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Biodegradation of diesel-contaminated soil: A soil column study

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Abstract

This study simulated *in situ* bioremediation for diesel-contaminated soil by a column operation. Several bioremediation approaches were conducted to examine the feasibility. Four lab-scale soil columns were operated specific to the following approaches: nutrient enhancement (NE), bioaugmentation (BA), biostimulation (BS), and sterilized soil (SS). Within 330 days of operation, the residual total petroleum hydrocarbon-diesel (TPH_d) was degraded from 10,290 mg TPH_d/dry soil to 3026, 1851, 4105, and 6506 mg TPH_d/kg dry soil by Columns NE, BA, BS, and SS, respectively. The diesel-degradation efficiency was 67%, 80%, 45%, and 24%, accordingly. Microbial diversity was monitored with molecular biotechnology DGGE. It was found that introduced bacteria CC-CF3 and CC-JG39 become undetectable after 90 days of operation, but another introduced bacteria, CC-RS1, evidently remained with small signals during the last time stage of operation.

Keywords: Bioremediation; Diesel-contaminated soil; Column test; Bioaugmentation; Biosurfactant

1. Introduction

Bioremediation of petroleum hydrocarbon contaminated soils has been recognized as an efficient, economic, versatile, and environmentally sound treatment (Margesin and Schinner, 2001). However, contamination problems increase when ages of relevant facilities, such as oil storage tanks and pipelines, increase over time. It has been reported that more than 1200 petroleum-hydrocarbon contaminated sites were under regulation in the United States, and about additional 32,000 sites were reported with potential contamination problems (Baker and Herson, 1994). In Taiwan, up to 2002, over 400 gas stations had encountered problems of being potentially contaminated with hydrocarbon petroleum, due to aged pipelines or storage tank leaking (Che, 2002).

Also, when treating the oil contamination problems with bioremediation approaches, inadequate bioavailability of the hydrocarbons to microorganisms due to low solubility has been addressed as a limiting step in biodegradation (Rahman *et al.*, 2002). Therefore, introducing surfactants to the contaminated soil in order to emulsify hydrocarbons, to increase their water solubility, and to reduce surface tension has been found to be a useful approach, particularly when a biosurfactant was utilized (Banat, 1993, 1995; Banat *et al.*, 2000; Moran *et al.*, 2000; Rahman *et al.*, 2002). According to Banat *et al.* (2000), applications of biosurfactants include being introduced to bioreactors and added to *in situ* contaminated sites to improve the solubilization and emulsification of hydrophobic contaminants. Thus, the bioavailability of the chemical compounds can be enhanced so as to enhance the resultant biodegradation (Banat *et al.*, 2000). Numerous existing research projects have addressed successful applications of biosurfactants to hydrocarbon-contaminated soil and enhanced degradation has been demonstrated (Bai *et al.*, 1997; Jain *et al.*, 1992; Moran *et al.*, 2000).

Addition of commercial microbial cultures (i.e., bioaugmentation) to contaminated soil was concluded it is not superior to just simply adding nutrients (i.e., biostimulation) to the contaminated soil (Alexander, 1999; Philp and Atlas, 2005; Van Hamme *et al.*, 2003). In the existing studies, bioaugmentation, compared with biostimulation, did not significantly enhance rates of oil biodegradation. However, a niche area for a successful bioaugmentation is possible when only small numbers of hydrocarbon-oxidizing bacteria could be found, particularly in pristine soils (Liu *et al.*, 2007; Philp and Atlas, 2005).

This study aimed at evaluating four different treatments employed: bioaugmentation (BA), biostimulation with biosurfactant (BS), nutrient enhancement (NE), and a control design with sterilized soil (SS). The objectives include using a lab-

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scale column study to determine the feasibility of the bioremediation approaches and to investigate the microbial community during the operation. In addition to the engineers' perspectives on biodegradation efficiency among the different treatments, parallel results of the molecular biotechnology were discussed.

Column BA was performed with the introduction of three isolates: *Comamonas testosteroni* CC-CF3, *Gordonia alkanivorans* CC-JG39, and *Pseudomonas aeruginosa* CC-RS1, which are proven effective diesel-degraders (Lin *et al.*, 2005; Young *et al.*, 2005). Column BS was designed by introducing a commonly isolated glycolipid biosurfactant produced by *P. aeruginosa* J4 (Cheng *et al.*, 2006; Whang *et al.*, 2008), rhamnolipid, to the contaminated soil. Rhamnolipid belongs to the most known biosurfactant class, glycolipids. Column NE was performed in a column where a Bushnëll Hass (BH) medium was provided in the influent (Bushnëll and Hass, 1941). Column SS was performed by controlling pH < 2 to limit the bioactivity in the influent and inside the column.

