Marine Pollution Bulletin 71 (2013) 269–275

Contents lists available at SciVerse ScienceDirect

Marine Pollution Bulletin

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

Rhamnolipids enhance marine oil spill bioremediation in laboratory system

Qingguo Chen [a,b], Mutai Bao [b,*], Xiaoning Fan [b], Shengkang Liang [b], Peiyan Sun [c]

[a] College of Marine Science and Technology, Zhejiang Ocean University, Zhoushan 316004, China
[b] Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao 266100, China
[c] North China Sea Environmental Monitoring Center of State Oceanic Administration, Qingdao 266033, China

ARTICLE INFO

Keywords:
Bioremediation
Rhamnolipids
Normal alkanes
Polyaromatic hydrocarbons
Biomakers

ABSTRACT

This paper presents a simulated marine oil spill bioremediation experiment using a bacterial consortium amended with rhamnolipids. The role of rhamnolipids in enhancing hydrocarbon biodegradation was evaluated via GC–FID and GC–MS analysis. Rhamnolipids enhanced total oil biodegradation efficiency by 5.63%, with variation in normal alkanes, polyaromatic hydrocarbons (PAHs) and biomakers biodegradation. The hydrocarbons biodegradation by bacteria consortium overall follows a decreasing order of PAHs > *n*-alkanes > biomarkers, while in different order of PAHs > biomarkers > *n*-alkanes when rhamnolipids was used, and the improvement in the removal efficiency by rhamnolipids follows another order of biomarkers > *n*-alkanes > PAHs. Rhamnolipids played a negative role in degradation of those hydrocarbons with relatively volatile property, such as *n*-alkanes with short chains, PAHs and sesquiterpenes with simple structure. As to the long chain normal alkanes and PAHs and biomakers with complex structure, the biosurfactant played a positive role in these hydrocarbons biodegradation.

1. Introduction

The demand of petroleum as an energy source increases with the increasing worldwide industrialization. Oil spill occurs frequently around the world in the petroleum exploitation and transportation (Brito et al., 2009; Joo et al., 2008). Large amount oil was released into the sea in oil spills and it has certainly done devastating effects on the marine environment. Physico-chemical methods are not completely effective in oil spill removal (Prince et al., 2003). Bioremediation, with the advantages such as low cost and environmentally-friendliness, bioremediation could be an alternative to solve oil pollution problem and is currently receiving favorite publicity (Rahman et al., 2002). Some studies have been reported to successfully use bioremediation for spilled oil (Atlas, 1995; Greenwood et al., 2009; Hii et al., 2009; Mearns, 1997; Radwan et al., 2005).

The bioavailability of petroleum hydrocarbons is limited since they are mostly insoluble in water. Surfactant amendment may enhance the oil mobility and thereby improve the biodegradation of petroleum (Laha et al., 1995). Microbial biosurfactants, as the naturally occurring surfactants, can also exert some influence on interfaces in both aqueous solutions and hydrocarbon mixtures (Bordoloi and Konwar, 2009). This will increase insolubility of these hydrocarbons and emulsify hydrocarbon–water mixtures (Banat, 1995), resulting in a growth of oil-degrading bacteria and an improvement in bacterial ability to utilize hydrocarbons (Ron and Rosenberg, 2002; Bao et al., 2012).

Rhamnolipid biosurfactants have been widely reported to enhance petroleum hydrocarbons biodegradation (Mulligan et al., 2001). They were used to improve the bioavailability of crude oil, diesel, or PAHs (Bordoloi and Konwar, 2008; Providenti et al., 1995; Whang et al., 2009) in different places such as wastewater, contaminated soil and marine environments (Nikolopoulou and Kalogerakis, 2008; Sponza and Gök, 2009; Whang et al., 2008). These studies mainly focused on the improvement of degrading efficiency on total *n*-alkanes or total PAHs by rhamnolipids, and none of them reported the function of rhamnolipids in specific *n*-alkane, PAH or biomarker biodegradation in oil samples.

