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Bioremediation of petroleum-contaminated soil by biostimulation amended with biochar

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ABSTRACT

In this study, the effects of rice straw biochar on soil contaminant biodegradation and microbial community compositions were investigated in the laboratory during a 180-day period. The results of soil microcosm experiments showed that contaminant degradation efficiency was significantly higher in soils amended with biochar than in soils without. The adding time of biochar had apparent effects on degradation efficiency. The removal efficiencies of total petroleum hydrocarbons (TPH) were 61.2%, 77.8% and 84.8%, in the soils without biochar, amended with biochar at the beginning or the 80th day respectively. When adding biochar at the 80th day, the TPH concentration decreased to below the threshold level required for Chinese soil quality for TPH (3000 mg kg^{-1} dry weight) in 140 days. The addition of biochar did not result in appreciable negative impacts on soil microbial community composition. It was speculated that when adding biochar at the 80th day, a large amount of metabolites could be absorbed onto the biochar, leading to significant reduction in soil toxicity and biodegradation enhancement.

1. Introduction

Spills, leaks and other releases of petroleum hydrocarbons can cause large amounts of soil spread pollution, representing a major environmental concern with serious consequences that has been drawing public concerns worldwide over the recent decades (Lu et al., 2010).

Bioremediation of petroleum contaminated soils is a hot area of soil restoration research, because of its relatively low costs and environmentally friendship compared to physical and chemical processes (Grace Liu et al., 2011). This technique has been shown to be effective for petroleum contaminated soils in both laboratory and field tests (Xu and Lu, 2010; Beškoski et al., 2011; Mukherjee and Bordoloi, 2011). However, the wide practice of bioremediation in the field is limited by its low efficiency and long-term maintenance (Trindade et al., 2005). This is especially true for historically contaminated sites where the pollutants mainly consisted of complex compounds with recalcitrant chemical structures and low bioavailability (Huesemann et al., 2004). The hydrophobic nature of oil retard mass transfer of air, water, and contaminants from soil particles to microorganisms, limiting the rate of uptake and metabolism of contaminants by hydrocarbon degraders (Semple et al., 2003).

Some auxiliary measures such as bioslurry treatment (Lu et al., 2009), chemical oxidation (Lu et al., 2010; Gong, 2012), surfactant enhancement (Mata-Sandoval et al., 2002; Urum et al., 2006), were incorporated into biological processes to enhance pollutant bioavailability and/or to reduce substrate toxicity. Biochar produced from biomass may sequester atmospheric CO_2 in soils for a long time, and thus reducing the carbon footprint of sorbent-based soil remediation in comparison with the use of coal-derived activated carbon (Bushnaf et al., 2011). Activated carbon or biochar amendments have been deployed in certain soil and sediment remediation purposes. The use of biochar could be cheaper in a remediation sense relative to AC because the waste source materials are essentially free and the production of biochar requires less energy and cost (Hale et al., 2011). The agronomic benefits of biochar in addition to sequestering C in plant based remediation may also be related to an increment of liming effects, water holding capacity, soil structure, cation exchange capacity, soil microbial activities and finally the plant growth (Glaser et al., 2002; Beesley et al., 2010). However, biochar amendment to degraded soil has

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also been shown to reduce the pollutant's availability for microbial break-down and increase persistence (Rhodes et al., 2008).

In the present work, the feasibility of the use of biochar in petroleum hydrocarbon contaminated soil remediation was assessed. To investigate the effect of adding time, biochar was amended at two different periods respectively, i.e. the beginning or the middle stage of the experiments.

2. Materials and methods

2.1. Contaminated soil

The petroleum-contaminated soil was collected from an oil spill site near a tank container in Shengli Oilfield, China. For sampling, surface litters were removed and soil samples were collected to a depth of 30 cm. The soil was air-dried and sieved through a 2-mm mesh sieve, homogenized by hand with a shovel, and then stored at 4 °C in the dark until used. The texture of the raw contaminated soil was classified as a clay loam, which contained (dry weight basis, d.w.): sand, 23.5%; silt, 51.2%; clay, 25.3%. The soil had the following characteristics: pH (1:2.5, soil/water ratio), 6.5; conductivity (1:10, soil/water ratio), 535 μS cm^{-1}; water holding capacity, 38.6 wt.%; humidity, 15.2 wt.%; total organic carbon (TOC), 5.42 wt.%; total nitrogen 85 mg kg^{-1}; total phosphorus 16 mg kg^{-1}; total heterotrophic bacteria, 7.50×10^6 colony-forming units (CFU) g^{-1}; diesel oil degrading bacteria, 3.60×10^4 CFU g^{-1}; total petroleum hydrocarbons (TPH), 16,300 mg kg^{-1} (saturated hydrocarbons, 8260 mg kg^{-1}; aromatic hydrocarbons, 5130 mg kg^{-1}; polar components, 2910 mg kg^{-1}).

