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Use of bacteria-immobilized cotton fibers to absorb and degrade crude oil

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ABSTRACT

Using enrichment culture technique, two isolates that brought a significant degradation and dispersion of crude oil were obtained from contaminated sediments of the Bohai Bay, China. 16S rRNA gene sequencing and phylogenetic analysis indicated that the two bacterial strains affiliated with the genera *Vibrio* and *Acinetobacter*. Subsequently, the bacterial cells were immobilized on the surface of cotton fibers. Cotton fibers were used as crude oil sorbent as well as a biocarrier for bacteria immobilization. Among the two isolates, the marine bacteria *Acinetobacter* sp. HC8-3S showed a strong binding to the cotton fibers, possibly enhanced through extracellular dispersant excreted by *Acinetobacter* sp. HC8-3S. Both planktonic and immobilized bacteria showed relatively high biodegradation (>60%) of saturated hydrocarbons fraction of crude oil, in the pH range of 5.6–8.6. The degradation activities of planktonic and immobilized bacteria were not affected significantly when the NaCl concentration reached 70 g/L. The immobilized bacterial cells exhibited an enhanced biodegradation of crude oil. The efficiency of saturated hydrocarbons degradation by the immobilized bacterial cells increased about 30% compared to the planktonic bacterial cells.

1. Introduction

Oil pollution originated from both natural and anthropogenic sources can have dramatic detrimental effects to the environment. Large amount of crude oil entering marine, groundwater, soil and other environment could cause significant damages to resident organisms. Recent disastrous oil spills, e.g. Deepwater Horizon oil spill in Gulf of Mexico in 2010 and Penglai 19-3 oil spills in Bohai Bay (China, 2011), become one of the major sources of oil pollution in the ocean. Cost-effective and environmentally benign strategies are urgently demanded for cleaning up spilled oil. Many techniques are utilized to mitigate and cleanse crude oil pollution in the environment (Obuekwe and Al–Muttawa, 2001). Conventional physical and chemical methods could rapidly remove the majority of leaked oil, but in most cases, the removal just transfer contaminants from one environment medium to another, even produce toxic by-products. More importantly, crude oil could not be completely cleaned up by physical and chemical methods (Gavrilescu, 2010).

Bioremediation is a technique utilizing biological organisms to aid in removal of hazardous substances from polluted area (Head et al., 2006). Biological treatments have been used to treat crude oil spills (Gentili et al., 2006), microorganisms are key players in the process (Röling et al., 2004). Compared to the planktonic bacteria, immobilized bacteria could not only stay away from predators and natural competition with the indigenous microorganisms, but also shield perturbations of environmental conditions, such as toxic compounds (Wang et al., 2012). Otherwise, in an open water system, immobilization also prevents bacteria from being washed away (Rahman et al., 2006). Many immobilized microorganisms have been successfully used for bioremediation of crude oil (Liang et al., 2009). Considering the broad chioces, low cost, simple process and less impact on microbial activity (Oh et al., 2000; Lee et al., 2010), immobilization have been proved to be an effective strategy to apply functional microorganism for bioremediation (Wang et al., 2007; Mollaei et al., 2010).

Herein, untreated cotton fibers were selected as a crude oil sorbent as well as a biocarrier for bacteria immobilization. Two high efficient crude oil-degrading bacterial strains were immobilized on the cotton fibers under mild conditions. Oil degrading efficiency of immobilized and planktonic bacterial cells was compared subsequently. The biodegradation rate of saturated

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hydrocarbons by planktonic and immobilized bacterial cells was analyzed using gas chromatography (GC).

2. Experimental

2.1. Isolation, identification of oil-degrading bacteria

The crude oil contaminated sediment was collected from Bohai bay, China. The isolation of oil-degrading bacteria was conducted as follows. 10 g of sediment samples and 1 g of crude oil were co-incubated in 100 mL mineral solution (7.01 mM K_2HPO_4, 2.94 mM KH_2PO_4, 0.81 mM $MgSO_4 \cdot 7H_2O$, 0.18 mM $CaCl_2$, 1.71 mM NaCl) for 7 days with shaking at 180 rpm and 30 °C. One milliliter of liquid culture was tenfold sequentially diluted to 10^{-7} and 100 μL aliquots of each dilution were spread onto mineral agar which was prepared by adding 1.5% agar into mineral solution. The plates were incubated at 30 °C for 48 h. Morphologically distinct colonies were streaked on mineral agar supplemented with crude oil as a sole source of carbon for purification. Single colony of each isolate was transferred to 10 mL of mineral solution. Aliquots (1.8 mL) of liquid culture were used for DNA extraction using ultra-clean microbial DNA isolation Kit (MoBio Laboratories, Carlsbad, CA) while 1 mL residual was cryopreserved at −80 °C with 1 mL of 60% glycerol. The 16S rRNA gene of each isolate was PCR amplified and sequenced using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-CGGYTACCTTGTTACGACTT-3′) as described previously (Enticknap et al., 2006). Sequences were analyzed using the BLASTn tool at the National Center for Biotechnology Information (NCBI) website. Isolates were presumptively identified according to the identity of the closest cultured relative in the top BLAST hits.

