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Bioaugmentation and biostimulation of hydrocarbon degradation and the microbial community in a petroleum-contaminated soil

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ABSTRACT

Nutrient additions can stimulate petroleum hydrocarbon degradation, but little is known about how these additions affect the microbial community involved in that degradation. A microcosm study was conducted to assess the impact of bioaugmentation with *Acinetobacter* SZ-1 strain KF453955 and biostimulation with nutrients nitrogen and phosphorus on petroleum hydrocarbon degradation efficiency and microbial community dynamics during bioremediation of an oil-contaminated soil. Soils were incubated without shaking at room temperature for 10 weeks, and petroleum hydrocarbon degradation efficiency, catalase activity, petroleum hydrocarbon degrader population, and bacterial community diversity were determined. Results showed biostimulation and bioaugmentation, respectively, promoted 60% and 34% degradation of the total petroleum hydrocarbons (TPH) after six weeks of incubation. A degradation plateau occurred in the seventh week. Catalase activity and the populations of oil degraders in soil were generally greater for biostimulation than for bioaugmentation. The inoculants survived into the seventh week for the bioaugmentation treatment, and bacterial diversity did not increase by biostimulation. The populations of TPH-degraders in soil were positively related to TPH degradation efficiency during bioremediation of petroleum-polluted soils.

1. Introduction

Petroleum hydrocarbons are composed of complicated mixtures of non-aqueous and hydrophobic components such as n-alkane, aromatics, resins and asphaltenes. Due to the adverse impact of these chemicals on human health and the environment, they are classified as priority environmental pollutants by the US Environmental Protection Agency (US EPA, 1986).

Oil pollution accidents have become a common phenomenon and have caused ecological and social catastrophes (Snape et al., 2001). When the oil spill accidents occur on land, degradation of petroleum hydrocarbons by indigenous microorganisms is often a slow process due to low microbial population and activity (Cerqueira et al., 2014; Abed et al., 2015). It is therefore a challenge to find an effective and efficient method to remediate these polluted soils.

Among a variety of remediation methods, bioremediation has been recognized as a cost-effective clean-up technology to treat oil-polluted soils and sediments (Al-Mutairi et al., 2008; Cerqueira et al., 2014). There are two main types of bioremediation technologies-bioaugmentation and biostimulation (Lladó et al., 2012; Simarro et al., 2013). Bioaugmentation involves inoculating exogenous degrading microorganisms to the soil (Ruberto et al., 2003; Maria et al., 2011; Taccari et al., 2012; Wu et al., 2013). Biostimulation stimulates the degrading capacity of the indigenous community by adding nutrients to avoid metabolic limitations (Yu et al., 2005; Kauppi et al., 2011; Sayara et al., 2011; Taccari et al., 2012; Abed et al., 2014). Currently, many reports have shown that bioaugmentation and biostimulation enhance the biodegradation of hydrocarbons in oil-polluted soil (Ruberto et al., 2003; Mancera-López et al., 2008; Tahhan et al., 2011; Taccari et al., 2012; Suja et al., 2014). However, the effects of bioaugmentation and biostimulation

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are case specific, and results are inconsistent and vary with inoculants and nutrients (Mancera-López et al., 2008; Abed et al., 2014; Suja et al., 2014). In general, it is considered that bioaugmentation and biostimulation are very promising methods for remediation of oil-contaminated soil.

Because the process of bioremediation is carried out by various microorganisms in soil, understanding how bioaugmentation and biostimulation influence the populations of hydrocarbon degraders, the diversity and activity of the microbial community, and the ability of inoculants to adapt to new environmental conditions are very important in ensuring effective bioremediation of petroleum-contaminated soil (Kaplan and Kitts, 2004; Kauppi et al., 2011; Liu et al., 2012; Taccari et al., 2012). However, little research has been conducted to investigate the relationship between petroleum hydrocarbon degradation efficiency and microbial community dynamics and microbial activity (Ruberto et al., 2003; Colombo et al., 2011; Taccari et al., 2012; Hassanshahian et al., 2014; Abed et al., 2015).

The objectives in this study were (1) to assess the efficiency of bioaugmentation with *Acinetobacter* SZ-1 strain KF453955 and biostimulation with addition of nutrients nitrogen and phosphorus, for petroleum hydrocarbon degradation and (2) to explore the relationship between hydrocarbon degradation efficiency and microbial community dynamics in petroleum-polluted soil.

2. Materials and methods

2.1. Soil analysis

A petroleum-contaminated soil was collected from the immediate area surrounding an oil well in Zichang county, Yan'an city, Shaanxi province, China. The site has a history of petroleum contamination over a period of years. The basic chemical and microbiological properties of this initial soil (IS) are shown in Table 1.

