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Effects of soil organic matter and bacterial community shift on bioremediation of diesel-contaminated soil

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ABSTRACT

Bioremediation of diesel-contaminated soils were applied to investigating effects of soil organic matter (SOM) and bacterial community shift. Soil samples were artificially contaminated with diesel oil, ranging from 4000 to 12000 mg/kg soil, remediated with laboratory-scale landfarming batch applications. The SOM levels in our experiment were 2.3% (presented as SOM15), 8.9% (SOM092), and 11.8% (SOM125). Based on each of the SOM levels, bioremediation approaches of bioaugmentation (BA015, BA092, and BA125) and using indigenous microorganisms as control groups (CT015, CT092, and CT125) were tested. After about 300-day operation, total petroleum hydrocarbon (TPH) degradation efficiency became 73%, 63%, and 59% in SOM015, SOM092, and SOM125, respectively. Their 1st order degradation rates also reduced with the increase of SOM. We preliminarily concluded that SOM affected the TPH degradation efficiency and 1st degradation rates. With a logarithm transformation, the degradation pattern of SOM092 and SOM125 found to resemble each other. No apparent improvement was found from the BA batches. Our Intergenic spacer (ITS) microarray result indicated the existence of diesel-degrading bacteria in the indigenous communities. Nonmetric multidimensional scaling (MDS) based on terminal restriction fragment length polymorphism (T-RFLP) data indicated that 1) CT community became similar to BA community, once the 1st degradation stage started, impling an activation of the indigenous bacteria; 2) the degradation stage affected the community dynamics more than the SOM or the remediation approaches could do, and 3) both BA092 and BA125 located in the same cluster on the MDS plot all the time, revealing the similar communities. The similar communities might cause the comparable degradation patterns in SOM092 and SOM125. The bacteria community shift found useful in explaining the TPH degradation performance.

1. Introduction

Contamination of total petroleum hydrocarbons (TPH) in soil from industrial sources and activities are producing ecological disasters and addressing public concerns. In Taiwan, over 400 gas stations had encountered problems of being potentially contaminated with petroleum hydrocarbons, due to aged pipelines or storage tank leaking (Che, 2002). Among a variety of the remediation methods, bioremediation has been recognized as an efficient, economic, versatile, and environmentally sound solution (Margesin and Schinner, 2001). However, during bioremediation processes, bioavailability of hydrocarbons to microorganisms could be a limiting factor (De Jonge et al., 1997). Limited bioavailability was defined as a limited pollutant degradation rate by microorganisms. A smooth biodegradation involves interactions between soil matrix, pollutants, and microorganisms. Particularly, soil–pollutant interactions have been described as influenced by factors including soil organic matters (SOM), 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). Especially when the pollutants in soil are hydrophobic, sequestration of the hydrophobic compounds was hypothesized as their partitioning into

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the organic fraction of soil, and the extent of the sorption is related to the percentage of SOM (Nam et al., 1998). From both viewpoints of biodegradability and risk assessment, predicting the different rate and extent of loss in bioavailability among soils with different SOM is important.

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, which has been useful in composting and facilitating soil remediation projects (Park et al., 2001; Joo et al., 2007; Ros et al., 2010). In the applied soils, humic substances are the most abundant component of SOM, defined as a series of relatively high molecular weight, yellow to black color substances formed by secondary synthesis reactions. The humified materials may include humic acids, fulvic acids, humins, polysaccharides, polypeptides, and altered lignins (Hayes et al., 2007).

While considerable information on the bioavailability of single hydrocarbon can be available in the literature, few were known about bioavailability and bioremediation strategies of TPH impacted by SOM. For example, the decrease in phenanthrene bioavailability supports the view that the sequestration caused by aging could be resulting from slow diffusion, partitioning, or a combination of both (Hatzinger and Alexander, 1995). However, probably due to the complicated mixtures of the total petroleum hydrocarbons, bioavailability limited by SOM is rarely discussed.