The soil contained a significant amount and proportion of an alkane (saturated) fraction-degrading population (0.48%), suggesting that biostimulation strategy was feasible for this soil matrix. Additionally, the oil contained a relatively high percentage of aromatic fractions (31.5%).

2.2. Biochar

Biochar was produced from rice straw at 500 °C using a slow pyrolysis under limited oxygen according to (Lou et al., 2013). Table 1 lists the characteristics of rice straw feed. Following charring, the biochar was lightly ground and sieved to obtain a fine (<0.16 mm) size fraction, which was then rinsed with distilled water to remove the ash content and dried at 60 °C for 5 days. The sample had a total surface area of 1053 m^2 g^{-1}, TOC content of 83.5 wt.%, C:N:S weight ratio of 325:3.9:1, and a pH of 8.9 ± 0.1 (1:2.5, biochar/water ratio).

Table 1
The main characteristics of rice straw feed.

Composition		Proximate analysis	
Component	Content (wt.%)	Component	Content (wt.%)
Cellulose	57.2	Water	5.3
Hemicellulose	29.6	Volatile matter	65.4
Lignin	13.2	Fixed carbon	16.6
		Ash	12.7
Elemental analysis		**Alkali metal concentration**	
Element	Content (wt.%)	Alkali metal	Content (mg kg^{-1})
C	38.5	Na	273
H	4.9	Mg	1942
N	1.8	Ca	3105
S	0.76	K	23,875
O	54.0		

2.3. Soil microcosm experiments

The soil was subjected to different treatments for an additional 180 days. For each treatment, three independent replicates (2-L glass receptacles covered with perforated parafilm) were prepared as microcosms, each containing 1000 g of soil. In all the treatments, the water content was adjusted to 60% of water holding capacity. Once a week, the microcosm contents were mixed and the soil water content was restored by controlling the weight. Every two weeks, $(NH_4)_2SO_4$ and K_2HPO_4 were added to produce a final C:N:P ratio of 100:10:5 (Gong, 2012). Four different trials were applied in triplicate: soil was supplemented with 2% (w/w) $HgCl_2$ to account for abiotic loss of pollutants (A); soil received no biochar (B); the soil was amended with 2% (w/w) biochar at the beginning (C), and the 80th day (D) of the experiment.

2.4. Analytical methods

Oil in soil was soxhlet-extracted with dichloromethane for 16 h. The extract was condensed to 1 mL in a rotary evaporator and fractionated by silica gel column chromatography to separate saturate, aromatic and polar fractions, following the methods of Bastow et al. (2007). The different elute was evaporated to dryness under N_2, and calculated gravimetrically.

The measurements of *n*-alkanes and polycyclic aromatic hydrocarbons (PAHs) were performed by gas chromatography–mass spectrometry (GC–MS), using a Thermo-Finnigan SSQ710 GC–MS (Thermo Finnigan, San Jose, CA, USA) with a HP-5MS elastic silica capillary columns (60 m × 0.25 mm × 0.25 μm). The carrier gas was helium at 37 kPa. Flow velocity was 1 mL min^{-1}. The analytical conditions were: initial temperature of 50 °C, with isothermal operation for 1 min; heating to 120 °C at a constant rate of 20 °C min^{-1}; and heating to a final temperature of 310 °C at a constant rate of 4 °C min^{-1}, with a 30 min isothermal. Mass spectrometer conditions were: electron impact, electron energy 70 eV; filament current 100 μA; multiplier voltage, 1200 V; full scan.

Concentrations of each *n*-alkane were calculated based on the standard calibration curve of each corresponding standard compound (Accu Standards Inc., New Haven, CT, USA). Individual PAHs were quantified based on the retention time and *m*/*z* ratio of an authentic PAH mixed standard (Sigma–Aldrich, St. Louis, MO, USA), and concentrations of each PAH were calibrated based on the standard calibration curve.

2.5. Microbiological enumeration

The heterotrophic bacteria were counted on nutrient agar after incubation at 30 °C for 2 days, and the results were expressed as CFU per gram of dry soil.

2.6. Microtox® toxicity assay

The toxicity of soil elutriate was determined using the Microtox® bioassay according to Xu and Lu (2010). Toxicity values were the average of five replicates of each filtrate sample, expressed as EC_{50} (15 min, 15 °C), which was defined as the effective concentration of pollutant for a 50% reduction of the luminescence of the bacterium *Photobacterium phosphoreum*.

2.7. DNA extraction, PCR amplification and 454 sequencing

High-molecular-weight DNA from the soil was extracted with a commercially available kit (Beijing Dingguo Biotechnology Ltd., China). The 16S rDNA genes were amplified by polymerase chain reaction (PCR) in a Techgene thermocycler (FTGENE 5D, 112757-4,