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Biodegradation of marine oil spill residues using aboriginal bacterial consortium based on *Penglai 19-3* oil spill accident, China

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ABSTRACT

Bioremediation, mainly by indigenous bacteria, has been regarded as an effective way to deal with the petroleum pollution after an oil spill accident. The biodegradation of crude oil by microorganisms co-incubated from sediments collected from the Penglai 19-3 oil platform, Bohai Sea, China, was examined. The relative susceptibility of the isomers of alkylnaphthalenes, alkylphenanthrenes and alkyldibenzothiophene to biodegradation was also discussed. The results showed that the relative degradation values of total petroleum hydrocarbon (TPH) are 43.56% and 51.29% for sediments with untreated microcosms (S-BR1) and surfactant-treated microcosms (S-BR2), respectively. TPH biodegradation results showed an obvious decrease in saturates (biodegradation rate: 67.85–77.29%) and a slight decrease in aromatics (biodegradation rate: 47.13–57.21%), while no significant difference of resins and asphaltenes was detected. The biodegradation efficiency of alkylnaphthalenes, alkylphenanthrenes and alkyldibenzothiophene for S-BR1 and S-BR2 samples reaches 1.28–84.43% and 42.56–86.67%, respectively. The efficiency of crude oil degradation in sediment with surfactant-treated microcosms cultures added Tween 20, was higher than that in sediment with untreated microcosms. The biodegradation and selective depletion is not only controlled by thermodynamics but also related to the stereochemical structure of individual isomer compounds. Information on the biodegradation of oil spill residues by the bacterial community revealed in this study will be useful in developing strategies for bioremediation of crude oil dispersed in the marine ecosystem.

1. Introduction

Oil spill has been a worldwide challenge in the modern society, which not only causes substantial economic loss, but also poses serious threats to the environmental and human health. Oil spills may be due to releases of crude oil from tankers, offshore platforms, drilling rigs and wells, as well as spills of refined petroleum products and their by-products. On June 4, 2011, the *Penglai 19-3* oilfield caused an oil spill from a sea floor leak that lasted until June 7, with 763 barrels of crude oil flowing into the Bohai Sea, China. It was reported that 840 square kilometers of clean coastal water were polluted due to this oil spill accident By June 17 (Liu et al., 2015). Due to the semi-enclosed characteristics of Bohai Sea, its water exchange with the open sea is limited, which in turn leads to a larger accumulation of pollutants for a long time (Hu et al., 2011). At present, petroleum hydrocarbons are considered as the third most common pollutant followed by nitrogen and phosphorus in this region (SOAC, 2015). Such oil pollutants not only damage the natural environment and ecological resources of Bohai Sea, but also pose a serious threat to human health (Li et al., 2015; Wang et al., 2018).

Biological treatments (bioremediation) is recognized as the most preferred measures on removal of oil because they are generally cost effective and environmentally friendly (Braddock et al., 1997; Ghazali et al., 2004; Camilli et al., 2010; Zhang et al., 2011; Bacosa et al., 2015; Borah and Yadav, 2017). Although a lot of efficient crude oil degrading bacteria have been isolated, such bacteria may thrive in one environment but may not be able to compete with other microorganisms in another complicated ocean environments. In the past few decades, a variety of crude oil degrading bacteria have been isolated from the oil-polluted locations (Pasumarthi et al., 2013; Wang et al., 2013; Hassanshahian and Boroujeni, 2016; Borah and Yadav, 2017). The bioremediation of the laboratory-cultured functional bacteria is limited to the marine environment (Wang et al., 2013). By contrast, the natural biodegradation processes of indigenous bacteria were believed to play a dominant role on the bioremediation. As we well known, 16 US EPA priority PAHs from oil spill may pose serious threats to coastal habitats

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due to more toxic effects (Pongpiachan et al., 2018). Detailed knowledge of PAHs biodegradation can provide some useful information for further understanding of their environmental fate and potential ecotoxicological effect.

A profound knowledge of the degradation mechanism, efficiency and biodegradation potential of spilled oil in marine ecosystem is thus essential for ecological risk assessment as well as for developing contingency plans for oil spill response. There are many studies about biodegradation of crude oil, but few of them focus on marine oil spill residues using aboriginal bacterial consortium based on a famous large oil spill accident, such as Penglai 19-3 oil spill accident, China. The use of natural microbial remediation on residual oil spill is one of the effective strategies for the restoration ecological environment in Bohai Sea. Overall, the main objectives of this study are (1) investigated the extent of degradation of *n*-alkanes and PAHs; (2) assess the biodegradation effectiveness of crude oil by indigenous bacteria based on *Penglai 19-3* oil spill accident; and (3) relative susceptibility to biodegradation of individual isomers (mono, di, and tri) within specific compound classes.