In addition to the bioavailability problem due to SOM, presence of useful and adequate numbers of hydrocarbon-degrading species is often another limiting factor. To overcome the possible drawback of lacking of important microbes, inoculation with high concentration of hydrocarbon degraders, so called bioaugmentation, is a recommended option (Boopathy, 2000; Mohn and Stewart, 2000; Mishira et al., 2001; Mohn et al., 2001).

Although ecological "black box" used to be shortfalls in the understanding of microbial community dynamics in remediation systems (Andrew and Mark, 2000), molecular technologies now successfully provide profiles of bacterial community dynamics in soils. Those culture-independent techniques including cloning, denaturing gradient gel electrophoresis (DGGE), terminal 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) have been applied to investigate the microbial community shifts in soils (Tiedje et al., 1999; Lukow et al., 2000; Torsvik and Ovreas, 2002; Muckian et al., 2007; Liu et al., 2009, 2011, 2012).

To interpret the collection of large amounts of molecular data, which does not necessarily lead to an adequate explanation of the community (Dollhopf et al., 2001), statistical methods including as principal component analysis (PCA) (Dollhopf et al., 2001; Kaplan and Kitts, 2004; Chaillan et al., 2004) and nonmetric multidimensional scaling (MDS) (Yannarell and Triplett, 2004; Liu et al., 2009, 2011, 2012) have been found useful in correlating the bacterial community shift with the TPH degradation.

The objectives of this paper are to investigate the effects of SOM on TPH degradation efficiency in diesel-contaminated soils, and at the same time, to evaluate the usefulness of a bioaugmentation approach in the soils with different SOM. Soils contaminated with maximum 10,000 mg TPH kg^{-1} were tested with about 300-day landfarming operation in a laboratory. Molecular biotechnologies of the terminal-restriction fragment length polymorphism (T-RFLP) and the oligonucleotide array targeting the ribosomal internal transcribed spacer (ITS) region were applied to profile the bacterial community shift, explained by a statistical tool MDS, and to present evidence of the association between the microbial ecology and the TPH degradation efficiency, in a variety of SOM.

2. Materials and methods

2.1. Soil contamination design

Contaminated soil samples from an oil storage site (named as KT) were mixed with peat from Mt. Shamao, northern Taiwan. The peat soil samples contained 27.3% of SOM, and the storage-site soil samples contained only 2.3% of SOM. Soil texture was identified as loamy sand with the particle size distribution of sand 89.4%, silt 4.5%, and clay 6.1% (w/w). The soils were mixed with different ratios in order to achieve SOM contents of 1.5%, 9.2%, and 12.5% for treatments SOM015, SOM092, and SOM125, respectively. About 0.4 kg dry weight soils were prepared in each glass serum at 30 °C constantly for the remediation course, and separately designed for bioaugmentation (BA) or control tests under different SOM levels (CT) (Table 1).

2.2. Bioremediation experimental design

Soils contaminated with 5000–10,000 mg TPH kg^{-1} were tested with about 300-day landfarming operation in a laboratory. Although the initial concentrations among the treatments were not the same, each of the batches was dedicated for different purpose. The soil moisture was controlled around 15–25%, and aeration was achieved by turning over twice a week.

In the BA batches (BA015, BA092, and BA125), the introduced bacterial consortia were composed of four strains that were isolated from different locations. Their diesel/fuel degradability has been individually verified. Two diesel degrading strains, *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 and *Serratia marcescens* KH1, were isolated from a same *ex situ* oil bioremediation site (Kao, 2008). The control batches (CT015, CT092, and CT125) were prepared with soils containing only indigenous microorganism, and operated with the same schedule regarding soil turning over and moisture control.

2.3. Total petroleum hydrocarbon and the fractional 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^{-1} and then remained constant for another 15 min. Fifty-

Table 1
Design of soil organic matters in different soil samples.

Batch explanation	SOM015 (BA015, CT015)	SOM092 (BA092, CT092)	SOM125 (BA125, CT125)
KT: Mt. Shamao (dry weight)	1.0:0	2.3:1	1.4:1
Designed SOM (%)	1.5	9.2	12.5
Measured SOM (%)	2.3	8.9	11.8
Initial TPH (mg kg^{-1})	10700 ± 2900	6100 ± 600	5000 ± 1000