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Bioremediation of diesel and lubricant oil-contaminated soils using enhanced landfarming system

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HIGHLIGHTS

- Enhanced landfarming was developed using unique operation and amendments.
- Enhanced landfarming could remediate lubricant and diesel contaminated soils effectively.
- Fern chips, compost, sludge could be applied to enhance TPH biodegradation efficiency.
- Chromatograph analysis is a useful indicator of the effectiveness of TPH biodegradation.

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ABSTRACT

Lubricant and diesel oil-polluted sites are difficult to remediate because they have less volatile and biodegradable characteristics. The goal of this research was to evaluate the potential of applying an enhanced landfarming to bioremediate soils polluted by lubricant and diesel. Microcosm study was performed to evaluate the optimal treatment conditions with the addition of different additives (nutrients, addition of activated sludge from oil-refining wastewater facility, compost, TPH-degrading bacteria, and fern chips) to enhance total petroleum hydrocarbon (TPH) removal. To simulate the aerobic landfarming biosystem, air in the microcosm headspace was replaced once a week. Results demonstrate that the additives of activated sludge and compost could result in the increase in soil microbial populations and raise TPH degradation efficiency (up to 83% of TPH removal with 175 days of incubation) with initial (TPH = 4100 mg/kg). The first-order TPH degradation rate reached 0.01 1/d in microcosms with additive of activated sludge (mass ratio of soil to inocula = 50:1). The soil microbial communities were determined by nucleotide sequence analyses and 16S rRNA-based denatured gradient gel electrophoresis. Thirty-four specific TPH-degrading bacteria were detected in microcosm soils. Chromatograph analyses demonstrate that resolved peaks were more biodegradable than unresolved complex mixture. Results indicate that more aggressive remedial measures are required to enhance the TPH biodegradation, which included the increase of (1) microbial population or TPH-degrading bacteria, (2) biodegradable carbon sources, (3) nutrient content, and (4) soil permeability.

1. Introduction

Lubricant and diesel oil-polluted sites are difficult to remediate because they have less volatile and less biodegradable characteristics compared to gasoline (Kuo et al., 2012; Silva-Castro et al., 2015). Total petroleum hydrocarbon (TPH) has been used to represent the mixture of hydrocarbons, which are found in crude oil. TPH is composed of volatile petroleum hydrocarbons (usually containing hydrocarbons from C_2–C_5) and extractable petroleum hydrocarbons (usually containing hydrocarbons from C_6–C_{40}) (Abdulsalam et al., 2011; Tang et al., 2012; Abed et al., 2014).

Bioremediation, which uses microorganism for pollutant biodegradation, has been applied for petroleum-hydrocarbon polluted site remediation. Because most of the petroleum hydrocarbons are comparatively biodegradable (Chien et al., 2011;

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Abbreviation list

BTEX	benzene, toluene, ethylbenzene, and xylenes
CEC	cation exchange capacity
CFU	colony forming unit
CPI	carbon preference index
DGGE	denaturing gradient gel electrophoresis
DI	deionized
EC	electric conductivity
GC	gas chromatography
MTBE	methyl-tert-butyl ether
NB	nutrient broth
ORP	oxidation-reduction potential
PCR	polymerase chain reaction
TPH	total petroleum hydrocarbon
UCM/R	unresolved complex mixture/resolved hydrocarbons
UST	underground storage tank

Gojgic-Cvijovic et al., 2012; Bahadure et al., 2013), bioremediation would be a feasible technology for petroleum-hydrocarbon polluted soil remediation (Nikolopoulou and Kalogerakis, 2010; Nikolopoulou et al., 2013; Thomé et al., 2014). To accelerate the site cleanup efficiency, application of soil excavation and on-site or ex-situ bioremediation system can be applied for soil treatment (Yu et al., 2007, 2014). Ex-situ bioremediation through landfarming is a remediation technique to treat a wide range of hydrocarbons (Besalatpour et al., 2011; Jho et al., 2014; Thomé et al., 2014). The efficiency of landfarming system can be promoted via the following activities: (1) indigenous bacteria stimulation by additives supplement (e.g., nutrients, oxygen) (also called biostimulation) (Whelan et al., 2015), and (2) specific contaminant-degrading bacteria inoculation (also called bioaugmation) (Ros et al., 2010; Liu et al., 2013; Jasmine and Mukherji, 2014).

