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Large scale bioaugmentation of soil contaminated with petroleum hydrocarbons using a mixed microbial consortium

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ABSTRACT

Singapore is a highly industrialised country with an important oil industry resulting in a significant environmental impact, creating vast amounts of hydrocarbon waste that needs to be remediated. Here we report on the commercial scale bioremediation of a soil contaminated with processed petroleum hydrocarbons at a petroleum facility located offshore from Singapore. Initial laboratory results indicated the efficacy of an adapted consortium in terms of degradation of petroleum hydrocarbons, with complete degradation of the contaminants occurring after 21 days. Microbial counts confirmed a bacterial increase during the degradation and an ecotoxicological assessment using *Brassica rapa* confirmed a reduction in the toxicity of the treated soil. In field studies for example, initial processed petroleum hydrocarbon concentrations of 26,240; 622,657; and 978,399 mg kg^{-1} in 250 tonnes of soil were degraded to <1000 mg kg^{-1} in 9, 12 and 17 weeks, respectively following the bioaugmentation treatment. A 100% germination rate of *Brassica rapa* was also observed in the field for all the treated soil batches except for soils further contaminated with oil tank bottom sludge. Moreover, further total petroleum hydrocarbons (TPH) analysis performed 3 years after the treatment confirmed that their concentrations have remained low (7–35 mg kg^{-1}) all over the site. To the author's knowledge, this is among the first studies to report large scale successful bioaugmentation of TPH-contaminated soil in Singapore.

1. Introduction

Singapore is one of the world's busiest ports and has the largest market for bunker fuel in the world. In addition, it has a significant petrochemical industry which brings in large quantities of crude oil and processed petroleum hydrocarbons (TPH) which are stored and processed into other products (Bai and Susanto, 2002). These industries generate substantial quantities of polluting waste by-products with implications for the environment given that Singapore is a city state with a small landmass and limited natural resources (Hamilton-Hart, 2012).

Waste management in Singapore relies on two core technologies: landfilling and incineration, where priority is given to all non-recovered, reused or recycled solid waste (Tan and Khoo, 2006). These pressures coupled with the very large quantities of TPH contaminated materials, including soil that has been generated by the petrochemical and shipping industry in Singapore make alternative technologies that require neither incineration nor the use of sanitary landfill space very attractive.

The bioremediation technology or the use of microorganisms to degrade petroleum hydrocarbons (monitored natural attenuation, biostimulation and bioaugmentation) to concentrations accepted by environmental agencies around the world provides an attractive cost effective alternative (Sheppard et al., 2011, Aburto et al., 2009, Aburto-Medina et al., 2012, Shahsavari et al., 2015).

There have been several studies comparing the efficacy of different bioremediation approaches including natural attenuation, biostimulation and bioaugmentation of TPH contaminated soil (Bento et al., 2005, Mancera-López et al., 2008, Xu and Lu, 2010).

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Natural attenuation relies on the catabolic potential of the indigenous microorganisms for reducing the mass and concentration of the contaminants while biostimulation aims to enhance those indigenous microbial communities with nutrients such as phosphates and nitrates. Bioaugmentation involves the introduction of adapted microorganisms capable of degrading the contaminants. The introduced microorganisms may be derived from a consortium already proven to degrade the contaminants or they may be isolated from the original contaminated environment and later adapted to be reintroduced.

The general finding has been that bioaugmentation in combination with biostimulation usually provides a more efficient rate of bioremediation than biostimulation on its own with a variety of hydrocarbon pollutants across a wide range of conditions (Calvo et al., 2009, Coulon et al., 2010a, Kauppi et al., 2011, Łebkowska et al., 2011, Grace Liu et al., 2011, Sheppard et al., 2011, Zhao et al., 2011).

In the present study we tested the inoculation of an adapted indigenous consortium (indigenous bioaugmentation treatment) with biostimulation into weathered soil heavily contaminated with hydrocarbons, specifically with bunker sludge, diesel and gasoline following successful laboratory studies. At high levels of contamination (>700,000 mg kg^{-1}) the soil and hydrocarbons formed a slurry. The rationale for the selection of an indigenous adapted consortium was based on the reduced likelihood that the consortia would be outcompeted by the natural microbial community within the soil slurry. The treatments were applied on a commercial scale with the aim to reduce the TPH concentrations to below 1000 mg kg^{-1}. Moreover, phytotoxicity assays with *Brassica rapa* were performed in order to confirm the reduced toxicity.

Thus, we suggest that bioaugmentation with an adapted consortium represents a low cost effective treatment for the bioremediation of commercial scale petroleum-contaminated soils.

2. Materials and methods

2.1. Site

The contaminated soil was generated from a petroleum facility in Singapore.

