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Remediation of petroleum hydrocarbon-contaminated sites by DNA diagnosis-based bioslurping technology

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HIGHLIGHTS

- Different molecular techniques were applied to evaluate the bioremdiation potential
- DNA microarray as screening tools was developed
- Based on DNA diagnosis, the remediation of contaminated soil was implemented sucessfully
- Bioslurping technology in treating petroleum-contaminated site was demonstrated

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ABSTRACT

The application of effective remediation technologies can benefit from adequate preliminary testing, such as in lab-scale and Pilot-scale systems. Bioremediation technologies have demonstrated tremendous potential with regards to cost, but they cannot be used for all contaminated sites due to limitations in biological activity. The purpose of this study was to develop a DNA diagnostic method that reduces the time to select contaminated sites that are good candidates for bioremediation. We applied an oligonucleotide microarray method to detect and monitor genes that lead to aliphatic and aromatic degradation. Further, the bioremediation of a contaminated site, selected based on the results of the genetic diagnostic method, was achieved successfully by applying bioslurping in field tests. This gene-based diagnostic technique is a powerful tool to evaluate the potential for bioremediation in petroleum hydrocarbon contaminated soil.

1. Introduction

The main cause of contamination of soil and groundwater is the leakage of petroleum hydrocarbons (PH), from underground storage tanks (USTs). PHs have densities lower than water, because of that they are classified as light nonaqueous phase liquid (LNAPL). Technologies for remediation of contaminated water and soil are classified as physical/chemical and biological processes. The selection and application of these technologies depend on the characteristics of the soil and contaminant, the desired effectiveness of each method, their economic viability, and the estimated time of effective results (Reddy et al. 1999; RAAG, 2000).

Some advantages of bioremediation include: low land requirements and low capital and operating costs (Hoeppel and Hinchee, 1994; Lei et al., 1994). However, due to certain limitations in the growth conditions of microorganisms capable of biodegrading PH, biological and physicochemical properties such as: the presence of the right microorganisms, substrate availability, and the biodegradation capabilities of the microbes present, must be examined to successfully bioremediate a contaminated site.

Among bioremediation technologies, bioslurping is the adaptation and application of vacuum-enhanced dewatering technologies to remediate hydrocarbon-contaminated sites (CPEO, 1998; FRTR, 1999; Khan et al., 2004). Bioslurping uses elements of bioventing and free product recovery to address two separate contaminant media (GWRTAC, 1996; MRI, 1998; Yen et al., 2003; Gidarakos and Aivalioti, 2007), simultaneously recovering free products and bioremediating vadose zone

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soils; thus, bioslurping can improve free-product recovery without extracting large quantities of groundwater.

In bioslurping, vacuum-enhanced pumping allows LNAPLs to be lifted off of the water table and released from the capillary fringe, minimizing changes in water table elevation and avoiding the creation of a smear zone. Bioventing of vadose zone soils is achieved by drawing air into the soil by withdrawing soil gas via the recovery well. The system is designed to minimize environmental discharge of groundwater and soil. When free-product removal is completed, the bioslurping system is converted easily to a conventional bioventing system to complete the remediation (RAAG, 2000; GWRTAC, 1996; MRI, 1998; Cresap, 1999). The final polishing steps are performed by aerobic hydrocarbon degrading bacteria (Beller et al., 2002; Chaillan et al., 2004; Mikesell et al., 1993).

Although some PHs can be degraded by microorganisms, their biological degradation is not possible at high concentrations, due to microbial toxicity (Das and Chandran, 2011). Microbe-based diagnosis methods are a prerequisite in evaluating the potential for biological remediation in petroleum-contaminated sites. However, isolation and identification of microorganisms that degrade PH compounds and qualitative and quantitative analysis of biodegrading enzymes are complex and time-consuming. In particular, it is nearly impossible to monitor degradation enzymes in environmental samples by conventional methods, because these enzymes are unstable under common environmental conditions, such as high temperature and humidity. Thus, DNA microarray targeting genes encoding for enzymes that can remediate contaminated sites can be used to analyze environmental functional gene arrays in contaminated soil and guide the selection of cost- and time-effective remediation methods before they are applied on a large scale.

A microbial DNA-based diagnostic method can assess the biodegradability of contaminated soil by detecting microorganisms or specific genes involved in the degradation of specific contaminants without having to culture the microorganisms. This approach can be used to verify the potential of bioremediation methods and can also be useful for assessing bioremediation progress.

Although microbe-based diagnosis methods for benzene, phenol, toluene, and naphthalene have been developed (Baldwin et al., 2003; Hendrickx et al., 2006; Kao et al., 2010; Movahedyan et al., 2009; Zylstra and Gibson, 1989), few studies have examined the application of these diagnosis methods in a field-scale bioremediation with mixtures of PHs, such as diesel and lubricants.

