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Assessing impediments to hydrocarbon biodegradation in weathered contaminated soils

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HIGHLIGHTS

- Impediments to TPH degradation were assessed using chemical and molecular assays.
- High TPH concentrations (68.9 g kg^{-1}) affected *alkB* bacterial community diversity.
- The lack of TPH degradation following ENA was due to limited TPH bioavailability.
- The presence of *alkB* genes does not always guarantee bioremediation success.

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ABSTRACT

In this study, impediments to hydrocarbon biodegradation in contaminated soils were assessed using chemical and molecular methodologies. Two long-term hydrocarbon contaminated soils were utilised which were similar in physico-chemical properties but differed in the extent of hydrocarbon (C_{10}–C_{40}) contamination (S1: 16.5 g kg^{-1}; S2: 68.9 g kg^{-1}). Under enhanced natural attenuation (ENA) conditions, hydrocarbon biodegradation was observed in S1 microcosms (26.4% reduction in C_{10}–C_{40} hydrocarbons), however, ENA was unable to stimulate degradation in S2. Although eubacterial communities (PCR-DGGE analysis) were similar for both soils, the *alkB* bacterial community was less diverse in S2 presumably due to impacts associated with elevated hydrocarbons. When hydrocarbon bioaccessibility was assessed using HP-β-CD extraction, large residual concentrations remained in the soil following the extraction procedure. However, when linear regression models were used to predict the endpoints of hydrocarbon degradation, there was no significant difference ($P > 0.05$) between HP-β-CD predicted and microcosm measured biodegradation endpoints. This data suggested that the lack of hydrocarbon degradation in S2 resulted primarily from limited hydrocarbon bioavailability.

1. Introduction

Hydrocarbon pollution of the natural environment is an important issue which has been exacerbated by increasing global demands for fossil fuel by emerging economies. Associated with this demand is greater exploration of new and existing world oil resources and increased risks of hydrocarbon pollution with such pollution having well documented adverse effects on the natural environment and severe economic consequences [1–3]. There are many methods for treating hydrocarbon contaminated environments for toxicity mitigation. These include incineration, stabilisation and solidification, thermal desorption and various bioremediation approaches including land farming, composting, biostimulation and bioaugmentation [4–6]. Bioremediation is considered a more suitable and widely accepted treatment technology for hydrocarbon contaminated environments (such as soil) because of its environmentally friendly nature (less intrusive) and comparatively cheaper cost [4,7].

The success of any bioremediation strategy is dependent on a variety of environmental and biological parameters. Factors such as temperature, pH, nutrients, and available microbial capacity can limit hydrocarbon removal from contaminated soils [8–10]. Other

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factors such as contaminant bioavailability/bioaccessibility, type and chain length of contaminating hydrocarbons can also affect the efficiency of the bioremediation process [4,11–13]. Therefore, the challenge in bioremediation is to determine how any of these factors could potentially impede the success of a chosen bioremediation strategy.

Data obtained from chemical analysis (such as contaminant bioavailability) can be useful for predicting the success or failure of a bioremediation strategy. However, assessment of microbial capacity, in concert with contaminant bioavailability, could provide a more reliable approach for predicting bioremediation efficacy. While hydrocarbon degrading microorganisms are ubiquitous, they may not always be present in sufficient numbers to achieve the desired contaminant reduction level [12]. Knowledge of available capacity would thus be invaluable in bioremediation. For example, the assessment of bacterial hydrocarbon degraders and heterotroph populations in addition to concentration of bioavailable hydrocarbon fractions has been useful in predicting the success of bioremediation [14].

Numerous biological methods can be used to assess soil microbial hydrocarbon degrading capacity. Labelled hydrocarbons may be used to assess hydrocarbon mineralisation rates in laboratory based soil microcosms [7–15]. This can provide useful information on microbial hydrocarbon mineralisation capacity under different environmental conditions (e.g. pH, temperature) and following nutrient amendment. Culture based methods may also be used to determine microbial composition and potential function in soils [14]. Inherent limitations of culture based methods (biased towards culturable microbial groups) may be overcome by the use of high resolution molecular tools which target both culturable and non culturable microbial groups. These include PCR, Quantitative PCR, molecular community fingerprinting (DGGE and TGGE), cloning and sequencing, microarray and next generation sequencing [6,16–18].

The use of molecular tools (especially when used to target hydrocarbon degrading genes) provides useful information on hydrocarbon degrading potential in different contaminated soils or sites prior to and during bioremediation [19,20]. Specific catabolic genes such as *alkB* (alkane degrading genes), *bssA* (toluene and ethylene) and *NidA* (pyrene) may be targeted using specific primers and quantified using DGGE and Quantitative PCR [8,15,20–22]. Higher concentrations of some of these genes have been linked to greater hydrocarbon removal. For example environments with elevated initial *bssA* levels have subsequently shown to have the greatest toluene degradation potential [21] while a reduction in hexadecane mineralisation has been associated with the inhibition of *alkB* genes [15].

