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Bioaugmentation efficiency investigation on soil organic matters and microbial community shift of diesel-contaminated soils

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ABSTRACT

This study investigated effects of microbial community shift, soil organic matter (SOM), and diesel contamination approaches with three independent batches. The SOM levels ranged from 2% to 55%. Diesel oil was artificially contaminated to soils with different approaches to achieve the highest level of 10000 TPH mg/kg. The three batches provided the same results regarding the negative influence of SOM on the TPH degradation rate (k). The results of Nonmetric multidimensional scaling indicated that (1) the bacterial community shift significantly associated with the TPH degradation stages; (2) when SOM levels were close to each other, their degradation performance and bacterial communities were similar to each other, and (3) dynamics of bacterial communities could influence the TPH degradability. In addition, the Intergenic spacer (ITS) microarray results emphasized the advantages of determining an effective bioaugmentation, confirming the successfulness of the inoculation, and identifying the survivors. Microbial community shift and SOM levels indeed influenced the TPH degradation.

1. Introduction

Contamination of total petroleum hydrocarbons (TPH) in soil from industrial sources and activities are producing ecological disasters and addressing public concerns. Spills, leaks and other releases of TPH have caused unavoidable soil contamination problems worldwide over the recent decades. In Taiwan, 45 gas stations, storage tanks, industrial parks have been regulated as remediation sites that had encountered problems of being potentially contaminated with petroleum hydrocarbons, due to aged pipelines or storage tank leaking (Chen, 2012). Among a variety of the remediation methods, bioremediation has been recognized as an efficient, economic, versatile, and environmentally sound solution, and has been shown to be effective for petroleum contaminated soils in several laboratory and field studies (Mukherjee et al., 2011; Coulon et al., 2012). However, bio-approaches may or may not be appropriate to meet the desired soil remediation end-point, due to limited bioavailability of hydrocarbons to microorganisms (De Jonge et al., 1997). Limited bioavailability includes (1) absence of hydrocarbon-degrading microbes, (2) inadequate numbers of important hydrocarbon-degrading microbes, and (3) unsuitable environments including lacking of nutrients or oxygen.

To overcome the first two limiting factors, inoculation with high concentrations of hydrocarbon degraders, so called bioaugmentation, is recommended (MacNaughton et al., 1999; Mishira et al., 2001; Mohn et al., 2001). However, some debates were observed over the usefulness of bioaugmentation. Alexander (1999) stated that inoculation has usually caused an increase of the bacterial concentrations in the beginning, but has no effect on the final stages. Another problem is the competition of introduced bacteria with well-adapted indigenous ones (MacNaughton et al., 1999). Therefore, to clarify the usefulness of the bioaugmentation approach, this study evaluated several batches with and without bioaugmentation approaches.

The third limited bioavailability, unsuitable environment, involves interactions among soil matrix, pollutants, and microorganisms. When a petroleum product is released into soil, the hydrocarbons might be changed with complicated physical, chemical, and biologically process through time. Particularly, soil

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organic matters (SOM) have been described as an important component affecting soil-pollutant interactions both in amount (Hatzinger and Alexander, 1995) and in their nature (Ortega-Calvo et al., 1997; Piatt and Brusseau, 1998). The influence of SOM has been proposed to be the most significant factor dominating the interactions (Reid et al., 2000). When hydrophobic pollutants such as petroleum hydrocarbons are sequestrated in soil, they were hypothesized as partitions into the organic fraction of soil, and the extent of the sorption is related to the percentage of SOM (Nam et al., 1998). Therefore, this study evaluated the TPH degradation rates and end-point concentrations among soils with different SOM levels. The SOM discussed in the study stands for the organic components naturally exist in soil matrix, rather than organic amendments such as manure or food waste. Humic substance is the major component in the evaluated SOM. The humified materials may include humic acids, fulvic acids, humins, polysaccarides, polypeptides, and altered lignins (Hayes et al., 2007).

To verify the SOM influence on the TPH degradation, the SOM percentage levels ranging from about 2% to 55% in our artificial contaminated soils were tested. To clarify the importance of the pollutant diffusion in the soil, two different approaches to artificially contaminate the soils with diesel oil were tested. To investigate the microorganism community shifting during the TPH degradation process, soil samples under the three circumstances were experimented: (1) indigenous communities, (2) introduced hydrocarbon degraders, and (3) sterilization.

In order to profile the microbial community shifts during the remediation, two culture-independent techniques were employed: internal restriction fragment length polymorphism (T-RFLP) and internal transcribed spacer (ITS) oligonucleotide array (Hsiao et al., 2005; Tung et al., 2006; Leaw et al., 2007). To interpret the large quantities of the molecular data, a nonmetric multidimensional scaling (MDS) (Yannarell and Triplett, 2004; Roling et al., 2004; Muckian et al., 2007) was applied to investigate the correlation between the bacterial community shift and the TPH degradation.