Biostimulation and bioaugmation are necessities if conditions of contaminated site soils are not appropriate for landfarming processes either due to the lack of nutrients, oxygen, substrates, or contaminant degrading bacteria (Kauppi et al., 2011; Zhao et al., 2011; Cerqueira et al., 2014; He et al., 2014). Bioaugmentation whereby petroleum degrading microorganisms are added to the soil matrix, and biostimulation, which introduces essential nutrients or biosurfactants to stimulate microbial petroleum degradation (Gomez and Sartaj, 2013; Pringault et al., 2015). Both biostimulation and bioaugmentation can be accomplished separately or in combination, by introducing hydrocarbon-degrading bacteria and/or amending contaminated soil or sludge with a heterogeneous additional material such as compost (Kriipsalu and Nammari, 2010; Adekunle, 2011; Lin et al., 2012).

Although landfarming system is a commonly used method to remediate TPH-polluted soils, supplement of fern chips of *Sphaeropteris lepifera* as the bulking agent (Ma et al., 2016), compost and activated sludge as the sources of organic materials and microorganisms, and specific TPH-degrading bacteria on the improvement of efficiencies of landfarming operation to cleanup diesel and lubricant oil contaminated soils has not been thoroughly discussed.

The fern chips is obtained from aerial roots stems of *Cyathea* sp. trunks (Chou et al., 2008; Chen et al., 2014). Fern chips could also be made from *Sphaeropteris lepifera* trunks (Chou et al., 2008; Chen et al., 2014). Because fern chips have characteristics of low clogging and pressure drop during packing and operation with easy control for operational conditions (Chou et al., 2008; Chen et al., 2014), fern chips were selected as the amendment in this study. Because compost and wastewater sludge and compost contain significant amounts of carbons and nutrients, supplement of compost or sludge as the additives has been applied to enhance the contaminants biodegradation efficiency (Chen et al., 2014; Li et al., 2015). Furthermore, compost and wastewater sludge contain higher microbial population and diversity, their application on contaminant biodegradation can be expected (Akbari and Ghoshal, 2014; Megharaj et al., 2011; Idemudia et al., 2014).

In the mechanism and effectiveness study, a modified landfarming system with the addition of different additives for efficiency enhancement was developed to bioremediate lubricant and diesel polluted soils. The main goals were to (1) perform microcosm and feasibility study to improve the effectiveness of bioremediating lubricant and diesel polluted soils with air replacement and with the addition of nutrients, activated sludge from aerobic basin of an oil-refining wastewater treatment plant, compost, TPH-degrading bacteria, and fern chips, (2) assess the change of bacterial population, dominant bacteria, and diversity during the modified landfarming process using DNA extraction, polymerase chain reaction (PCR), and nucleotide sequence analyses, and (3) perform chromatograph analysis to assess the variations in chromatograms of petroleum hydrocarbons during biodegradation.

2. Materials and methods

2.1. Site description

A leaking spent lubricant and diesel-oil underground storage tank site was discovered in 2010 in southern Taiwan. Based on results of our preliminary site investigation study, the TPH concentrations of the contaminated soils ranged from 3820 to 4390 mg/kg within the polluted zone. Because the soil TPH exceeded Taiwan's soil remediation standard (1000 mg/kg) (TEPA, 2003), site soils needed to be treated to meet the soil standard. Thus, TPH became the target compound in this study.

TPH (lubricant and diesel)-contaminated soils were collected for laboratory microcosm studies. Soil analyses included the following: TPH concentrations, pH, organic and moisture contents, nutrient concentrations, cation exchange capacity (CEC), and total (cultivable) heterotrophs, electric conductivity (EC). Analyses were performed using methods described in Standard Methods (APHA, 2011). The soil formation was mainly silty loam and the soil characteristics were as follows: pH = 7; ORP = 245 mv; organic matter content = 1.01%; CEC = 1.02 meq/100 g; total nitrogen = 0.53 g/kg; total phosphorus = 0.12 mg/kg; total heterotrophs = 3.5×10^3 CFU/g.

2.2. Microcosm study

Microcosms were constructed to study the effectiveness of the application of enhanced landfarming for TPH-polluted soil cleanup with the supplement of different additives under different biodegradation conditions. The treatment efficiency of the TPH-contaminated soils was evaluated in this study. Table 1 presents the characteristics of seven groups of microcosms, which contained kill control microcosm (A1), live control microcosm (A2), nutrient supplement microcosm (B), fern chips supplement microcosm (C), activated sludge supplement microcosm (D), TPH-degrading bacteria supplement microcosm (E), and compost supplement microcosm (F). Three-hundred mL serum bottle was used to construct the microcosm, and it contained 50 mL deionized (DI) water [or nutrient medium (Group B)], certain amount of amendments, and 150 g of polluted soils. Each bottle was capped with Teflon-lined rubber septa, and 150 mL of air in headspace. The nutrient