The four different column treatments were operated for 330 days. The soil packed in the columns was a contaminated soil sample collected near a diesel-storage tank in the LY petrochemical park. The initial TPH_d in the contaminated soil was 3980 mg/kg, and additional diesel was introduced to the soil during Time Stages 3 and 4 in order to make the designed TPH_d to be about 10,000 mg/kg.

2. Material and methods

2.1. Soil column operation

Water quality, including dissolved oxygen (DO), pH values, total inorganic carbons (TIC), nitrate–nitrogen (NO_3–N), nitrite–nitrogen (NO_2–N), was monitored throughout the operation period. The degradation efficiency was evaluated by analyzing the total petroleum hydrocarbon-diesel (TPH_d) using a gas chromatograph with a flame ionization detector (GC-FID). The chemical organics with carbon number ranging from 10 to 28 (C10-C28) were defined as diesel-related organics (DRO) and the integrated area within the corresponding range on the chromatograph was calculated for the TPH_d analysis. The microbial population diversity was measured with molecular biotechnology DGGE. Finally, the optimum treatment was determined and a mass balance of TPH_d was achieved.

2.2. Soil column description

The soil column was made of glass with a volume of 348–358 cm^3. The column provided an inlet and outlet for the nutrient solution, which was aerated and sterilized before entering the column. Four sampling pores were set vertically for the purpose of taking samples. The four columns were kept upright to allow vertical flow of the influent nutrient solutions in a bottom-top direction. The contaminated soil samples were packed in the middle section of the column, with a small amount of quartz sand (6.6 cm^3) in the top and the bottom sections to prevent soil loss (Fig. 1). Contaminated soil with an average wet weight of 447 g and average bulk density of about 1.2 g/cm^3 was packed into the four columns (Table 1). The soil samples were sieved (<2 mm) before each packing. The soil property was analyzed, and a texture of sandy loam was concluded (Table 2). The operations were conducted with four different time stages, and the specific conditions in each time stage are explained in Table 3.

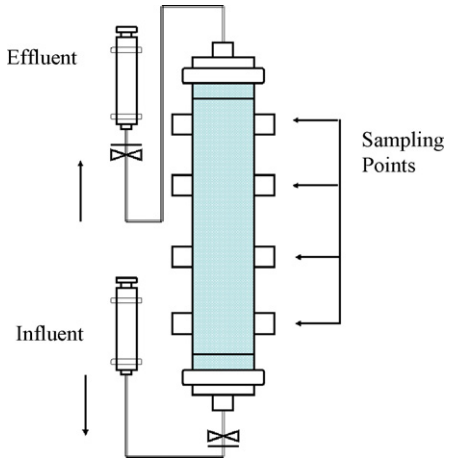

Fig. 1. Soil column schematic.

During Time Stage 1, to enhance the bioactivity of indigenous microorganisms in the soil, influent nutrient of BH medium was provided for the four columns. After about 2 months acclimation to the BH medium, synthetic groundwater (KCl 18.6 mg/L, $FeSO_4$ 0.5 mg/L, $MnCl_2$ 0.2 mg/L, NaCl 58.5 mg/L, NH_4NO_3 12.9 mg/L, Na_2HPO_4 1.47 mg/L, $CaCO_3$ 100 mg/L, $MgCl_2$ 50.8 mg/L) was provided during the rest of the operations in order to mimic a groundwater condition. The flow rate was set as 50 mL/day (13.89 m^3/year) by syringe pumps, in order to simulate a groundwater flow condition. The resultant hydraulic retention time ranged from 3.64 to 4.14 days. In addition to the influent of synthetic groundwater, during the last two time stages, 3200 mg/kg diesel oil was spiked to the top of the four columns to provide substrates.

In column BA, in addition to the indigenous microbes, several isolates that were proven to be diesel-degraders were mixed with the contaminated soil. The introduced bacterial consortia was composed of *C. testosteroni* CC-CF3, *G. alkanivorans* CC-JG39, and *P. aeruginosa* CC-RS1, which were isolated from soil samples of restaurant sewage, oil storage tanks, and campus soil, respectively (Lin *et al.*, 2005; Young *et al.*, 2005). Each of the isolates, prior to being mixed with the contaminated soil, was enriched with LB medium at

Table 1
Porosity of soil packed in the columns

Column	Soil wet weight (g)	Soil volume (1/cm)	Bulk density (g/cm^3)
NE	470	358	1.3
BS	438	356	1.1
BA	424	351	1.1
SS	455	348	1.2