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In situ microbial remediation of crude oil-soaked marine sediments using zeolite carrier with a polymer coating

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ABSTRACT

Marine oil spill pollution is an important environmental problem in the world, especially crude oil-soaked marine sediments, because they are difficult to be remediated. In this study, *in situ* bioremediation of oil-soaked sediment was performed in the middle of the Bohai Sea. Oil-degrading bacteria were adsorbed on powdery zeolite (PZ)/granular zeolites (GZ) surfaces and then wrapped with poly-γ glutamic acid (γ-PGA). Settling column and wave flume experiments were conducted to model marine conditions and to select appropriate biological reagents. The optimal conditions were as follows: the average diameter of GZ 3 mm, mass ratio of GZ/PZ 2:1, and concentration of γ-PGA 7%. After bioremediation, over 50% of most oil-spilled pollutants *n*-alkanes (C_{12} to C_{27}) and polycyclic aromatic hydrocarbons were degraded in 70 days. This work resulted in a successful trial of *in situ* bioremediation of oil-soaked marine sediments.

1. Introduction

With the development of offshore oil exploration and ocean transportation, crude oil spills have already caused global concern and become an important source of marine environmental pollution (Guo et al., 2013). In recent years, the accidental oil spill in the Gulf of Mexico (2010) led to the release of nearly 400 million barrels of oil directly into the sea (Lin and Guo, 2015). The event is considered as the largest offshore oil spill in the world and was disastrous for the Gulf Coast environment. In China, the Dalian Port blast oil spill (2010), Penglai 19-3 oil spills in the Bohai Bay (2011), and oil pipeline blast in Qingdao (2013) caused tremendous damage to the local marine environment (Wang et al., 2015). Marine oil spills have become an important factor among those endangering the marine environment and human health (Lan et al., 2015). Marine oil spills not only damage the surrounding coastal environment, but also pose even broader danger for the entire marine system (Peterson, 2001). The migration and transformation of leaking oil in the ocean may affect marine fish hearts and eyes (Xu et al., 2016). It can also cause serious damage to fish foraging patterns and threaten the safety of other marine animals and plants (Nyankson et al., 2016). In addition to causing direct harm to the marine flora and fauna, primary or secondary pollutants generated by the oil spill may indirectly harm human health through food chain enrichment (Lei et al., 2015). Furthermore, the evaporation and dissolution of oil floating on sea surface form a dense layer of scattered ions that aggregate to form tar balls, or get adsorbed onto other particulate matter. These finally settle on the sediments, resulting in severe pollution of the seabed sediment (Yavari et al., 2015; Morales-Caselles et al., 2008; Peterson et al., 2003). Generally, spilled oil will persist in the natural environment for > 30 years after an oil spill accident (Petkewich, 2002). Under the turbulent mixing of ocean currents and waves, the oil or oil oxidation products sinking into the sea can return to the sea surface, causing secondary pollution (Ranjbar et al., 2014).

For remediation of oil spill tar balls pollution, a variety of advanced clean-up technologies have been applied. Special abrasive paper can be used to clean up the oil spill (Shi et al., 2015), and the oil can be collected by oil skimmers or simply be burnt off (Song et al., 2014). Chemical sorbents and dispersants are also common methods for treating spilled oil (Lim et al., 2016). However, these kinds of physical and chemical methods can be harmful to the environment and aquatic life (Schaum et al., 2010; Kujawinski et al., 2011), and most of these clean-up methods can only be applied to remedy surface oil spills. Compared to physical and chemical techniques, bioremediation is a well-described technology for the remediation of oil spill pollution (Pavitran et al., 2006; Dua et al., 2002; Okoh and Trejo-Hernandez, 2006; Fernandez-Luqueno et al., 2011). Although this method can be

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applied to remedy floating oil on the seawater or on the beach and in soils, it has limitations for the remediation of oil-soaked sediments on the ocean bottom under deep water. This is because it is difficult to spread bioremediation remedy agents (RAs) on the contaminated marine sediments. Therefore, few technologies have been reported for the remediation of oil-polluted sediment in deep sea areas. Although oil dispersants have been used to remedy deep sea oil pollution, both options and efficacy suffer from great uncertainty (Carriger and Barron, 2011).

Application of functional microorganisms like bacteria is a useful technique for oil pollution remediation. Previous studies reported that the bacterial genus *Bacillus* spp. has high efficacy for crude oil biodegradation (Tao et al., 2017; Chettri et al., 2016). Poly-γ glutamic acid (γ-PGA) and chitosan are natural biopolymers that are widely used as wrapping agents (Shi et al., 2016), flocculants, or bioremediation agents in environmental engineering (Wang et al., 2014) because of their biodegradability (Liu et al., 2015; Matsusaki et al., 2002). Zeolite is a natural and economic material used for pollution control because it has high specific surface area, medium pH and no secondary pollution (Shi et al., 2009; Javed et al., 2016; Castaldi et al., 2005; Kumpiene et al., 2008). If the oil-degrading bacteria could be loaded onto powdery zeolite (PZ) or granular zeolites (GZ) and then wrapped with γ-PGA or chitosan, they could be distributed on the bottom without the bacteria being washed away by currents to realize sediment bioremediation.