Therefore, the aim of this study was to utilise both chemical and molecular methodologies to assess the potential and performance of bioremediation for the treatment of weathered hydrocarbon-contaminated soil. This was performed using ^{14}C-labelled hydrocarbons, bioaccessibility assays and by targeting *alkB* degrading genes in laboratory based soil microcosms.

2. Materials and methods

2.1. Hydrocarbon-contaminated soils

Hydrocarbon contaminated soils were sampled from a former oil refinery site in Australia. Historically contaminated soils (~30 kg) were collected from stockpiled material on-site with bulk soil samples being collected from the top 20 cm of the stockpile. Bulk soils (<2 mm) had an initial hydrocarbon concentration of 16.5 g kg^{-1} (C_{10}–C_{40}: soil S1) and 68.9 g kg^{-1} (C_{10}–C_{40}: soil S2): the concentration of various equivalent hydrocarbon molecular weight ranges and other soil properties are listed in Table 1. Determination of soil type was carried out using the methodology described by McDonald et al. [23] while soil moisture content, water holding capacity, pH and organic matter content were determined using standard methods.

Table 1
Physico-chemical characteristics of soil used in this study.

Property	Soil S1	Soil S2
Soil type	Sandy loam	Sandy loam
Sand, silt, clay (%)	70, 14, 16	68, 15, 17
Bulk density (g cm^3)	1.5	1.5
Moisture content (%)	17.0 ± 0.2	9.3 ± 0.2
pH (1:5, water)	7.2 ± 0.1	7.6 ± 0.1
pH (1:5, $CaCl_2$)	6.8 ± 0.2	7.0 ± 0.2
Organic matter (%, LOI)	14.7 ± 0.7	20.4 ± 0.9
Nitrate (mg kg^{-1})	<2.0	<2.0
Phosphate (mg kg^{-1})	<2.0	<2.0
Sulphate (mg kg^{-1})	480	270
Hydrocarbon fractional range (mg kg^{-1})		
C_{10}–C_{14}	938 ± 704	6146 ± 165
C_{15}–C_{29}	9327 ± 69	44,558 ± 414
C_{29}–C_{36}	5121 ± 120	16,328 ± 225
C_{37}–C_{40}	1066 ± 406	1823 ± 137

2.2. Mineralisation assays

Mineralisation of ^{14}C-hexadecane by indigenous soil microorganisms in hydrocarbon-contaminated soil was determined in replicate biometer flasks (Bellco Glass). Contaminated soil (50 g moistened to 60% water holding capacity) was supplemented with 1.0 μCi of ^{14}C-hexadecane and mineralisation (evolution of $^{14}CO_2$) monitored for up to 98 days (timeframe based on pilot investigations; data not shown). The effect of nutrient amendments on the enhancement of ^{14}C-hexadecane mineralisation was investigated by the addition of nitrogen and phosphorus to effect a C:N:P molar ratio of 100:10:1. Control flasks, to assess abiotic hexadecane mineralisation, consisted of contaminated soil (±amendments) to which 1% $HgCl_2$ was added. Soils were incubated at 24 ± 2 °C and the evolution of $^{14}CO_2$ (trapped in 1 M NaOH) monitored routinely over the incubation period. Aliquots (1 ml) from $^{14}CO_2$ traps were combined with scintillation cocktail (ReadySafe, Beckman-Coulter, USA) and the samples were counted and quantified by liquid scintillation counting (Beckman LS3801) using standard counting protocols and automatic quenching correction [24].

2.3. Soil microcosms

Biodegradation experiments were conducted in 2 l glass microcosms (Silverlock packaging, Dudley Park, SA, Australia) containing ~800 g of moist soil. For all treatments, the soil–water content was adjusted to ~60% water holding capacity and maintained throughout the experiment on a weight basis with additional distilled water added if required. For natural attenuation experiments, no additional amendments were added to soil microcosms, however, for enhanced natural attenuation (ENA) treatments, nutrients ($(NH_4)_2SO_4$, KH_2PO_4 and K_2HPO_4) were added to each soil to achieve a C:N:P molar ratio of 100:10:1, based on hydrocarbon loading as a measure of C. Control soils, to assess the extent of abiotic hydrocarbon removal over the incubation period were prepared by supplementing soils with mercuric chloride (1% w/w). Microcosms were incubated at 24 ± 2 °C over a 98 day treatment period with samples removed at 0, 7, 14, 21, 42, 70 and 98 days for the assessment of hydrocarbon concentration. Both natural attenuation and ENA microcosms were aerated on a weekly basis. Soil samples were stored at −20 °C prior to extraction and analysis of hydrocarbon concentration.

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