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Bioremediation of oil sludge contaminated soil by landfarming with added cotton stalks

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ABSTRACT

A field bioremediation of oil sludge contaminated soil was conducted by landfarming treatment with added cotton stalks in the Shengli oil field. The ability of landfarming treatment was evaluated to reduce petroleum hydrocarbons and restore soil quality. For 39-month landfarming, the initial concentration of total petroleum hydrocarbons (TPH) was 12.57 mg g^{-1} for oil sludge contaminated soil. The removal efficiency of TPH, saturated fraction and aromatic fraction was 68.48%, 90.04% and 85.55%, respectively. Degradation of TPH followed first order exponential decay kinetics. Soil physic-chemical properties of soil pH, saline alkali degree, nutrients, organic matters and hydrocarbon degraders were greatly improved. The results of Biolog and PCR-DGGE analysis revealed the improvement of soil microbial quantity and diversity, and the isolated predominant 23 strains showed a shift in soil community structure toward the hydrocarbon degrading species including *Streptococcus* sp., *Shewanella* sp., *Bacillus* sp., *Pseudomonas* sp., *Marinobactera* sp., *Thermoanaerobacter* sp., etc.

1. Introduction

Oil sludge was mainly from drilling, refining, transportation, and storage of crude oil, which has been classified as a priority environmental pollutant. The annual volume of oil sludge that produced by large petrochemical facilities was about 10,000 m^3 (Gafarov et al., 2006). In China, the environmental risk of oil sludge contaminated sites has been verified through the investigation of vadose zone and groundwater (Jin et al., 2011). In the current technical systems of cleaning up petroleum contaminated sites with large areas, bioremediation appears more practical applicability when compared with other techniques such as solvent washing, ozonation, incineration, landfill and thermal desorption in the consideration of cost-effect and secondary pollution (Ting et al., 1999; Ouyang et al., 2005). Landfarming treatment for petroleum hydrocarbon-contaminated soils has relatively low cost ranging from US$30 to US$70 per ton (Pope and Matthews, 1993; Giasi and Morelli, 2003), and this cost decreases with the development of technology. The previous studies revealed that adding organic matters can enhance bioremediation effectiveness by improving the soil physicochemical properties and indigenous soil microbial activity (Rhykerd et al., 1999; Kriipsalua et al., 2007). However, to date, only a few long-term landfarming projects have been implemented (Mikkonen et al., 2012). In China, there are about 28.63 million tons of cotton stalks produced by 4.57 million planting hectares every year (China Cotton Association, 2013). The available large amount of cotton stalks provides the potential as organic amendments in landfarming treatment of petroleum oil contaminated soils. Wang et al. (2012) proved the primary advantage of biopile constructed with cotton stalks in enhancing TPH removal in soil, as well as the other benefits including lowering remediation cost and reuse of cropping wastes.

In this study, the landfarming treatment of oil sludge contaminated soil was conducted, and the cotton stalks were added for enhancing remediation effectiveness. The objects were to evaluate the ability of this technique to reduce petroleum hydrocarbons and improve soil physical and biological properties. For those purposes, the natural attenuation treatment was used as blank control. The parameters regarding soil quality including physicochemical properties, quantity of total heterotrophic bacteria and petroleum degrading bacteria, microbial diversity, soil residual TPH and petroleum fractions were determined before and after landfarming treatment.

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2. Materials and methods

2.1. Site characterization, contaminated soil and cotton stalk

The demonstrated landfarming of oil sludge contaminated soils located in the Shengli Oil Field in Shandong Province. The site of 13,750 m^2 has been used for open storage of oil sludge for over 43 years. The local climate conditions were as follows: the average annual precipitation was about 642.0 mm, and the average annual temperature was about 12.8 °C ranging from −10.3 °C in January to 35.6 °C in July.

The soil type of demonstration site was sandy clay loam that was composed of 13.7% clay (<0.002 mm), 29.6% silt (0.02–0.002 mm) and 56.7% sand (2–0.02 mm). The total petroleum hydrocarbon (TPH) content in the topsoil (0–60 cm) ranged from 0.2 to 452,000 mg kg^{-1}.

The cotton stalks used in this study were collected from the local planting region and mechanically shredded into a variety of length ranging from 10 to 30 mm. The organic matter content of cotton stalk was about 78.6% by mass ratio, and the total N, P and K content was of 1.09%, 0.15% and 1.68%, of which the available content of N, P and K was 865.6, 687.4 and 5325.1 mg g^{-1}, respectively.

2.2. Landfarming process

Oil sludge contaminated soil was mixed with cotton stalks, and the ratio of soil to cotton stalks was 1:4 (w/w). There were three 100-m^2 plots prepared for landfarming treatment, and one 100-m^2 plot was set for natural attenuation as blank control. Oil sludge contaminated soils and cotton stalks were mixed and laid on the plots with 30 cm layer depth. The remediation duration lasted from June 2009 to November 2012, and the 0~30 cm topsoil was tilling with the frequency of every 2 months to keep soil moisture at 20 ~ 40%.

