Marine Pollution Bulletin xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Studies on crude oil removal from pebbles by the application of biodiesel

Wen-xiang Xia [a], Yan Xia [a], Jin-cheng Li [a,*], Dan-feng Zhang [a], Qing Zhou [b], Xin-ping Wang [b]

[a] *College of Environmental and Municipal Engineering, Qingdao Technological University, Qingdao 266033, China*
[b] *Key Laboratory of Marine Spill Oil Identification and Damage Assessment Technology, North China Sea Environmental Monitoring Center of State Oceanic Administration, Shandong, Qingdao 266033, China*

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Bioremediation
Petroleum contamination
Pebble
Biodiesel
Nutrients

ABSTRACT

Oil residues along shorelines are hard to remove after an oil spill. The effect of biodiesel to eliminate crude oil from pebbles alone and in combination with petroleum degrading bacteria was investigated in simulated systems. Adding biodiesel made oil detach from pebbles and formed oil–biodiesel mixtures, most of which remained on top of seawater. The total petroleum hydrocarbon (TPH) removal efficiency increased with biodiesel quantities but the magnitude of augment decreased gradually. When used with petroleum degrading bacteria, the addition of biodiesel (BD), nutrients (NUT) and BD + NUT increased the dehydrogenase activity and decreased the biodegradation half lives. When BD and NUT were replenished at the same time, the TPH removal efficiency was 7.4% higher compared to the total improvement of efficiency when BD and NUT was added separately, indicating an additive effect of biodiesel and nutrients on oil biodegradation.

1. Introduction

Oil spills are one of the major reasons to ocean pollution, causing dramatic damages to the sea and shorelines. The spilled oil may persist in coastal areas for years, transport through the food chains (Lee, 1976; Teal et al., 1992) and exhibit long-term toxicity (Southward, 1982). Oil stranded on rocky shorelines is extremely difficult to remove especially since the shorelines are lack of seawater washing. A layer of fuel oil may stick firmly to the surface of rocks or pebbles due to the accumulation of the recalcitrant components and act as a slow-release source of contamination.

High pressure flushing with hot water has been used in shorelines affected by the Prestige oil spill. Nevertheless, it is labor intensive and time consuming, and a large amount of wastes was produced with high energy consumption (Fernández-Álvarez et al., 2006). Bioremediation has the advantage of low cost and high efficiency under favorable conditions without causing environmental damage (Beolchini et al., 2010; Mohajeri et al., 2010; Pritchard and Costa, 1991; Venosa and Zhu, 2003; Wang et al., 1998). It is an efficient alternative for the removal of oil from rocks or pebbles by physical means.

Petroleum degrading bacteria (PDB) are ubiquitous in the marine environment (Atlas, 1993). They require not only carbon but also nitrogen and phosphorus for incorporation of biomass. The application of nutrients has proven to be an effective way to stimulate oil biodegradation in the bioremediation of petroleum contaminated shorelines (Santas et al., 1999; Venosa et al., 1996). However, mineral oil attached to the pebbles has low water solubility, leading to difficulties for microbes to utilize it as an energy source. Therefore, the bioavailability of petroleum hydrocarbons to oil degraders is a major factor influencing the bioremediation effect.

Biodiesel is composed primarily of fatty acid methyl esters. It is an environmentally benign addition since it is readily degraded by microorganisms (Makareviciene and Janulis, 2003; DeMello et al., 2007) and less toxic than mineral oils (Birchall et al., 1995). In recent years, biodiesel has been used to eliminate petroleum contamination in laboratory and field tests. For example, Taylor and Jones (2001) reported that the addition of biodiesel to soil containing coal tar increased the degradation of coal tar polycyclic aromatic hydrocarbons (PAHs), which was ascribed to tar solubilization and dispersion thereby increasing the PAHs bioavailability. Fernández-Álvarez et al. (2006) applied biodiesel for removal of crude oil that contaminated rocks and sand after the Prestige oil spill. They found it not only accelerated the clean-up of the polluted surface but also enhanced the degradation of the residual oil. However, neither the added microorganisms nor the nutrients accelerated the degradation rate of the residual oil in the affected shorelines in their studies, which was quite different from other reports (Hozumi et al., 2000; Oh et al., 2001).

* Corresponding author. Tel.: +86 532 85071262; fax: +86 532 85015375.
E-mail address: 1145985449@qq.com (J.-c. Li).

http://dx.doi.org/10.1016/j.marpolbul.2014.11.026
0025-326X/

On the whole, the removal of petroleum contamination by the addition of biodiesel is both a physicochemical and a biochemical process. These dual actions were discussed in previous studies (Taylor and Jones, 2001; Fernández-Álvarez et al., 2006). However, the role of biodiesel played alone on the removal of fuel oil was unclear. Furthermore, when biodiesel was applied together with nutrients, their combined influence on the hydrocarbon degrading microorganisms need to be further explored.

Laboratory studies are valuable in assessing the effectiveness of a shoreline cleaning strategy since it is not practical to replicate the true conditions of the shoreline environment (Mearns, 1997; Santas et al., 1999). In this study, we use a simulated system to explore the effect and mechanism of biodiesel on removing oil from shorelines. First, biodiesel (BD) was applied to crude oil contaminated pebbles in a sterilization system without inoculum (INO) in order to study the physiochemical effects of biodiesel alone. Secondly, with INO present in the system, BD, nutrients (NUT) and BD + NUT were replenished respectively to study the biochemical effects of biodiesel in combination with oil degrading bacteria.

