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Bioremediation of petroleum-contaminated soil using aged refuse from landfills

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ABSTRACT

This study explored the effects and mechanisms of petroleum-contaminated soil bioremediation using aged refuse (AR) from landfills. Three treatments of petroleum-contaminated soil (47.28 $mg \cdot g^{-1}$) amended with AR, sterilized aged refuse (SAR) and petroleum-contaminated soil only (as a control) were tested. During 98 days of incubation, changes in soil physicochemical properties, residual total petroleum hydrocarbon (TPH), biodegradation kinetics, enzyme activities and the microbial community were investigated. The results demonstrated that AR was an effective soil conditioner and biostimulation agent that could comprehensively improve the quality of petroleum-contaminated soil and promote microbial growth, with an 74.64% TPH removal rate, 22.36 day half-life for SAR treatment, compared with the control (half-life: 138.63 days; TPH removal rate: 22.40%). In addition, the petroleum-degrading bacteria isolation results demonstrated that AR was also a petroleum-degrading microbial agent containing abundant microorganisms. AR addition significantly improved both the biotic and abiotic conditions of petroleum-contaminated soil without other additives. The cooperation of conditioner addition, biostimulation and bioaugmentation in AR treatment led to better bioremediation effects (half-life: 13.86 days; TPH removal rate: 89.83%). In conclusion, AR amendment is a cost-effective, easy-to-use method facilitating in situ large-scale application while simultaneously recycling huge amounts of AR from landfills.

1. Introduction

Spillage of petroleum contaminants into soil often results in diminished soil porosity and permeability, and reduced microbial biomass and nutrient availability (Atlas, 1985, Nie et al., 2011, Tara et al., 2014), posing potential threats to natural fauna and flora. In recent years, bioremediation technology to treat petroleum-contaminated soil has been well developed because it is a thorough, cost-effective and environment-friendly technique compared with other physical and chemical remediation efforts (Couto et al., 2010, Liu et al., 2010).

Most bioremediation treatments of petroleum-contaminated soil fall into two main categories: bioaugmentation and biostimulation. Bioaugmentation refers to the introduction of exogenous petroleum-degrading microorganisms or indigenous petroleum-degrading strains after being isolated and cultured into contaminated soil (Madueño et al., 2011), while biostimulation enhances the metabolic activities of indigenous petroleum-degrading strains by adding nutrients and/or organics amendments (Wu et al., 2016). Soil conditioners are also used to promote the biodegradation of petroleum contaminants by improving soil pH, aeration, water availability, and electron acceptor and pollutant accessibility (Varjani and Upasani, 2017). The biodegradation capacities of petroleum contaminants is affected by many physicochemical and biological factors such as soil porosity, moisture, pH, nutrients, the presence of petroleum-degrading bacteria, pollutant concentration and bioavailability, etc (Akbari and Ghoshal, 2015, Sun et al., 2015, Varjani and Upasani, 2017, Warr et al., 2009). Therefore, it is difficult to achieve effective bioremediation of petroleum-contaminated soil if only a single soil conditioner, bioaugmentation or biostimulation approach is used. To improve the effectiveness of bioremediation, researchers often implement several soil conditioner amendment, bioaugmentation and biostimulation measures simultaneously (Varjani and Upasani, 2017, Xu et al., 2016), which substantially increases the cost of bioremediation. In addition, there are many problems with bioremediation, such as expensive microbial agents, long-term maintenance and poor effects in severely petroleum-contaminated soil. These problems limit the large-scale application of bioremediation. Therefore,

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further studies are needed to reduce the cost and enhance the effects of bioremediation.

Previous studies showed that adding substances with large specific area, high porosity and abundant organic matter, nutrients and microorganisms, such as cinder powder (Huang et al., 2015), activated carbon (Liang et al., 2009), sawdust, agricultural wastes (Chen et al., 2016, Shahsavari et al., 2013, Wang et al., 2016), and sewage sludge (Aburto-Medina et al., 2012), can significantly enhance the efficiency of bioremediation. With these amendments, the bioavailability of petroleum, microbial living environment, and/or catabolic activity are subsequently improved. A number of studies demonstrated that aged refuse (AR, bio-stabilized through years in the landfill) (Xie et al., 2012) has high porosity, high moisture retention, large specific area, and abundant organic matter in addition to essential nutrients for microbial growth. AR also provides pH buffering capability and additional microbial biomass and diversity for soil, which favors the biodegradation of petroleum substances. Therefore, AR is a potential medium to remediate petroleum-contaminated soil.

Furthermore, sanitary landfill is one of the most popular municipal solid waste treatments (Renou et al., 2008). Recycling AR to remediate polluted soil can not only reclaim the land area and volume of landfills, but also reduce the cost of soil remediation. Because AR is abundant, it can be added to petroleum-contaminated soil in a high proportion to dilute the petroleum level and this technique can be applied on a large scale. However, AR has mainly been investigated as an adsorbent (Lou et al., 2009), an organic fertilizer (Li et al., 2008) and a medium for wastewater treatment (Sun et al., 2017, Wang et al., 2012). Using AR from landfills to remediate polluted soil has rarely been considered.

