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Study on bioadsorption and biodegradation of petroleum hydrocarbons by a microbial consortium

Nana Xu [a], Mutai Bao [a,*], Peiyan Sun [b], Yiming Li [a]

[a] Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao, Shandong Province, China
[b] Key Laboratory of Marine Spill Oil Identification and Damage Assessment Technology, North China Sea Environmental Monitoring Center of State Oceanic Administration, Qingdao, Shandong Province, China

HIGHLIGHTS

- Surface adsorption and cell uptake of crude oil and PAHs by microbe were studies.
- Microbe was tolerant to 6.2 mM Cu^{2+}, 2.7 mM Zn^{2+} and 9.5 mM Pb^{2+}.
- Degradabilities of periplasmic, cytoplasmic and extracellular enzymes were different.
- Adsorption by dead microbe was constant for PAHs, while decreased for crude oil.
- Stability of adsorption and uptake by live microbe followed NAP > PHE ≈ PYR > crude oil.

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ABSTRACT

A microbial consortium isolated from Shengli oilfield polluted sludge was capable of degrading naphthalene (NAP), phenanthrene (PHE), pyrene (PYR) and crude oil. It performed high biodegradation activity and emulsifiability for petroleum hydrocarbons, and was tolerant to 6.2 mM Cu^{2+}, 2.7 mM Zn^{2+} and 9.5 mM Pb^{2+}. Biodegradation rates of NAP, PHE, PYR and crude oil were 53%, 21%, 32% and 44% in the presence of heavy metal (Cu^{2+}, 1.7 mM and Zn^{2+}, 2 mM), respectively. Exploration on the adsorption and uptake of petroleum hydrocarbons by microbe suggested the stability of surface adsorption and cell uptake by live microbial consortium followed a decreasing order of NAP > PHE ≈ PYR > crude oil. The adsorption by heat-killed microbial consortium was constant for PAHs, while decreased for crude oil. Experiments on enzymatic degradation indicated that the metabolic efficiency of periplasmic, cytoplasmic and extracellular enzymes secreted by the microbial consortium for diverse substrates was different.

1. Introduction

Petroleum is one of the most important energy resources and raw materials of chemical industry. With the growth in demands for energy, a large number of petroleum hydrocarbons enter our environment in the process of exploitation, transportation and refining, especially accidental spills. Among the complex compounds of petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) exhibit carcinogenic, teratogenic, mutagenic and other toxic properties such as bioaccumulation, biomagnification and persistent toxicity (Balachandran et al., 2012). The USEPA has listed 16 PAHs as priority pollutants. Oil pollutants, especially PAHs contaminate our groundwater, atmosphere, terrestrial soil, marine water and sediment causing extensive environmental problems; posing serious threat to marine life, human health, marine ecological resources and systems; and destroying the ecological balance which may take years or even decades to recover (Park and Park, 2011; Cohen, 2013). The most recent examples of large-scale spills are the Deepwater Horizon blowout in Gulf of Mexico on April 2010 and the oil pipeline explosion in Dalian, China on July 2010 which caused massive damages to the ocean and coast. Consequently, petroleum hydrocarbons contaminations have raised great environmental concern all over the world.

Although petroleum hydrocarbons released into the environment may undergo chemical oxidation, photolysis, volatilization and adsorption on sediment and soil particles, the main pathway for their removal is probably through microbial transformation and degradation. Compared with physical and chemical methods such as combustion, photolysis and adding dispersant, biodegradation is expected to be an efficient, economic and environmentally friendly alternative for removal of oil pollution from contaminated environments and reducing damages caused by oil spills (Zhang et al., 2011). Thus, more and more research interests are turning

* Corresponding author. Address: 238 Songling Road, Qingdao 266100, Shandong, PR China. Tel./fax: +86 532 66782509.
E-mail address: mtbao@ouc.edu.cn (M. Bao).

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to the biodegradation of crude oil and PAHs (Zhang et al., 2011; Jin et al., 2012; Moscoso et al., 2012). At present, many studies have focused on the bioremediation potential of pre-adapted microbes and the improvement of biodegradation efficiency by bioaugmentation and biostimulation. There have contaminations from a variety of sources in the environment. Heavy metals are common constituents of crude oil and petroleum derivatives such as pb^{2+}-leaded gasoline, lubricating oils and greases; Zn^{2+}, Cd^{2+} compounds amended engine oil etc. (Máthé et al., 2012). In addition, heavy metals pollution specially Cu^{2+}, Zn^{2+} and Pb^{2+}, frequently happened in many rivers, seas or lakes etc. (Meybeck et al., 2007; Kwon et al., 2010). Facing multiple contaminations in the environment, some researchers only focused on biodegrading crude oil and PAHs, while ignoring the other existed disadvantageous conditions such as the presence of heavy metal. So the effects of heavy metals on petroleum hydrocarbons biodegradation including the resistance of microorganism to heavy metal are essential to be taken into account.