2.3. Soil sampling and analysis of TPH and petroleum fractions

The remediation plot was divided into 16 different sampling cells based on 2.5 m × 2.5 m grid. The representative 20 cm surface soils were collected, and the sampling intervals were of 0 month (June, 2009), 1 month (July, 2009), 2 months (August, 2009), 4 months (October, 2009), 9 months (March, 2010), 13 months (July, 2010), 16 months (October, 2010), 26 months (August, 2011) and 39 months (September, 2012), respectively.

Soil samples were freeze-dried, grounded, 2-mm mesh sieved and stored at −4 °C. The accelerated solvent extractor (ASE300, Dionex, USA) and trichloromethane were used to extract petroleum hydrocarbons from 10 g soil. Referring to the previous study, the TPH content was determined by gravimetric method, and the petroleum fractions were analyzed by Thin-Layer Chromatography (MK-6S, Tokyo, Japan) (Wang et al., 2010).

2.4. Enumeration of cultivable bacteria and determination of microbial activity

The quantities of total heterotrophic bacteria and petroleum degrading bacteria in the soil samples were assessed by the plate count method. Tryptone Soya Agar (TSA) was used to determine the total heterotrophic bacteria (THB), and the mineral medium with the addition of sterile crude oil was used to determine the petroleum degrading bacteria (PDB) (Chaîneau et al., 1999). The colony-forming units (CFU) were recorded after incubation in the dark at 25 °C on days 5 and 21, respectively.

Microbial community level physiological profiles (CLPPs) were reflected by the patterns of sole-carbon-source utilization using a BIOLOGTM system. In this method, the average well color development (AWCD) was introduced to reflect soil microbial activity, and Shannon index were used to reflect soil microbial diversity (Zak et al., 1994).

2.5. Soil microbial community analyses by PCR-DGGE

The total soil DNA was first extracted using a Power Soil™ DNA Isolation Kit according to the manufacturer's instructions (MoBio Lab Inc., Carlsbad, CA, USA), and then the integrity and purity of DNA were measured using electrophoresis and Nanodrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). PCR amplification targeting bacterial of the V3 region within 16S rDNA was performed with the 357F-GC and 517R primer set (Muyzer et al., 1993). The nested PCR was conducted for amplification of 16S rDNA using primers 27F and 1492R (Lane, 1991).

Denaturing Gradient Gel Electrophoresis (DGGE) for analysis of resulting DNA fragments (15 μL) were conducted according to the method used by Muyzer et al. (1993). The denaturing gradient varied from 45% to 75% for DGGE analysis. Gels were run at 60 V for 16 h in a D-Code System (Bio-Rad) filled with 8% (w/v) polyacrylamide (acrylamide: bisacrylamide, 37:1). The gels were stained in 1:10,000 (v/v) Sybr Green I (Cambrex, Rockland, USA) nucleic acid solution for 30 min. The DGGE images were obtained from the Gel Doc XR system (Bio-RadLab, Segrate, Italy) under UV light. The selected DGGE bands were excised and purified by a TaKaRa Mini BEST DNA Fragment Purification Kit (TaKaRa Bio Inc., Shiga, Japan), and then unidirectional sequenced by the reverse primer. A sequences alignment of the purified 16S rDNA was then conducted with a BLAST program. After entering query sequences, nucleotide collection that was composed of GenBank, EMBL, DDBJ, PDB and RefSeq sequences was chose as the alignment database (http://blast.ncbi.nlm. nih.gov, the accession number (NCBI) of all 23 samples were shown in Table 2).

2.6. Data processing

All data except local mean temperature were expressed as mean ± standard deviation, and the statistical testing of the data was performed with SPSS v. 13.0. The local temperature shown in Fig. 3 was daily maximum temperature, daily mean temperature and daily minimum temperature, respectively. The correlation analysis of residual TPH in soil with remediation time and quantity of hydrocarbon degrading bacteria was performed by Metlab software (v. R2014a), and the optimal regression model with the smallest relative residual standard deviation was chosen.

3. Results and discussion

3.1. Changes of soil physicochemical properties after remediation

Soil properties including texture, cation exchange capacity (CEC), nutrient status, and soil bacteria types and numbers are important factors in influencing the remediation efficiency of petroleum contaminated soils (Atlas, 1981). Significant changes of soil properties were observed after 39-month landfarming (Table 1). Previous studies showed that the improvement of soil properties facilitated biodegradation of petroleum hydrocarbons, for example, Zhang et al. (2008) found that the biodegradation rate of hydrocarbons increased as salinity decreased; and Warr et al. (2009) found that higher soil CEC stimulated the mineralization of aromatic, resin and asphaltene; and Brook et al. (2001) found that the biodegradation of hydrocarbons was enhanced if the C:P ratios were adjusted in the range of 60–800; and low salinity increases biodegradation efficiency of petroleum compounds (Garcia and