Biochemical Engineering Journal 112 (2016) 170–177

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Comparison of phytoremediation, bioaugmentation and natural attenuation for remediating saline soil contaminated by heavy crude oil

Bin Cai [1], Jie Ma [1], Guangxu Yan *, Xiaoli Dai, Min Li, Shaohui Guo

State Key Laboratory of Heavy Oil Processing, Beijing Key Laboratory of Oil & Gas Pollution Control, China University of Petroleum—Beijing, 18 Fuxue Road, Changping District, Beijing 102249, PR China

ARTICLE INFO

Article history:
Received 18 December 2015
Received in revised form 8 March 2016
Accepted 19 April 2016
Available online 23 April 2016

Keywords:
Bioremediation
Heavy crude oil spill
Saline soil
Biodegradation
Microbial growth
Waste treatment

ABSTRACT

Bench-scale pot culture systems were used to investigate the effectiveness of phytoremediation (*Testuca arundinacea*), bioaugmentation (5 oil-degrading strains), and natural attenuation for remediating saline soil contaminated by Venezuela heavy crude oil. GC–MS was used to have a comparative characterization for chemical composition changes of aromatic groups (polycyclic aromatic hydrocarbons (PAHs), sulfur-containing heterocycles (SCHs) or polycyclic aromatic sulfur heterocycles (PASHs), and aromatic biomarkers) during different remediation processes. Bioaugmentation had faster startup (higher total petroleum hydrocarbon (TPH) removal efficiency on Day 30) while phytoremediation had higher TPH removal efficiency at the end of experiments (90 days). The 90-day TPH removal efficiency of phytoremediation ($64.0 \pm 1.6\%$) was significantly higher ($p < 0.05$) than that of bioaugmentation ($54.6 \pm 1.3\%$) which was significantly higher ($p < 0.05$) than that of natural attenuation ($20.7 \pm 2.8\%$). GC–MS analysis shows that the removal efficiencies of most of individual PAH and SCH compounds by phytoremediation were also significantly higher ($p < 0.05$) than bioaugmentation which were significantly higher ($p < 0.05$) than natural attenuation. GC–MS analysis also shows that the removal efficiencies of individual PAH and SCH compounds for all three treatments (phytoremediation, bioaugmentation, and natural attenuation) decreased with increases in ring number and degree of alkyl substitution. Overall, this study shows that phytoremediation with *T. arundinacea* and bioaugmentation with a halotolerant microbial consortium are two effective approaches for remediating saline soil contaminated by heavy crude oil.

1. Introduction

The use of heavy crude oil is rapidly growing to meet the escalating energy demand all over the world. Compared to conventional crude oil, heavy crude oil has much higher density, and viscosity, and much higher contents of high molecular weight (HMW) hydrocarbons, heterocyclic compounds and heavy metals [1]. Incidental and accidental oil spill is a common occurrence and the likelihood of encountering heavy oil in the spill is increasing [2–4]. Heavy oil spill may cause serious environmental damages and is extremely difficult to clean up [3,5]. Therefore, there is an urgent need to develop effective remediation techniques for addressing heavy oil spills.

Bioremediation is cost-effective, environmental-friendly, simple to maintain and applicable over large areas [6,7]. The successful application of bioremediation techniques including bioaugmentation, biostimulation and phytoremediation for remediating oil spills has been reported by numerous studies [8–11]. However, most of these studies focused on conventional crude oil (e.g. Exxon Valdez spill and Deepwater Horizon spill) or refined fuel oil (e.g. Prestige spill) [7,12–14], which obviously are more readily degradable and easier to be cleaned up. Relatively few studies investigated the effectiveness of bioremediation in remediating heavy crude oil spills [4,15–17].

Another knowledge gap is that previous bioremediation studies on heavy crude oil usually monitored bulk parameters such as total petroleum hydrocarbons (TPHs) concentrations [18], or focused on the negative impact on microbial populations and plants [4]. Detailed characterization on changes in chemical composition of heavy oil during remediation process has never been reported. Considering the highly complex components of heavy crude oil, a more in-depth characterization of its chemical composition would undoubtedly reveal previously unrecognizable level of chemical

* Corresponding author.
E-mail address: yangx0919@163.com (G. Yan).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bej.2016.04.018
1369-703X/

diversity, thus providing a more complete and accurate picture of *in situ* chemical transformation process in impacted environments.

Heavy crude oil has much higher sulfur contents than conventional crudes and fuels. Most of the sulfur is present in aromatic structures, especially as sulfur-containing heterocycles (SCHs) or called polycyclic aromatic sulfur heterocycles (PASHs), which are structurally analogous to toxic polycyclic aromatic hydrocarbons (PAHs) [19,20]; see chemical structures illustrated in Fig. S2. Since SCHs are usually the most abundant heterocycles in heavy crude oil, improved understanding on their fate and transport process especially biodegradation process is of great importance. However, biodegradation of these SCHs of heavy crude oil was neglected by previous studies. In particular, degradation of SCHs during phytoremediation process has never been reported.