The two objectives of this study were: to use DNA-based methods to select a candidate site for bioremediation, and to demonstrate their applicability in a full scale bioremediation approach. To achieve these objectives, we designed, manufactured, and used DNA chips to diagnose contaminated soil; used genomic information to select and further study a site that had a good prognostic for biological degradation, and remediated the selected contaminated sites by bioslurping.

2. Materials and methods

2.1. Site survey

2.1.1. Soil samples and analysis

Three soil samples from the Pusan region in Korea were analyzed, two of which were contaminated. Two contaminated samples (P2, C1) were obtained from separate sites of a former gas-generating plant and were severely contaminated with petroleum fuels; the other soil sample (P1) was uncontaminated. All soil samples were taken from a depth of about 0.4 m (from the surface), mixed 5 times samples in plastic bags and stored at 4 °C in sterile universal containers.

Compared with soil conservation laws in Korea, we measured PHs, such as benzene, toluene, ethylbenzene, xylene (BTEX), and total petroleum hydrocarbon (TPH). At sites P2 and C1, the concentrations of petroleum hydrocarbons (BTEX and TPH) were higher than the reference values in the soil conservation law, and site P1, as expected had undetectable pollutant levels (Table 1). Sites P2 and C1 were considered positive controls with regard to contamination with petroleum hydrocarbon, and site P1 was used as the negative control with regard to contamination.

Table 1
BTEX and TPH content in soils.

	TPH (mg/kg)	BTEX (mg/kg)
Site P1	ND	ND
Site P2	13,100 ± 3200	226 ± 34
Site C1	24,600 ± 4350	386 ± 41

2.1.2. Genomic DNA extraction

Soil samples from site C1 and P2 were used to inoculate minimal medium of petroleum hydrocarbons (MMPH) (Bushnell-Haas Broth 3.27 g/L, yeast extract 0.1 g/L, 0.25% Tween 80, and 1% diesel), and cultures were enriched to verify the microbial community capable of degrading PH for 5 d at 150 rpm and 30 °C in a shaking water bath. DNA was extracted from each soil and enrichment cultures using the Ultra CleanTM Soil DNA kit as described (MoBio Laboratories Inc., Solana Beach, CA, USA).

2.2. PCR-DGGE

Denaturing gradient gel electrophoresis (DGGE) was used to assess microbial diversity in the soil samples. The V3 region of the 16S rRNA genes (corresponding to positions 341–534 in *Escherichia coli*) from soil and enrichment cultures DNA was PCR-amplified using the following primers: GC341(f) (5′-CCTACGGGAGGCAGCAG-3′) and PRUN518(r) (5′-ATTACCGCGGCTGCTGG-3′). A GC clamp was added to the forward primer at the 5′-end (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG-3′). Detailed conditions for PCR are as previously described (Muyzer et al., 1993; He et al., 2005).

DGGE was performed using the Dcode Universal Mutation Detection System (Bio-Rad, USA). The PCR samples were loaded onto 8% (wt/vol) polyacrylamide gels in 0.5× TAE (20 mM Tris, 10 mM acetate, and 0.5 mM Na-EDTA, at pH 7.4). Gels (bisacrylamide gel stock solution, 37.5:1) were prepared with a 30% to 60% gradient of denaturant (7 M urea and 40% deionized formamide) and run at 60 °C for 3 h at 200 V. The gels were then stained for 45 min with SYBR Green I (Molecular Probe, Netherlands), inspected under UV light, and photographed on a Gel Doc 2000 (BIO-RAD).

2.3. Detection of catabolic enzyme-encoding genes

2.3.1. PCR primers

Table 2 summarizes selected PCR primers used to probe for specific genes encoding for catabolic enzymes of interest, such as: alkane hydroxylase, naphthalene dioxygenase, toluene dioxygenase, and catechol 2,3-dioxygenases (Table 2).

2.3.2. PCR conditions

PCR amplification was performed in 50-μL reaction mixtures, containing 10 mM Tris–HCl (pH 8.0), 50 mM KCl, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 1 U *Taq* DNA polymerase, 10 pM forward primer, 10 pM reverse primer, and 10 ng template DNA. The PCR (GeneAmp® PCR system 9700, PE Biosystems) amplification conditions were as follows: 94 °C for 5 min; 30 cycles, comprising 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min; a final extension at 72 °C for 5 min; and cooling to 4 °C. Amplicons were analyzed by horizontal electrophoresis in an agarose gel (1% to 1.5%) and visualized with ethidium bromide per Sambrook et al. (1989). Fragment sizes were visualized and calculated on a Gel Doc 2000 (BIO-RAD).