The goal of this work was to perform *in situ* bioremediation engineering of oil-soaked marine sediments using the combination of oil-degrading bacteria (*Bacillus* spp., patent protection), bacterial adsorbent (zeolite), and wrapping agents (γ-PGA or chitosan). The objectives were 1) to find and evaluate a suitable microorganism wrapping agent to decrease the loss of effective (living) bacteria during the delivery process, and 2) to investigate the efficiency of petroleum hydrocarbon degradation after bioremediation. In this work, the use of a feasible technology was explored and new thought provided about the remediation of oil-soaked marine sediments.

2. Method and materials

2.1. Materials preparation

In order to put liquid petroleum degrading bacteria (microbial inoculum) onto the sea floor efficiently, different absorbing and wrapping agents were selected in this study. Microporous zeolites (GZ and PZ) were used as the adsorbents and carriers for the oil-degrading bacteria. With zeolite, the bacteria could be fully adsorbed and the wrapping agents loaded, which were purchased from a local market. The GZ and PZ used in the present study was clinoptilolite, which was natural zeolites with atypical unit cell formula $Na_6[(AlO_2)_6(SiO_2)_{30}]\cdot 24H_2O$. The clinoptilolite has a characteristic tubular morphology that shows an open reticular structure of easy access, formed by open channels of 8–10 membered rings (Paola et al., 2018). The different GZ diameters tested were 1 mm (mm), 3 mm, 5 mm, and ≥7 mm. The average diameter of the PZ was ~30 μm, and its specific surface area was 230–320 $m^2\,g^{-1}$. The bacterial adsorption rates of different zeolite materials (GZ and PZ) were determined after being soaked for 30 min in microbial inoculum. Potential wrapping agents used in this study were chitosan and γ-PGA. Chitosan (content > 90%, white powder crystalloid, molecular weight of *ca.* 1450 kDa) was purchased from Jinan Tianben Biological Technology Co., Ltd., Jinan, China. Chitosan was dissolved in 2% (volume ratio) acetic acid solution prior to use. The γ-PGA (content > 99%, agricultural level, molecular mass 700–1100 kDa, white, granulate, free-flow particle crystalloid) was purchased from Freda Biotechnology Co., Ltd., Shandong, China. The oil-degrading bacteria (in solution) were selected and cultured from oil-polluted sludge; the dominant bacterial genus was *Bacillus* spp. (patent protection, preserved in China General Microbiological Culture Collection Center, Beijing, China). The initial concentration of the oil-degrading bacteria was 1×10^9 CFU mL^{-1}. Mass production of the absorbent (mixture of GZ and PZ) and the wrapping (γ-PGA) agent was authorized to a local biological technology company.

2.2. Experimental design

2.2.1. Optimal GZ diameter and mix ratio investigation

In order to ensure a sufficient count of effective (living) bacteria in zeolite, three groups were set with GZ diameters of 3 mm, 5 mm, or 7 mm. Each group had six parallel variations with different mass ratios of GZ to PZ, which were set as 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8. The same concentration of a wrapping agent (chitosan or γ-PGA) was added to the different ratio variants prior to testing. The concentration of chitosan was set as 1% or 2% (dissolved in 2% acetic acid solution). The concentration of γ-PGA was set as 1%, 3%, 7%, or 14% (dissolved in deionized water). The pH values of the different concentrations of chitosan and γ-PGA were measured prior to use. Because the microbial inoculum is colorless and transparent, red dye was applied to trace the migration path of the oil-degrading bacteria prior to determination of the bacterial concentration.

2.2.2. Modeling test procedure

Based on the results of Section 2.2.1, GZ and PZ were mixed in a beaker (with optimal mass ratio); then, microbial inoculum was added to the beaker. After 30 min of agitation for adsorption (saturated adsorption rate was 22% wt/wt, established in a preliminary experiment), superfluous microbial inoculum solution was discarded. Next, either chitosan or γ-PGA (at optimal concentration) was added to the beaker, with agitation for ~5 min to produce flocculation block. When the block inclusions were in the process of settlement (settling column, Fig. 1), they first maintained a polymeric block at the top of the settling

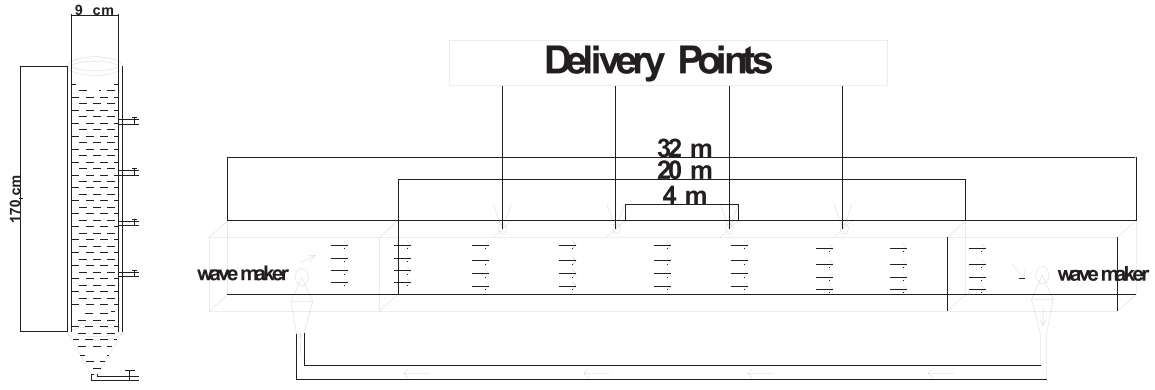

Fig. 1. Diagrammatic sketches of settling column (left) and wave flume (right).