In addition, oil spill sometimes occurs in saline soil environments [15,21]. This will pose more constrains on degrading microorganism/plant species to tolerate both contamination and salinity, and may have negative impacts on degradation of heavy crude components. This may further raise the challenge for bioremediation attempts. To improve our understanding on these questions, pot-culture experiments were conducted to evaluate the effectiveness of a halotolerant microbial consortium and a salt-tolerant turf grass, tall fescue (*Testuca arundinacea*) in remediating saline soil contaminated by heavy crude oil. TPHs removal efficiency and microbial growth in soil were monitored for three different treatments (phytoremediation, bioaugmentation, and natural attenuation). Gas Chromatograph-Mass Spectrometer (GC–MS) was used to further characterize changes in chemical composition of aromatic groups (PAHs, SCHs and aromatic biomarker compounds) during these processes.

2. Materials and methods

2.1. Microbial consortium for bioaugmentation

A halotolerant microbial consortium, which consists of five oil-degrading strains, was used as bioaugmentation agent in this study. These oil-degrading microorganisms were isolated from oil-contaminated saline sediments in Xingang port of Dalian City in China. Artificial seawater medium (ASM) containing 1% (w/w) of heavy crude oil was used as a saline enrichment culture for strain isolation. The heavy crude oil, which was imported from Venezuela, contained 27% aliphatics, 48% aromatics, 17% resins, and 8% asphalthenes. The oil degradation efficiency of the isolated strains was measured. The taxonomy of these strains was identified by 16S rRNA sequencing (Table S1). Details on strain isolation, oil degradation efficiency measurement, and 16S rRNA sequencing are summarized in supplementary materials. More details on strain isolation and characterization were provided in another manuscript in preparation [22].

Inoculum of microbial consortium was prepared just before the experiments began. Five selected strains were pre-grown aerobically in Luria Bertani (LB) broth at 28 °C in a rotary shaker (150 rpm) for 12–24 h. Cell growth was monitored by measuring the optical density at 600 nm (OD_{600}) using a spectrophotometer (UV-2800, UNICO, USA). Pure cells were harvested and suspended in sterile NaCl solution (0.9%, w/w). Then the consortium was prepared by mixing equal proportions of cell suspensions of the five strains (OD_{600} value of 0.9).

2.2. Plant species for phytoremediation

Tall fescue (*T. arundinacea*) is a cool-season turf grass with moderate tolerance to salinity and hydrocarbon contaminants, so it was selected as the plant for phytoremediation [16,23]. Seeds were obtained from the Jintudi Research Institute for Agricultural Technology (Beijing, China). Seeds with uniform plumpness were selected for phytoremediation treatment.

Table 1
Characteristics of contaminated soil.

Soil property	Value
pH	7.6
Electrical conductivity (EC)	4.7 ms cm^{-1}
Organic matter	31.2 ± 2.3 g kg^{-1} dry soil
Total organic carbon	16.1 ± 0.8 g kg^{-1} dry soil
Total nitrogen	540 ± 23 mg kg^{-1} dry soil
Carbon/nitrogen ratio	29.8:1
Total phosphorus	2.3 ± 0.4 mg kg^{-1} dry soil
Total potassium	270 ± 41 mg kg^{-1} dry soil
Total sulfur	247 ± 14 mg kg^{-1} dry soil
Total petroleum hydrocarbons (TPHs)	$12{,}113 \pm 68$ mg kg^{-1} dry soil

2.3. Soil sample

Clean coastal soil from uncontaminated area in Xingang port was collected. The soil was air-dried at room temperature for 2 days and sieved to remove particles larger than 2 mm. Then the soil was spiked with Venezuelan heavy crude oil sample, which was also used for strain isolation. The spiked-soil was mechanically homogenized in a fume hood under the darkness for two weeks to mimic oil weathering process. It allowed loss of some volatile hydrocarbons and enhanced contact of oil components with soil matrix, thus making the properties of artificially contaminated soil more like real weathered soils. The final total petroleum hydrocarbons (TPHs) concentration in soil was 12114 ± 68 mg kg^{-1}, which represent the high end of TPHs concentrations at Xingang port site. Other characteristics of the artificially contaminated soil can be found in Table 1.

2.4. Pot-culture experiment

Bench-scale pot-culture experiments were conducted to investigate the effectiveness of bioaugmentation (by microbial consortium) and phytoremediation (by *T. arundinacea*) for remediating saline soils contaminated by heavy oil. Plastic pots, each packed with 1.5 kg of artificially contaminated soils (12114 ± 68 mg TPH kg^{-1}), were prepared for three different treatments: bioaugmentation, phytoremediation and natural attenuation. Each treatment was carried out in triplicate pots. Bioaugmentation treatment was prepared by inoculating with 10 mL oil-degrading microbial consortium culture in each pot. Phytoremediation treatment was prepared by sowing with 30 tall fescue seeds in each pot. The seeds were surface-disinfected by immersing in NaClO solution (0.3% m/v) for 5 min and washed with distilled water before sowing [24]. Natural attenuation treatment was prepared with artificially contaminated soil without adding microbial consortium or growing tall fescue. Pots of all three treatments were placed randomly in a greenhouse with natural light at room temperature (17–29 °C) and irrigated to keep a water holding capacity of 60–70% throughout the experiment. All pots were fertilized with Hoagland solution (its component was given in supplementary materials) every two weeks. The pot-culture experiment lasted for 90 days.

2.5. TPHs measurement and microbial cell counting

To measure TPH concentration and microbial cell number in the soil, triplicate soil samples were collected from each pot on Day 0, 30, 60 and 90 of the experiment. In the phytoremediation pot, soil in the rhizosphere zone was carefully collected to avoid any damage