2. Experimental

2.1. Materials and biodegradation experiment setup

Two different microcosm assemblages were set up to conduct the oil bioremediation experiment in this study. The used crude oil was collected from the oil platform in oil-gas mining area of central Bohai Sea. The sediment sample collected from the sea area near *Penglai 19-3* oil platform was used for culture isolation. TOC contents of surface sediments in Bohai Sea is 0.23 wt%. The sediment components are mainly composed of sand (53.26%) and silt (38.28%). For biodegradation experiment 1, aliquots (2% w/v) of crude oil and 10 g sediments were co-incubated in 100 mL mineral solution. Refer to the natural living conditions of bacteria in studied area, aerobic bacteria and inorganic salt concentration of aqueous medium were chosen in this study. The mineral media composition was as specified (concentration in g/L): $(NH_4)_2PO_4$ (1), KH_2PO_4 (0.5), Na_2HPO_4 (0.075), $MgSO_4{\cdot}7H_2O$ (0.2), $NaCl_2$ (30) and $CaCl_2$ (0.02). Furthermore, the assemblage (experiment 2) with additional Tween-20 (1%, v/v) was simultaneously performed in order to evaluate the effects of surfactant on bioremediation. Such mixtures in flask were incubated on a constant temperature shaker (120 rpm) and 30 °C for 28 days, and then 15 mL inoculum was transferred to a fresh mineral salt medium and incubated for another cycle. Triplicate treatments were maintained for every sample as well as biotic and abiotic controls. After consecutive transfers, hydrocarbon degraders were isolated by plating on LB solid plates. The effective degradation strains of spilled oil were screened. At the end of the experiment, the original sediments adding crude oil (S0) and samples collected at the end of the incubation for both bioremediation experiments 1 (S-BR1) and experiments 2 (S-BR2) were kept at − 80 °C for further analysis. Bacterial populations associated with oil bioremediation were detected by the analysis of 16S rRNA gene based DGGE and clone library analysis. The detail information on the changes of bacterial communities associated with oil degradation will be discussed in other papers.

2.2. Extraction, fractionation

Treatments were freeze-dried, extracted and analyzed after biodegradation experiment. Samples were extracted and analyzed according to the methods that were established at the National Laboratory for Environmental Testing, Environment Canada (1994). Detailed information on sample collection, treatment and analysis were also reported in previous studies (Hu et al., 2011; Wang et al., 2011, 2017). Briefly, extraction was performed using Soxhlet method with dichloromethane and methanol (93:7) as the solvent mixture for 72 h. The aliphatic hydrocarbons, aromatic hydrocarbons and resins were separated by column chromatography using activated alumina and silica gel, and gradient solvents as eluent: ligarine, ligarine/dichloromethane (3:9, v/v) and MeOH, respectively. Prior to analysis of the fractions by the instrumental analysis, the samples volume were adjusted to 1 mL by evaporation using a stream of filtered N_2 gas. The recoveries of crude oil group varied from 89.54% to 93.75%. In addition, the recovery of crude oil components has increased by 4.16% affected by the addition of Tween-20.

2.3. Instrumental analysis

The instrumental analysis was performed on a Hewlett-Packard 6890 gas chromatograph interfaced with a Hewlett-Packard 5973 mass-selective detector made by Agilent (USA). Helium was used as the carrier gas. *n*-Alkanes and some PAHs were identified by GC-MS in full scan mode. Sample extracts were injected in a splitless mode onto H-5 fused capillary column (30 m × 0.2 mm i.d) coated with 0.25 μm thick. The gas chromatograph oven temperature was programmed at 80 °C for 2 min and ramped at 4 °C/min to a final temperature of 290 °C held for 30 min. The mass spectrometer was operated at an electron energy of 70 eV with an ion source temperature of 250 °C.

Individual *n*-alkanes were identified based on the retention time of the authentic standards ($nC_{10–40}$, Sigma). On the other hand, individual PAHs were quantified based on the retention time and *m/z* ratio of an authentic PAH mixed standard (Sigma). The minimum method detection limits for an individual aliphatic or aromatic compound is 10 ppb. Relative abundance was calculated from respective mass chromatogram peak areas. Five surrogate standards (naphthalene-d8, phenanthrene-d10, dibenzothiophene-d10, fluoranthene-d10, pyrene-d10) were added to all samples to monitor matrix effects. The calibration curves of PAHs were fitted by peak area of the blank samples spiked with 10, 20, 40, 60, and 80 ng/g standards. The average recoveries of surrogate standards varied from 81.6% to 92%. For detailed analytical procedure, conditions, and quality control refer to previous studies (Hu et al., 2011; Wang et al., 2017).

2.4. Statistical analysis

Repeat values of the sample were presented as the mean ± standard deviation (SD). Statistical analysis was performed with SPSS Version 11.5 analysis. Differences were considered significant at $p < 0.05$. The tests were considered statistically significant if $P < 0.05$. In this paper, we use the average value of triplicate samples in order to better use graph to display the law of data change.

3. Results and discussion

3.1. Bioremediation of crude oil fractions

Crude oil can be divided into four main fractions with a silica gel G column: saturated hydrocarbons (Sat), aromatic hydrocarbons (Aro), resins (Res) and the asphaltenes (Asp) according to their polarizability and polarity. In this study, the relative degradation values of total petroleum hydrocarbon (TPH) are 43.56% and 51.29% for sediments with untreated microcosms (S-BR1) and surfactant-treated microcosms (S-BR2), respectively. The relative abundance of saturated hydrocarbons decreased sharply from 45.30% to 26.28% and 21.60% in S-BR1 and S-BR2 samples, respectively. For aromatic hydrocarbons fraction, the relative abundance decreased slightly from 26.10% to 24.9% and 23.46% in the S-BR1 and S-BR2 sediments, respectively. In contrast, the relative abundance of resins and asphaltene fractions increased dramatically (Fig. 1a). The aromatic fraction (biodegradation rate: 47.13%, 57.21%) is generally more resistant to biodegradation than the fraction of saturated hydrocarbons (biodegradation rate: 67.85%, 77.29%). The saturated hydrocarbons fraction showed the