This article describes the results of our experimental studies.

2. Materials and methods

2.1. Samples

Clean seawater collected from Jinsha Beach near Qingdao, China was filtered through 0.22 μm membrane to eliminate the impact of micro-organisms. Pebbles (diameter 4–5 cm) collected from Qingdao Huiquan Bay were soaked with hydrochloric acid and sterilized before use.

Crude oil samples were purchased from Huangdao oil depot. The density, kinetic viscosity and API value of the oil at 20 °C was 0.987 (g/cm^3), 86.55 (mm^2/s) and 11.9 respectively. In order to avoid the influence of oil volatilization, the crude oil was weathered for 4 weeks in a fume hood before use, losing 10.8% of its initial weight.

Biodiesel in the form of rapeseed oil methyl esters was purchased from local suppliers in Qingdao, China. The density and kinetic viscosity at 20 °C was 0.836 (g/cm^3) and 5.6 (mm^2/s) respectively.

2.2. Microorganism acclimatization

Since no single strain of bacteria has the metabolic capacity to degrade all components found in crude oil, a mixture of bacterial suspension was used in the experiment. A medium containing 1 g/L NH_4NO_3, 1 g/L KH_2PO_4, 1 g/L K_2HPO_4, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.05 g/L $FeCl_3$, and 0.02 g/L $CaCl_2$ was used to culture bacteria (Dutta and Harayama, 2000; Mohajeri et al., 2010). Oil-polluted seawater containing oil degrading bacteria was collected from Dagang Wharf No. 6 in Jiaozhou Bay was added to 100 mL of medium and incubated on a rotary shaker at 25 °C, 200 r/min for 2 weeks. Then enriched solution was added to fresh medium and incubated at 25 °C for 1 week. To obtain standard inoculum, the mixed cells were harvested by centrifugation, rinsed three times in sterile saline before being re-suspended in sterile liquid basal medium to yield an absorbance reading of 0.5 at 540 nm.

2.3. Experimental design

Plastic containers (400 × 260 × 200 mm) were prepared and sterilized pebbles were put into them, and seawater was poured into the container to attain a height of 150 mm which is about 10 mm higher than the top of pebbles. Twelve plastic tubes fitted with a flow control device was inserted through holes around the containers at a height of 0, 5 and 10 cm from the top of the pebbles to allow seawater to be drained through the system and analyzed (Fig. 1). The containers were placed indoor and the temperatures is 20 °C. In order to mimic the washing of the wave and replacement effect of seawater, fish pump with 4 outlets was installed under the bottom of the pebbles, and seawater (800 mL) was flushed and drained through the holes with the same amount of fresh seawater replenished every day after the seawater samples were taken.

2.3.1. Effects of biodiesel on residual oil clean-up without hydrocarbon degraders

Four containers were used in each group, one for control, and three for experiments which were treated with biodiesel. To each of the containers, 20 g weathered crude oil was applied to the surface of the pebbles as uniformly as possible to simulate pollution density occurred along Qingdao shorelines in 2013. Results from our previous studies (dada not shown) indicated the earlier the biodiesel was added, the higher the oil removal efficiency. Since it needs some time for the response action to take place when large area of pollution occurs in real situations, 3 h is chosen in our experiment to mimic real situations and biodiesel (6, 12 and 18 mL) was sprayed respectively in 3 experimental tests. The washing effect of the tide was simulated by filling the container with seawater until it submerged the pebbles with fish pump switching on and off. No biodiesel was added in the control.

Seawater samples (20 mL) containing the biodiesel-oil mixture was taken at different position from sample outlets in 1, 2, 3, 4, and 7 days after the addition of the biodiesel, and 4 samples at the same height were mixed and treated to analyze the total petroleum hydrocarbon (TPH) content. Seawater 240 mL was replenished in the container after sampling. When the experiment ended, oil remaining on pebbles was analyzed.

Another two groups were used as parallel experiments and the above process was repeated three times.

2.3.2. Effects of biodiesel on residual oil biodegradation in the presence of hydrocarbon degraders

In order to study the subsequent biodegradation of oil detached from pebbles after the addition of biodiesel, five containers were prepared as a group, 1 control and 4 experimental. The arrangements are shown in Table 1.

Crude oil contaminated pebbles are prepared as previously mentioned. One control test was set up with only seawater added, and 4 experimental tests were prepared with the addition of inoculum (INO) as well as biodiesel (BD), nutrients (NUT) or BD + NUT (Table 1). NO_3–N and PO_4–P were amended according to the results of former study (Xia et al., 2005), and the N:P ratio is 10:1. Oxygen was supplied by fish pump in order to simulate the aerobic condition along shoreline and make inoculum distribute evenly, and the dissolved oxygen (DO) of the seawater varied between 3.8 and 4.5.

Seawater samples (20 mL) in water surface were obtained from each outlets on day 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 following the addition of the biodiesel, and 4 seawater samples from different positions were mixed and prepared to analyze TPH, petroleum degrading bacteria (PDB), dehydrogenase activity (DHA) and DO. When the experiment ended, the residual oil still attached to the pebbles was analyzed.

Another two groups were used as parallel experiments and the above process was repeated three times.

2.4. Sample analysis

2.4.1. Total petroleum hydrocarbon (TPH) in seawater

The mixture of biodiesel and crude oil in the seawater were extracted using carbon tetrachloride. The biodiesel–oil in carbon