kg^{-1}, 454 pyrosequencing detected the overrepresentation of potential hydrocarbon degrading genera such as *Pseudomonas*, *Burkholderia*, *Mycobacterium* and *Gordonia* whilst amplicons for PCR-DGGE were detected only with *alkB* primers targeting *Pseudomonas*. This indicated the presence of potential microbial hydrocarbon degradation capacity in the soil. Using non-exhaustive extraction methods of 1-propanol and HP-β-CD for hydrocarbon bioaccessibility assessment combined with biodegradation endpoint predictions with linear regression models, we estimated 33.7% and 46.7% hydrocarbon removal respectively. These predictions were validated in pilot scale studies using an enhanced natural attenuation strategy which resulted in a 46.4% reduction in soil hydrocarbon content after 320 days. When predicted biodegradation endpoints were compared to measured values, there was no significant difference ($P = 0.80$) when hydrocarbon bioaccessibility was assessed with HP-β-CD. These results indicate that a combination of molecular and chemical techniques that inform microbial diversity, functionality and chemical bioaccessibility can be valuable tools for assessing the suitability of bioremediation strategies for hydrocarbon-contaminated soil.

1. Introduction

Hydrocarbon pollution of the natural environment has well documented adverse effects on the abiotic and biotic components of the ecosystem (De La Huz et al., 2005; Kostka et al., 2011; Levy and Gopalakrishnan, 2010). Consequently, re-use of such polluted environments, or materials derived from these environments for other beneficial processes such as land redevelopment for residential or commercial purposes or as waste derived fill requires prior detoxification. There are different physical and chemical methods for detoxifying hydrocarbon polluted environments (Riser-Roberts, 1998), however, bioremediation strategies are preferred because of their lower cost and minimal impacts on the environment (Boopathy, 2000; Sanscartier et al., 2009).

Despite their widespread use (Aspray et al., 2007; Makadia et al., 2011; Mohn et al., 2001; Yergeau et al., 2012), the application of bioremediation strategies for the treatment of contaminated soil is

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not a guarantee for successful hydrocarbon removal. This is because hydrocarbon biodegradation can be impaired by a variety of factors such as soil type, pH, temperature, nutrient and contaminant availability (Boopathy, 2000; Leahy and Colwell, 1990; Nikolopoulou and Kalogerakis, 2009; Pollard et al., 1994). These factors therefore make it difficult to confidently predict the endpoints of hydrocarbon biodegradation (Diplock et al., 2009) prior to initiating bioremediation. As a result, developing strategies that can predict the efficacy of bioremediation especially in pilot scale studies is an imperative.

Two factors that are critical for developing this strategy are microbial capacity and edaphic (environmental) factors. Microbial capacity refers to the inherent ability of soil microbial groups such as bacteria and fungi, to degrade contaminating hydrocarbons. Knowledge of the available capacity is crucial in any degradation strategy. For example, evaluation of heterotrophic numbers, hydrocarbon degraders, soil respiration rates and response of developed microbial biosensors has been useful in predicting the efficiency and success of bioremediation (Diplock et al., 2009). Molecular tools such as functional gene based PCR, quantitative PCR and fingerprinting and sequencing from cloning are widely used in assessing microbial community structure and function (Erkelens et al., 2012; Muyzer et al., 1993). These tools can also be used to reveal the type and scope of microbial catabolic capacity in the environment and can be useful in strategy development as catabolic capacity may be correlated with actual biodegradation (Beller et al., 2002).

Less widely used for this purpose are high throughput shotgun sequencing methods such as those on the Illumina and 454 platforms (Kostka et al., 2011; Mason et al., 2012; Yergeau et al., 2012). These platforms allow for the provision of detailed information on the taxonomy and metabolic potential of microbial communities in contaminated environments (Mason et al., 2012; Yergeau et al., 2012). Whilst traditional PCR based tools provide information on a target group of microorganisms such as either bacteria or fungi, high throughput sequencing methods give more detailed information on the different microbial capacities available with genomic DNA or amplicon based sequencing. They are therefore more effective in assessing microbial catabolic capacities than PCR based methods.