2.2. Experiment design for the microcosm study

The petroleum-contaminated soil was air-dried, ground and sieved through a 2-mm sieve prior to use. Afterwards, the soil was subjected to three different treatments. (1) CK, sterile water was added to soil to maintain 20% water content. (2) BA; bioaugmentation with *Acinetobacter* SZ-1 strain KF453955, a TPH-degrader, which was isolated from a petroleum-polluted soil by Yang et al. (2014). This strains was inoculated into soil to achieve a density of 10^8 cfu g^{-1} of soil as suggested by Abalos et al. (2004). Sterile water was added to soil to maintain 20% water content. (3) BS; biostimulation with $(NH_4)_2SO_4$ and KH_2PO_4 that were added to soil to achieve a C:N:P ratio of 100:10:1. Sterile water was added to soil to maintain 20% water content.

For each treatment, three microcosms (three replicates) were prepared, each containing 500 g soil, 100 g sterile water (20% water content) plus the bioaugmentation or biostimulation treatment materials in a 10 × 10 × 10 cm plastic box without a lid. The microcosms were then incubated at room temperature for 10 weeks without shaking to mimic an oil spill situation. Soil moisture was periodically monitored and water was supplied to the microcosms to keep the 20% water content. The soils inside the microcosms were stirred weekly to ensure sufficient air and oxygen.

Table 1
Selected chemical and microbiological characteristics of the petroleum-contaminated soil.

Main characteristics	Values
Total petroleum hydrocarbons (TPH) (mg/kg)	44,600
pH	7.5
Total carbon (g/kg)	789
Total nitrogen (mg/kg)	102
Total phosphorus (mg/kg)	160
Total bacterial population (cfu/g)	1.0×10^8
TPH degrader population (MPN[a]/g)	1.2×10^5
Alkane degrader population (MPN/g)	4.6×10^4
PAH[b] degrader population (MPN/g)	ND[c]

[a] MPN: Most probable number.
[b] PAH: polycyclic aromatic hydrocarbon.
[c] ND: Not detected.

2.3. Total petroleum hydrocarbon (TPH) degradation

A 5 g soil sample was collected weekly from each microcosm for 10 weeks for TPH analysis. The samples were dried for 24 h at room temperature in a fume hood immediately after sampling. Each soil sample was then extracted three times in an ultrasonic ice-bath (15 min for each extraction) with 15 mL of carbon tetrachloride and then centrifuged at 6000 rpm for 15 min. The extracts were combined to obtain the total organic extract. After filtration, the extracts were dried over Na_2SO_4 and adjusted to a 50 mL volume. The TPH in the extracts was analyzed using an Infrared Photometer Oil Content Analyzer (OCMA-350, Japan).

2.4. Catalase activity determination

Catalase activity was measured by the method of Lin et al. (2009). Briefly, 1 g air-dried soil was suspended in 40 mL distilled water and shaken for 30 min on a rotary mixer at 30 rpm, and then 5 mL of 0.3% H_2O_2 was added. The mixture was reacted with shaking for a further 10 min at 20 ± 2 °C, and then 5 mL of 3 M H_2SO_4 was added to stabilize the undecomposed H_2O_2. Finally, the mixture was filtered and titrated using 0.02 M $KMnO_4$. Catalase activity was expressed as ml $KMnO_4$ g^{-1} dry soil h^{-1}.

2.5. Determination of TPH-, alkane- and PAH- degrading microbial populations

The TPH-, alkane- and PAH-degrading microbial populations in soils collected from the microcosms at weeks 0, 1, 2, 6, and 7 of incubation were enumerated by a modified most probable number (MPN) procedure as described by Wrenn and Venosa (1996). Briefly, one gram fresh soil was homogenized in 9 mL PBS buffer (0.27 g KH_2PO_4, 1.4 g Na_2HPO_4, 0.8 g NaCl, 0.2 g KCl, 1 L distilled water, pH 7). Then 0.2 mL of the soil solution was transferred to a 5-mL-snap-cap culture tube containing 1.8 mL Bushnell-Haas medium containing 2% NaCl and the specific selective growth substrate. For TPH-degrading microbial population, the growth medium contained 56 μL standard petroleum hydrocarbons as the selective growth substrate. For alkane-degrading microbial population, the growth medium contained 56 μL n-hexadecane as the selective growth substrate. For PAH-degrading microbial population, the growth medium contained three PAHs (800 μg anthracene, 800 μg phenanthrene, and 400 μg pyrene) as the selective growth substrates. Tenfold serial dilutions were made until a dilution occurred where microbial growth was no longer evident.

The alkane- and TPH- degrader cultures were incubated by shaking at 180 rpm at room temperature (25 °C) for 1 week, and the PAH-degrader cultures were incubated for 3 weeks. For enumerating the alkane and TPH-degrader populations, iodonitrototnzolium violet (INT) was used to identify positive cultures. After 1 week of incubation, 100 μL of filter sterilized INT (3 g L^{-1}) was added to each culture tube. If red precipitate occurred due to INT reduction, the culture tube was positive. For PAH-degrader