This work presents a simulated experiment using rhamnolipids to enhance oil bioremediation with a bacterial consortium. The function of rhamnolipids in the main petroleum hydrocarbon components biodegradation was explored by gas chromatography.

2. Materials and methods

2.1. Materials

2.1.1. Oil sample for biodegradation

The crude oil was obtained from Shengli Oilfield of China. This light crude oil has a viscosity of 22.2 m Pa s (determined at 50 °C in 50 r min^{-1}) and a density of 0.855 g cm^{-3}. It was pretreated following these steps before used: (1) 10 g crude oil was dissolved by 200 ml petroleum ether (boiling point of 30–60 °C, after aromatics

* Corresponding author. Tel.: +86 532 66782509; fax: +86 532 66782540.
E-mail address: mtbao@ouc.edu.cn (M. Bao).

0025-326X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.marpolbul.2013.01.037

removal treatment) and was filtered by qualitative filter paper in order to remove insoluble material, (2) the filtrate was dried by sodium sulfite for 12 h and filtered again to remove sodium sulfite hydrates, (3) the filtrate was heated in water bath (temperature of 65 ± 5 °C) to distill most of the petroleum ether, and (4) the treated oil sample was placed in vacuum desiccation chamber at 65 °C to remove the remaining petroleum ether.

2.1.2. Seawater sample

The seawater used for oil spill bioremediation experiment was collected from the Bathing Beach of Qingdao, Shandong Province, China. It has a pH of 8.1 and a salinity of 32.1‰.

2.1.3. Rhamnolipids

Rhamnolipids were produced by *Pseudomonas* sp.0-2-2, which was previously isolated in our laboratory, the purification of the rhamnolipids used in this study has been described previously (Wang et al., 2005). Rhamnolipids solutions (0.1 g L^{-1}) was prepared by dissolving 0.1 g of purified Rhamnolipids into 1 L of distilled water.

2.1.4. Bacterial consortium used for bioremediation

The bacterial consortium used in this study was isolated from seawater near Qingdao port of China, which consists of four strains including N1, N2, N3 and N4. N1 is affiliated to *Ochrobactrum* sp., while N2, N3 and N4 are affiliated to *Brevibacillus* sp. The nucleotide sequences of 16S rDNA of N1, N2, N3, and N4 were stored in the GenBank database with an accession number of HQ231209, HQ231210, HQ231211 and HQ231212. The optimum growth conditions for the four bacteria are pH of 7.5–8.5 and temperature of 15–30 °C. They show high crude oil degradation efficiency at salinity of 20–35‰. The consortium of the four strains has a strong ability to utilize the crude oil, and the oil removal efficiency was up to 74.8%.

The bacterial cells were obtained by centrifugation at 6150 times gravitational acceleration centrifugal force after the bacterial consortium incubated in LB culture broth for 3 days. The obtained cells were washed by distilled water and centrifuged for 3 times to remove the residual nutrition in the culture broth. These purified cells was mixed with phosphate buffer to get the bacterial suspension, which was 1.5 of absorbance adjusted via absorbance at 600 nm (Bao et al., 2010), then the bacterial suspension was ready for use.

2.1.5. Media

The medium for biodegradation is 1 L of seawater sample spiked with 2 g of pre-treated oil sample. They were sterilized in an autoclave at 120 °C before use. Three media used in oil spill bioremediation include: (1) 1 L of the described medium for control (MC), (2) 1 L of MC with 10 mL bacterial consortium suspension (prepared in Section 2.1.4) for bacterial control (BC), and (3) 1 L of BC with 10 mL rhamnolipids solutions prepared in Section 2.1.3 for bacterial control added with rhamnolipids (BCR). The oil in these media on the surface of the seawater formed an oil slick.

