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Insights into the biodegradation of weathered hydrocarbons in contaminated soils by bioaugmentation and nutrient stimulation

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HIGHLIGHTS

- Improved biodegradation of weathered hydrocarbon residues.
- Nutrient addition is a key parameter for promoting biodegradation.
- Soil grinding reduced effectiveness of biostimulation.
- Reduction of petroleum hydrocarbons in soil not directly correlable to reduction in toxicity.

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ABSTRACT

The potential for biotransformation of weathered hydrocarbon residues in soils collected from two commercial oil refinery sites (Soil A and B) was studied in microcosm experiments. Soil A has previously been subjected to on-site bioremediation and it was believed that no further degradation was possible while soil B has not been subjected to any treatment. A number of amendment strategies including bioaugmentation with hydrocarbon degrader, biostimulation with nutrients and soil grinding, were applied to the microcosms as putative biodegradation improvement strategies. The hydrocarbon concentrations in each amendment group were monitored throughout 112 days incubation. Microcosms treated with biostimulation (BS) and biostimulation/bioaugmentation (BS + BA) showed the most significant reductions in the aliphatic and aromatic hydrocarbon fractions. However, soil grinding was shown to reduce the effectiveness of a nutrient treatment on the extent of biotransformation by up to 25% and 20% for the aliphatic and aromatic hydrocarbon fractions, respectively. This is likely due to the disruption to the indigenous microbial community in the soil caused by grinding. Further, ecotoxicological responses (mustard seed germination and Microtox assays) showed that a reduction of total petroleum hydrocarbon (TPH) concentration in soil was not directly correlable to reduction in toxicity; thus monitoring TPH alone is not sufficient for assessing the environmental risk of a contaminated site after remediation.

1. Introduction

Land contamination from poor historical industrial practices or incidents is a widespread and well recognised environmental issue. In the UK alone, it has been estimated that ca. 300,000 ha of land could be affected by industrial activity leading to contamination (Environment Agency, 2009). Petroleum hydrocarbons are one the most common contaminant, though a wide range of chemicals may be present (Towell et al., 2011). Once released into the environment, petroleum hydrocarbons are subject to abiotic and biotic weathering reactions e.g. physical and biochemical transformations, interactions with soils, that will change their composition and toxicity, and will influence their fate and biodegradation (Brassington et al., 2007; Stroud et al., 2007; Maletić et al., 2011). The extent of these transformations will vary according to the type of petroleum products present, the soil conditions (e.g. organic

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matter content, soil grain size and clay-type at the sites) (Stroud et al., 2007), and the bioavailability and susceptibility of the different compounds (Stroud et al., 2007; Maletić et al., 2011).

Bioremediation has become the preferred method for the remediation of petroleum hydrocarbon contaminated soils, because it is considered cost effective and sustainable, and can accelerate naturally occurring biodegradation processes through the optimisation of limiting parameters (Vidali, 2001; Coulon et al., 2012). To be effective, it is important to investigate and understand all factors (e.g. soil and contaminant characteristics, bioavailability, stage of weathering) that might effect the efficacy of the process. For example, aliphatic hydrocarbons of intermediate length (ranging between C_{10} and C_{25}) tend to be readily degradable by microorganisms despite their low solubility, whereas longer chain alkanes (C_{25}-C_{40}), especially those with branched or cyclic chain structures, are more resistant to biological degradation (Maletić et al., 2011).

Heavily weathered hydrocarbons are difficult to biodegrade and have relatively low toxicity, but high residual concentrations can severely alter the physical and chemical properties of the soils, thus reducing soil fertility (Coulon et al., 2012; Wu et al., 2013). Remediation outcomes using biological methods for the treatment of weathered hydrocarbons are often unpredictable, and in some instances contaminated soil may be regarded as 'untreatable' via bioremediation (Brassington et al., 2007). Debate around the benefits of bioaugmentation and its capacity to increase the microbial degradation of weathered hydrocarbons after indigenous microorganisms are no longer effective continues, and only a few studies demonstrate continued biodegradation after introduction of specific hydrocarbon degraders (Coulon et al., 2012; Gallego et al., 2001). Biodegradative performance via bioaugmentation can be further improved by the addition of appropriate nutrients; a process referred to as biostimulation (Xu and Lu, 2010). Due to the limited number of studies on the subject, and the complexity of weathered petroleum hydrocarbon products, there is a need for investigation into the potential for bioaugmentation coupled with biostimulation to enhance biotransformation and reduce residual toxicity.