2.2. Pretreament of cotton fibers

Cotton fibers (absorbent, Taizhou Xinkang Medical Materials Co., Ltd. Jiangsu, China) were selected as biocarrier because of their large surface area and high absorption capacity. In order to remove potentially toxic compounds, the cotton fibers were immersed into chloroform for 3 days, rinsed with distilled water and dried at room temperature.

Crosslinking cotton fabric containing carboxyl groups were prepared according to previous literature (Ibrahim et al., 2007). Cotton fibers were immersed into 100 g/L of 1,2,3,4-butantetracarboxylic acid (BTCA) solution. After drying at 85 °C for 30 min, the treated cotton fibers were washed by 50 °C distilled water to remove excess reactants for 10 min. Carboxyl groups which from the crosslinked cotton fibers were activated by immersing into a 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimidemetho-*p*-toluen-sulfonate (CMC) solution at 45 °C overnight. Followed by thoroughly rinsing, cotton fibers were immersed in an alkaline polyethyleneimime (PEI) and diethylamine (DEA) solutions for 24 h, respectively. Subsequently, the cotton fibers were dipped into a fixing agent containing glutaraldehyde solution (2%) for 2 h, washed with distilled water and dried.

2.3. Immobilization of oil-degrading bacteria

The functional bacteria were incubated in 50 mL Zobell 2216E medium at 30 °C, 180 rpm for 12 h first. Then, aliquots (1 mL) of two bacterial cultures were inoculated into 100 ml Erlenmeyer flasks, respectively. The PEI-treated, DEA-treated and untreated cotton fibers were used as biocarriers. The flasks containing 50 mL sterilized Zobell 2216E medium with 0.25 g biocarrier in a rotatory shaker at 30 °C, 180 rpm for 24 h, which allowed the self-adhesion of the two bacteria strains. Cotton fibers immobilized with functional bacteria were dehydrated with a series of ethanol solutions (30–100%) and dried. Then, the bacteria were fixed with 2.5% (w/v) glutaraldehyde solution at 4 °C for 12 h and washed twice with phosphate buffered saline (PBS, pH = 7.4) for 10 min (Khondee et al., 2012). Scanning electron microscopy (SEM) was used to observe the immobilized bacteria on cotton fibers.

2.4. Biodegradation of crude oil by immobilized and planktonic bacteria

The crude oil which used in studying the aerobic biodegradation is from Shengli oil field, Dongying, China. Microbial degradation of crude oil was compared between planktonic and immobilized bacteria. The combination (0.25 g cotton fibers and 1.14×10^{10} cfu bacterial culture) were mixed with crude oil (0.5 g) placed into a 100 mL Erlenmeyer flask with 50 mL sterilized nutrient supplements. Control flask was set up with co-incubation of crude oil (0.5 g), bacterial culture (1.14×10^{10} cfu) and nutrient supplements without cotton fibers. The experiments were carried out at 30 °C in a rotatory shaker (180 rpm). The reaction was maintained a pH of 7.6 throughout the entire experiment.

2.5. Effect of pH and sodium chloride concentration on crude oil biodegradation

The effect of pH on biodegradation was assessed by modifying pH values in medium to 4.6, 5.6, 6.6, 7.6, 8.6 and/or 9.6 individually, and pH was adjusted using either NaOH or HCl solutions. The influence of NaCl concentration on crude oil biodegradation was investigated by using different NaCl concentrations set at 30, 40, 50, 70, 90, 120 g/L, respectively. After 5 days' co-inoculation with crude oil at 30 °C and 180 rpm, the biodegradation efficiency of planktonic and immobilized bacteria was measured.

2.6. Crude oil extraction and components analysis

The 4 components (saturated hydrocarbons, aromatic hydrocarbons, the more polar, non-hydrocarbon components, and asphaltenes) of crude oil were separated using typical column chromatography (Head et al., 2006). The residual oil was extracted from the cultures with 10 mL *n*-hexane for three times. The extracts were collected and dried in anhydrous sodium sulfate (Bost et al., 2001). After being concentrated with vacuum rotary evaporation, the extracts were fractionated by column chromatography. Multilayer column was carried out as following from the bottom: 3 g silica gel (activated at 110 °C for more than 4 h), 2 g alumina (activated at 400 °C for more than 4 h) and 1 g sodium sulfate anhydrous (activated at 400 °C for more than 4 h). Subsequently, the column was applied to separate components of the extracts crude oil. The saturated hydrocarbons were eluted under gravity with 25 mL *n*-hexane, aromatic hydrocarbons with 15 mL *n*-hexane and dichloromethane (v/v = 1:2) mixture, and polar compounds with 20 mL methanol (Bastow et al., 1999). Asphaltene was precipitated in *n*-hexane before washing. The saturated hydrocarbons of extracted oil hydrocarbons were analyzed by GC (Agilent 7890A) with a capillary column (HP 5 model, 30 m long × 0.32 mm diameter × 0.25 μm thick). The GC condition were as follows: injector temperature was 280 °C; column temperature was 80 °C for 2 min, with a ramp to 280 °C at a rate of 5 °C/min at 280 °C maintained for 20 min; detector temperature was 300 °C. The carrier gas (nitrogen) flow rate was 2 mL/min.