2.2. Oil spill bioremediation simulated experiment

The simulation of marine oil spill bioremediation was carried out in three aquariums. Two of the aquariums were filled with 1 L MC and the other was filled with 1 L BCR. The aquariums were put in clean bench (YT-CJ-IND, Beijing Yatai-Kelong instrument technology Co., LTD., China) to prevent contamination at ambient temperature for 30 d. The temperature was allowed to fluctuate in the range of 16–27 °C in order to make sure the temperature condition close to real marine environment. During this experiment, distilled water was added at regular intervals as the supplement of the evaporated water in the aquariums. No aeration equipment or agitating device was used in this work.

2.3. Oil sample processing and gas chromatography analysis

Oil samples in the biodegraded samples and the control were extracted by chloroform and concentrated by a rotary evaporator. The oil samples were vacuum dried at 65 °C to remove the rest of chloroform. The oil samples were accurately weighed and dissolved in hexane, and then centrifuged at 3000 r min^{-1} speed. 0.2 mL of above oil solution was quantitatively loaded onto a 3 g silica gel microcolumn (200 mm × 10.5 mm I.D.) which was preconditioned with 20 mL of hexane. The saturated hydrocarbons were eluted with 12 mL of hexane. The eluent was concentrated with a stream of nitrogen and then adjusted to accurate 1.0 mL. This fraction was analyzed by chromatography–flame ionization detector (GC–FID) analyses and gas chromatography–mass spectra (GC–MS) for the aliphatic compounds (Sun et al., 2006, 2009).

The analyses of individual *n*-alkanes, pristane and phytane were performed on a Shimadzu GC-2010 (Kyoto, Japan) with a FID detector. Analyses for polycyclic aromatic hydrocarbons (PAHs) and biomarkers were performed on a Shimadzu GC–MS-QP2010. A HP-5 capillary chromatographic column (30 m × 0.32 mm I.D.) and a DB-5MS capillary column (60 m × 0.25 mm I.D.) were used, respectively for the GC–FID and GC–MS. The biomarkers of sesquiterpenes, tricyclic and pentacyclic terpanes, steranes were determined at m/z 123, m/z 191 and m/z 217, respectively.

Quantitation of the *n*-alkanes, unsubstituted PAHs and their alkylated homologues, and biomarker compounds were accomplished using internal standards. C_{24} D_{50}, D_{14}-trichlorodiphenyl, 5-α-Androstane and 17β(H), 21β(H)-hopane were used as the internal standards for the analysis of the *n*-alkanes, PAHs and biomarker compounds, respectively. System control and data acquisition was achieved with a GC solution and GC–MS solution software, respectively. Chromatographic conditions and quality control refer to Sun et al. (2009).

2.4. Calculation of hydrocarbon compounds removal efficiency

The removal efficiency of the hydrocarbon compounds was calculated by the difference of the concentration between the control and biodegraded sample divided by the concentration of the control.

3. Results and discussion

As seen in Fig. 1, the oil slick in BC and BCR was significantly altered after 30d's bioremediation, compared with it in MC. Especially for BCR, the oil slick was completely destroyed and dispersed in the medium, which mainly due to the emulsification by rhamnolipids. Those three samples were processed as described in Section 2.2. The recovered oil from MC, BC and BCR was 1.67 g, 1.22 g and 1.13 g, respectively. After bioremediation, the removal efficiency of oil by the bacterial consortia alone was 26.72%, and the oil removal efficiency through combined effect of the bacterial consortia and the amendment of rhamnolipids was up to 32.3%. The rhamnolipids existed in BCR enhanced the oil biodegradation efficiency by 5.63%.

In order to better understand the specific role of rhamnolipids in the degradation of target hydrocarbon compounds by the bacterial consortium, *n*-alkanes, PAHs and biomakers in the three oil samples were detected by gas chromatograph.

3.1. n-Alkanes, pristane and phytane

It was observed that only *n*-C13 to *n*-C30 were detected in these three oil samples. The GC chromatograms of oil samples in MC, BC and BCR were shown in Fig. 2a, and the comparison of *n*-alkanes of