2.2. Bioaugmentation

The bioaugmentation treatment consisted of the addition of 22 bacterial strains (Table 1).

Each of the aerobic bacterial strains was systematically isolated from a biological trickling filter (biofilter) collected from an operational wastewater treatment plant (WWTP) located within a petroleum facility. The isolation procedure was as previously reported (Zhao et al., 2011). The wastewater contained high phenol concentrations (>3,000 mg L^{-1}), petroleum hydrocarbons and high chemical oxygen demand (COD) values (>10,000 mg L^{-1}). The strains were originally isolated to establish a collection of bacterial cultures capable of phenol degradation (Banerjee and Ghoshal, 2010, Singh et al., 2013). These cultures were isolated using a positive end dilution approach to capture part of an ecological community that constituted the dominant indigenous microorganisms able to survive and persist in an environment with high concentrations of TPHs. These strains were tested for TPH degradation because they were adapted to high TPH concentrations but also because they were isolated from the original wastewater that is contaminated with a wide range of compounds such as phenol and TPHs. The isolated cultures were identified via Gram staining, Microbac^{TM}, API kits (bioMérieux) (Bouchez Naïtali et al., 1999) and Sanger sequencing as previously described (Aburto and Ball, 2009).

2.3. Inoculum preparation

Pure bacterial strains retrieved from Microbank^{TM} vials were cultured overnight in 50 mL of nutrient broth and later mixed into a 1 L baffled flask for a further overnight incubation (35 °C) to create the desired consortium ready for the laboratory experiments.

A modified protocol (from the one used in the laboratory studies) was used for the translation and scale-up due to the requirement of large volumes. Pure strains were cultured in nutrient agar, and then incubated overnight at 37 °C in 50 mL of production broth (PB) media (glucose 10 g, yeast extract 8 g, and NaCl 5 g per L) with agitation (150 rpm). These were then used to inoculate 500 mL of PB media that was incubated for 24–48 h at 37 °C with agitation (500 rpm). Each pure culture was then mixed with 500 mL of phosphate buffer and all cultures were pooled together in a Braun fermenter which was stirred (150 rpm) for 5 min and aerated for better mixing. The consortium was then ready for use and stored in 4 L carboys at 4 °C until required. while that for the translation and scale-up for the 250 tonnes of soil bioreactor pit was approximately 0.1% for each batch once every 3 weeks over 9, 12 or 17 weeks according to the initial TPH concentration.

2.4. Laboratory experiments

Preliminary laboratory experiments were conducted to test the efficacy of the bacterial consortium used in the bioaugmentation of oily TPH contaminated soil slurry (>700,000 mg kg^{-1}) and oil contaminated soil (<700,000 mg kg^{-1}) to reduce the TPH content. The bench scale experiments were based on materials sourced from a stockpile of aged and weathered TPH contaminated soil at the petrochemical facility.

A 5 kg polyethylene bag of weathered TPH contaminated soil that included bunker fuel, diesel and gasoline was sourced from a stockpile of approximately 2,500 plus metric tonnes, stacked to a height of approximately 3 m. There was no covering above the exposed materials or lining below at the site of the stockpile. Approximately 5 kg of the TPH contaminated soil was placed into a large plastic tray and mixed repeatedly. The soil was then divided into plastic containers (500 g each) for use in the bench scale work performed at the laboratory as described below.

A combination of bioaugmentation (addition of microbial consortium) and biostimulation was applied to four lots of TPH contaminated soil labelled as A and C accordingly, each containing approximately 500 g. The application of the bacterial consortium was applied as Treatment A (in duplicate), while the application of sterile water combined was applied as Treatment C (control), in duplicate. The treatment regime involved an injection of 1.0% (v/v) inoculum (or sterile water in the case of the control) once a week for a period of 4 weeks. Soils were incubated at 30 °C to emulate the field temperature at the industrial site as in previous studies with periodic manual agitation (Chemlal et al., 2012, Diplock et al., 2009). Sampling was performed every week after treatment by taking 50 g of sample per lot and stored at 4 °C.

2.5. Field experiments

Approximately 250 metric tonnes of materials were excavated from the stockpile of aged and weathered TPH contaminated soil and transported into a pit specially prepared with an aeration grid (Fig. 1). An existing grease pit was used as the bioremediation reactor pit. The dimensions of the bioreactor were 11 m × 20 m × 1 m, similar to that used by Łebkowska et al. (2011); however field conditions at the island site were not conducive for windrowing due to issues with labour and a lack of suitable equipment. Leachate of TPH was prevented from entering into the groundwater by using an old grease pit with a concrete base coated with epoxy paint as