Therefore, the primary objectives of this study were to evaluate the bioremediation effects of petroleum-contaminated soil with AR addition, and to study the underlying mechanisms of AR on petroleum degradation. We conducted a lab-scale experiment in a walk-in environmental chamber and monitored changes in residual petroleum contents, physicochemical properties, enzyme activities, microbial biomass and microbial community structure in treatments with and without AR addition. High-throughput sequencing and real-time quantitative PCR (RT-qPCR) technologies were applied to identify and quantify bacteria and fungi in samples. Further, to identify the role of exogenous strains in the degradation of petroleum contents, petroleum contaminated soil with sterilized AR (SAR) was also investigated.

2. Materials and methods

2.1. Materials

Clean soil samples were collected from the top 20 cm of an agricultural field located in Jiangyou, China (latitude, 31°44′N; longitude, 104°44′E). The soil was sandy loam and consisted of 66.75% clay (<0.002 mm), 21.86% silt (0.02–0.002 mm) and 11.39% sand (2–0.02 mm). Used crude oil, with a 69.41% saturated fraction, 3.27% aromatic fraction and 27.32% polar fraction, was collected from the East Sichuan Drilling Branch of China National Petroleum Corporation. Before the experiment, clean soil samples were air dried and sieved through 2 mm screens. Then, clean soil was spiked with crude oil at concentrations of 50 mg·g^{-1} dry soil, air dried for 4 days to remove the volatile components of petroleum, and homogenized. The AR was stabilized domestic waste and excavated from the Yibin municipal sanitary landfill in Sichuan, China, and had been buried for 8 years. AR were sieved to remove particles larger than 10 mm in diameter and stored in polyethylene bags before use. It was odorless and had a uniform particle size. The heavy metal contents of AR were lower than the limitation of II level standard of environmental quality standard for soils (GB15618-1995). The AR characteristics are shown in Table 1.

2.2. Experimental set-up

Experiments were conducted in 1 L plastic tubes 14 cm in height and 11 cm in diameter. SAR was prepared via autoclaving AR at 121 °C and 15 psi for 1 h. Three experimental treatments were selected, with each treatment triplicated:

- Control treatment (Ctrl, with 500 g (dry weight) petroleum-contaminated soil only);
- SAR treatment (Exp-SAR, with 250 g (dry weight) petroleum-contaminated soil + 250 g (dry weight) SAR for biostimulation);
- AR treatment (Exp-AR, with 250 g (dry weight) petroleum-contaminated soil + 250 g (dry weight) AR for biostimulation and bioaugmentation)

All treatments were incubated in a walk-in environmental chamber at 25 °C for 98 days. During incubation, the content of each vessel was tilled once every four days for aeration, and the moisture content was maintained at 15% by weight method with the periodic addition of sterile distilled water. Samples (40 g) were taken by five-point cross sampling method every two weeks over the incubation period. Half of the sample was freeze-dried and sieved through 0.25 mm screens to analyse petroleum hydrocarbons. The other half of sample was air dried and sieved through 2 mm screens to analyse enzyme activities.

2.3. Soil physicochemical properties and ecotoxicity analyses

Total porosity was calculated from bulk density assuming a particle density of 2.65 g·cm^{-3} and 98% saturation (Bao, 2005). Maximum water holding capacity was measured by the gravimetric method according to Bao (2005). Soil pH was determined from a soil water suspension (1:5 w/v) by using a pH meter (Cornfield, 1960). Wet oxidation and titration was used to measure organic matter (Nelson and Sommers, 1982). Total P was measured by the phosphovanado-molybdate method according to Page et al. (1982). Total N was measured by a modified Kjeldahl method (Bremner and Mulvaney, 1982). The methods of ammonium acetate, alkaline hydrolysis diffusion and sodium bicarbonate were

Table 1
Physicochemical properties of the experimental AR.

Parameters	Value
Water content (%)	28.33 ± 0.79
Bulk density (g·cm^{-3})	0.79 ± 0.02
pH	7.63 ± 0.10
CEC (mmol·kg^{-1})	398.73 ± 6.14
Total P (g·kg^{-1})	1.51 ± 0.02
Total N (g·kg^{-1})	3.60 ± 0.02
OM (%)	13.89 ± 0.74
Extractable humus C (g·kg^{-1})	34.52 ± 2.61
Biologically degradable matter (%)	8.21 ± 0.43
Total Hg (mg·kg^{-1})	0.25 ± 0.16
Total Pb (mg·kg^{-1})	115.15 ± 3.88
Total Cd (mg·kg^{-1})	Not detected
Total Cr (mg·kg^{-1})	193.72 ± 11.56
Total Cu (mg·kg^{-1})	129.30 ± 1.71
Total Ni (mg·kg^{-1})	49.32 ± 5.68
Total Zn (mg·kg^{-1})	267.49 ± 14.14
FDA hydrolytic activity (μg·g^{-1}·h^{-1})	125.34 ± 3.93
Dehydrogenase activity (μg·g^{-1}·h^{-1})	0.18 ± 0.01
Polyphenol oxidase activity (mg·g^{-1}·h^{-1})	1.16 ± 0.06

Data represent the mean and standard deviation of triplicate samples.
"OM" represents organic matter; "CEC" represents cation exchange capacity.

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