In this study, we investigated the potential for biotransformation of weathered hydrocarbon residues in soil. To do so, we determined whether it was possible to improve biodegradation with the simultaneous application of bioaugmentation and biostimulation on two soil types. Soil A was taken from a windrow where bioremediation had been completed and soil B was taken from a site prior remediation where oil drums had leaked contaminating the soil. Soil A treatment was deemed completed as no further degradation could be achieved. This research provides valuable knowledge concerning chemical and toxicological changes on a soil type not previously investigated and could be used to support the development of bioremediation strategies. Finally, we discuss the relationship between chemical change, toxicity, and total petroleum hydrocarbons (TPH) measurements in the context of risk assessment, highlighting the effects that remediation might have on soil toxicity.

2. Materials and methods

2.1. Physical and chemical characterisation of soil samples

Two different soils collected at a depth of 5–20 cm from two commercial oil refinery sites located in the UK were labelled A and B (to maintain owner anonymity). Soil A is a sandy soil which was heavily contaminated with weathered hydrocarbons (TPH = 50,000 mg kg^{-1}). After 6 month windrow treatment, TPH concentration was decreased to 22,700 mg kg^{-1} where it was believed no further degradation was possible. Soil B is predominantly clay soil contaminated with weathered hydrocarbons (TPH = 31,500 mg kg^{-1}) taken from a more recently contaminated site where there was no history of any remedial activity. The soils were air-dried for 24 h and sieved through 2 mm mesh to remove stones, plant material, and to facilitate mixing. Prior to air drying the field moisture content was determined in triplicate by oven drying at 105 °C for 24 h. Soils were then stored at 4 °C in the dark before use.

For both soil samples, a routine set of characterisation was carried out. Soil pH was measured using a pH meter (Jenway 3540) in a distilled water slurry (one part soil: two parts water) after a 30 min equilibration period (Black, 1965). Maximum water holding capacity (WHC) was determined in duplicate by flooding the wet weight equivalent of 100 g of dry soil in a filter funnel and allowing it to drain overnight (Black, 1965). Particle size analysis was performed by a combination of wet sieving (sand) and sedimentation (silt and clay), as described by Gee and Baude (1986). The organic matter content as indicated by loss on ignition (LOI) of each soil was measured by combustion at 450 °C in a furnace for 24 h, according to ASTM Method D297487. Total organic carbon (TOC) was analysed by potassium dichromate oxidation, as described by Schnitzer (1982).

For nitrate, phosphate, and ammonium determination, 10 g of soil was first extracted in 0.5 M potassium bicarbonate (adjusted to pH 8.5). The extractant was then analysed by high-performance liquid chromatography (HPLC) for nitrate and phosphate as described by Brenner and Mulvaney (1982) and Olsen and Sommers (1982), respectively. Ammonium was analysed using the colorimetric test described by Reardon et al. (1966).

2.2. Microcosm experiment design

Soil microcosms were established using 700 g of either soil A and B in sterile 1 L, wide-mouth amber glass jars. Four different microcosm conditions for each soil were established and tested in triplicate (Table 1). Soil grinding was done using mortars and pestles made from hard chemical-porcelain ware. The mortars had a lip and were glazed on the outside. The pestles were glazed to the grinding surface. Soil aliquots of about 70 g were ground for about 15 min in the mortar with the pestle to pass the soil through a 42.5 μm sieve. The ground soil aliquots were then combined for additional sample preparation. Nutrients were added in the form of ammonium nitrate and potassium orthophosphate to obtain a C:N:P ratio of 100:1:0.1. The hydrocarbon-degrading inoculum was composed of three bacterial isolates supplied by Remedios Limited (Aberdeen). The bacterial isolates were isolated from an attenuated enrichment culture from No.6 oil impacted soil (Alzahrany, 2011). Two bacterial isolates were related to Pseudomonas sp. (100% match) and one to Klebsiella sp. (99% match). Inoculum was grown using a minimal medium supplemented with diesel (equivalent to 5.5 mg C l^{-1}) as carbon source. The cell concentration added to each microcosm was such as to give 5×10^7 CFU g^{-1} soil. For each amendment, a few woodchips were added to 10 ml of Bushnell-Haas broth supplemented with 1 g l^{-1} salicylic acid and 1% ethanol, adjusted to pH 7. The mixture was placed in an orbital shaker at 150 rpm in the dark at 20 °C and left overnight, after which 1 ml was added to 100 ml of fresh medium and grown to a stationary phase (about 24 h, checked by optical density readings at 600 nm). The cell number at stationary phase was 10^8 cells ml^{-1}. The inoculum solution was then added to the soils at 0.01 ml g^{-1} dry wt soil to achieve 10^6 cells g^{-1} dry wt soil. The moisture content of each microcosm was adjusted to 80% of the soil's water holding capacity using deionised water. The microcosms were incubated in the dark at 15 °C. High humidity was