The objectives of this paper are to investigate the effects of SOM on TPH degradation efficiency in diesel-contaminated soils, to clarify usefulness of the bioaugmentation approach, to correlate microbial community shift with TPH degradation performance, and to evaluate the diesel contamination approaches.

2. Materials and methods

2.1. Experimental design

The soils with different SOM were designed by mixing the soils from with oil contamination site (S site, T site, and KT), non-contamination site (Gr), and peat (from Shamao Mountain) (Table 1). The SOM in the soils varied from 2.2 to 55.1% (w/w). The soil properties are explained with textures (mostly sand, loamy sand, and loam), SOM contents, total nutrients, exchangeable metals, and cation exchange capacity (Table 1). The maximum weight of one kilogram dry soil was tested in a glass serum bottle at room temperature.

The initial TPH achieved ranging from 3000 to 10,000 mg/kg, after artificially contaminated with diesel oil (spray or TCLP) (Table 2). The bioaugmentation treatments in Batch II and III were conducted with inoculation of bacteria and fungi. The microorganisms in Batch III were inoculated with pure cultures after sterilization, which was conducted with methanol in the TCLP process. The soils were turned over every other day to keep sufficient oxygen and to simulate landfarming operations. Moisture was maintained between 15 and 30% and monitored periodically along with the TPH analyses.

Table 1
Soil physical and chemical properties in the three batches.

Soils	Batch I		Batch II		Batch III	
	S Site	T Site	KT	Shamao Moutain	Gr	Peat
Soil Texture	Sand	Loamy Sand	Loamy Sand	Loam	Sandy Clay Loam	Loam
Soil Organic Matters	1.9	2.6	1.5	27.3	2.2	49.3
Total Nitrogen (%)	0.030	0.052	0.062	0.453	0.0596	0.823
Total Phosphorus (%)	0.046	0.049	0.0416	0.0503	348	558
Exchangeable Ca (mg/kg)	2510	2941			46.4	155
Exchangeable Ma (mg/kg)	45	47			88.7	421
Exchangeable K (mg/kg)			36.7	72.6	791	2548
Cation Exchange Capacity (cmol/kg)	3.7	4.1	4.37	47.8	53.9	61.8

2.2. Bacterial and fungal consortia

To resolve the problem of degradation plateau (U.S. EPA, 2002) or residual concentration problems, a bioaugmentation approach using oil-degrading bacteria and fungi inoculums was conducted in Batch II and III (Table 3). The introduced bacteria consortia were isolates from environmental samples, and their diesel or fuel oil degradability were verified. Among which, the diesel degrading bacteria consortia: *Gordonia alkanivorans* CC-JG39 (Young et al., 2005; Lin et al., 2008) and *Rhodococcus erythropolis* CC-BC11 (Lin et al., 2005), were isolated from a gas station oil storage tank and from a campus soil sample, respectively. The fuel oil degrading strains, *Acinetobacter junii* CC-FH2, *Serratia marcescens* KH1, and *Exiguobacterium aurantiacum* CC-LSH-4, were isolated from a same *ex situ* oil bioremediation site. *Pseudomonas aeruginosa* CC-RS1 was isolated from recycled sewage sludge in a wastewater treatment plant. Also, two fungal species, *Candida guilliermondii* (Bioresource Collection and Research Center 21559) and *Candida parapsilosis* (BCRC 20515) were added to the bioaugmentation treatments in Batch III.

2.3. Rhamnolipid biosurfactant

In Batch II, biosurfactant rhamnolipid was used to increase solubility of the petroleum oil in soil so as to enhance the hydrocarbon bioavailability to the microorganisms. The rhamnolipid biosurfactant was produced by *Pseudomonas* spp. S2 cultured with glucose as the carbon source (Chen et al., 2007). Rhamnolipid used in the study belongs to anionic surfactant glycolipid, with mixed structure of mono- and di-rhamnolipid. The corresponding emulsion index was detected to be 70%.

2.4. Total petroleum hydrocarbon analysis

The total petroleum hydrocarbon was quantified as hydrocarbons with carbon number between 10 and 40 (TPH). After being dehydrated with sodium sulfate anhydrous, the soil sample was extracted with dichloride methane in an Ultra-sonic apparatus (DELTA DC400H, Taiwan) (US EPA, 1996). TPH in the soil samples was quantified using a gas chromatograph with a flame ionization detector (GC-FID, Shimadzu GC-2010, Japan) equipped with a 30 m capillary column (DB-1(HT), 0.32 mm inner diameter, 0.1 μm film thickness, Agilent, USA) (US EPA, 1995). The operation program of the GC-FID for the TPH analysis was started with injector and detector temperatures of 350 °C. Oven temperature was programmed from initial 50 °C (held for 5 min) to 350 °C in the speed of 10 °C/min and then remained constant for another 15 min. Fifty-seven minutes were spent for each analysis. H_2 was used as carried gas with flow rate was 40 mL/min and air flow rate was 400 mL/min.

The QC reference sample was diesel oil containing the following concentrations in weight: 750, 1000, 1500, 2000, 3000, 4000, and