Since the expression of microbial capacities is influenced by a variety of factors especially contaminant bioavailability, the concentration of substrates available for microbial interactions is crucial in the assessment of contaminant biodegradability. Assays that can predict bioavailable fractions have the potential to reduce the time and costs associated with bioremediation (Dandie et al., 2010). Different chemical assays may be used to quantify biodegradable contaminant fractions from the soil matrix (bioaccessible fraction). These include non-exhaustive extraction techniques with 1-butanol and 1-propanol (Semple et al., 2007) and chemical oxidation by potassium persulfate (Dandie et al., 2010; Semple et al., 2007). Hydroxypropyl-β-cyclodextrin (HP-β-CD) extraction which provides a good estimate of bioaccessible hydrocarbon fractions has been successfully used to predict the efficacy of hydrocarbon degradation in aged contaminated soils (Dandie et al., 2010). Linear relationships have been established between biodegradable hydrocarbon fractions (at concentrations <10 g kg^{-1}) and HP-β-CD extractable hydrocarbons (Diplock et al., 2009).

Therefore, the aim of this study was to utilise molecular and chemical assays to predict the potential performance of bioremediation strategies for the treatment of weathered hydrocarbon-contaminated soil. This was performed by using a combination of PCR-DGGE (*alkB*), 454 pyrosequencing methods to assess microbial hydrocarbon degrading potential and bioaccessibility assays to predict contaminant degradation endpoints during bioremediation. Endpoint predictions were then compared to hydrocarbon biodegradation data from pilot scale trials to determine the suitability of the molecular and chemical bioremediation pre-screening approach for predicting hydrocarbon biodegradation efficacy.

2. Materials and methods

2.1. Hydrocarbon-contaminated soils

Hydrocarbon contaminated soils were obtained from a former oil refinery site in Australia. Historically contaminated soil (~500 kg) was collected from multiple points on the stockpiled material on-site. These samples were then mixed together to generate a bulk sample which was used for physico-chemical and microbial analysis. Bulk soils (<2 mm) had an initial hydrocarbon concentration of 10.3 ± 0.6 g kg^{-1} (C_{10}–C_{36}) with the concentration of various equivalent hydrocarbon molecular weight ranges and other soil properties being listed in Table 1. Determination of soil type, soil moisture content, water holding capacity, pH and organic matter content were performed using standard methods (Rowell, 1994).

2.2. DNA extraction

DNA was extracted from soil samples using two commercially available kits. PowerSoil (MoBio laboratories Carlsbad, CA, USA) was used to extract genomic DNA used for PCR based DGGE analysis whilst PowerMax® Soil DNA Isolation Kit (MoBio laboratories, Carlsbad, CA, USA) was used for genomic DNA extraction for 454 pyrosequencing. In both cases, the extraction processes were carried out according to the manufacturer's protocol.

2.3. PCR and DGGE

PCR amplification of *alkB* genes was performed using different primer sets targeting different phylogenetic groups. The primer sets TS2S, DEG1RE and DEG1RE-GC (Smits et al., 1999) and P1f485, P1r851 and P1r851GC (Hamamura et al., 2008) were used to target *Pseudomonas oleovorans* GPo1 and *Pseudomonas aeruginosa* groups respectively in semi-nested PCR reactions. Amplicons from the first PCR (for example TS2S and DEG1RE) reactions were used as templates for the second reaction (TS2S and DEG1RE-GC). The *AlkB* group in the lineage of *Rhodococcus* spp were also evaluated with R1f438, R1r835 and R1r835GC whilst those belonging to *Burkholderia* and *Acinetobacter* were assessed with BCf577, BCr837 and BCr837GC and Acf532, Acr872 and Acr872GC respectively via semi-nested PCR reactions (Hamamura et al., 2008). DGGE was carried out on selected PCR amplicons on a Universal Mutation Detection System D-code apparatus (Biorad, CA, USA) with a 9% polyacrylamide gel using a 45–60% denaturing gradient at 60 °C for 20 h. DGGE gels were silver stained (Girvan et al., 1993), scanned and saved as Tiff files with an Epson V700 scanner. Digitised gel images were then analysed with TotalLab analysis package (Nonlinear Dynamics, USA). Unweighted Pair Group with Mathematical Averages (UPGMA) dendrograms were then generated with Total lab.

Table 1
Hydrocarbon concentration in contaminated soil at the start of pilot scale treatment and following 320 days of natural attenuation and enhanced natural attenuation.

Hydrocarbon fraction	Initial hydrocarbon concentration (mg kg^{-1})[a]	Final hydrocarbon concentration (mg kg^{-1})[b]	
		NA	ENA
C_{10}–C_{14}	119 ± 34	<50	<50
C_{15}–C_{28}	4759 ± 91	4665 ± 162	2520 ± 85
C_{29}–C_{36}	5350 ± 382	4580 ± 103	2935 ± 79
C_{10}–C_{36}	10185 ± 629	9245 ± 163	5455 ± 77

[a] The initial hydrocarbon concentration is the average of time zero values for each of the biopiles.
[b] Residual concentration at the end of the treatment period (320 days).