Moreover, microorganisms play an important role in the fate of contaminations in the environment, including bioadsorption and biodegradation (Stringfellow and Alvarez-Cohen, 1999). In the process of bioremediation, microorganisms can be seen both as a biosorbent that accommodates organic pollutants and as a bioreactor that degrades pollutants (Chen et al., 2010). So far, the bioadsorption of heavy metal, dyes, and pesticides by biological materials such as bacteria, fungus and algae were extensively reported (Sharma et al., 2011; Khosravihaftkhany et al., 2013; Maciel et al., 2013). Recently, the influences of bioadsorption and biodegradation on the transport, fate and pollution control of persistent organic pollutants in the environment have received increasingly more attention. Most research have mainly focused on the factors affecting the adsorption of a single of bacteria, fungi or algae; the relevance of adsorption, cell polarity or the partition coefficient; the contributions of bioadsorption and biodegradation to biodissipation of dissolved and adsorbed pollutions; and the influence of continuous culture on adsorption (Chen et al., 2010; Ke et al., 2010; Chen and Ding, 2012; Ding et al., 2013). Few researchers focused on the surface adsorption and cell uptake of different organic pollutant by microorganism. Thus, it is very significant and interesting to explore the fate of contaminations in microorganisms' cell and surface for understanding the mechanism and transport of degrading contaminations.

The main objective of this present work was to study the biodegradation, bioadsorption and uptake of petroleum hydrocarbons (crude oil and PAHs) by a microbial consortium. It could provide a theoretical support for the bioremediation of petroleum hydrocarbons contaminations. The investigation was primarily focused on the transport of surface bioadsorption and cell uptake by live or heat-killed microbial consortium. Meanwhile, study on heavy-metal resistance, biodegradation activity and emulsifiability of the microbial consortium, as well as the metabolic efficiency of periplasmic, cytoplasmic and extracellular enzymes secreted by the microbial consortium were also explored.

2. Methods

2.1. Materials

2.1.1. Chemicals

Phenanthrene (PHE, purity $\geqslant$97%) and pyrene (PYR, purity $\geqslant$97%) were obtained from Aladdin Chemistry Co. Ltd. Naphthalene (NAP) was purchased from Sinopharm Chemical Reagent Co. Ltd. Other reagents were analytical grade and purchased from various commercial sources. Crude oil was obtained from Shengli oilfield, which was dehydrated and removed undissolved precipitate.

2.1.2. Solutions and media

The single substrate solutions were prepared in advance, respectively. The concentration of each PAHs (NAP, PHE and PYR) was 2 g L^{-1} in cyclohexane. The concentration of crude oil was 10 g L^{-1} in petroleum ether. The concentration of each heavy metal ($CuSO_4$, $ZnSO_4$ and $Pb(NO_3)_2$) was 100 g L^{-1} in distilled water.

The mineral salts medium (MSM) was composed of (g L^{-1} distilled water) NaCl 0.5, $(NH_4)_2SO_4$ 0.1, $MgSO_4{\cdot}7H_2O$ 0.025, $NaNO_3$ 0.2, KH_2PO_4 0.4, $K_2HPO_4{\cdot}3H_2O$ 1.0. The degradation medium was composed of MSM and single substrate (crude oil, NAP, PHE and PYR). Beef extract peptone medium was composed of (g L^{-1} distilled water) beef extract 0.5, peptone 1.0, NaCl 0.5, agar 15–25 (solid). All media were adjusted to pH of 7.2–7.4 with either HCl or NaOH solutions and sterilized in an autoclave at 121 °C for 20 min before used.

2.1.3. Microorganism and culture condition

A microbial consortium isolated from Shengli oilfield polluted sludge was capable of degrading crude oil and PAHs including NAP, PHE and PYR, and used them as the sole carbon source. The optimum growth conditions of microbial consortium are temperature of 30 °C, pH 7–8, NaCl concentration 5–10 g L^{-1}, NAP 800 mg L^{-1}, PHE 40 mg L^{-1}, PYR 40 mg L^{-1} and crude oil concentration 3 g L^{-1}. The degradation efficiency of NAP, PHE, PYR and crude oil reached up to 80%, 30%, 56% and 48%, respectively. These results were obtained from early exploration experiments in our laboratory.

The microbial consortium was cultivated in beef extract peptone medium at 30 °C with shaking at 120 rpm. Cells pellets were harvested by centrifugation (6000 rpm for 5 min at 4 °C) and washed 3 times with MSM for removal of impurities. Then pellets were resuspended in MSM. After these procedures, the pellets could be used.

2.2. Identification of bacteria isolates

2.2.1. Biochemical characterization

To identify and characterize the bacteria isolates, biochemical tests such as Gram staining, spore staining and tests for oxidation/fermentation, M.R, V-P, indole and the hydrolysis of cellulose were performed according to manual of common bacterial identification (Dong and Cai, 2001).

2.2.2. Molecular identification

Analysis of the 16S rDNA gene was performed for the taxonomic characterization of the isolated bacteria. Total DNA was extracted from the bacterial strains using the TIANamp Bacteria DNA extraction Kit (Tiangen Biotech Co., Ltd., Beijing, China). The bacterial 16S rDNA loci were amplified using the domain-specific universal forward primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and the universal reverse primer 1492R (5′-AAGGAGGTGATCCAGCCGCA-3′). The amplification reaction was performed in a total volume of 30 μL containing 17.8 μL of H_2O, 3 μL of reaction buffer, 3 μL of each forward and reverse primer, 2 μL of dNTPs, 0.2 μL of Taq polymerase and 1 μL of DNA template. Amplification program was performed with initial denaturation step at 95 °C for 5 min; followed by 30 cycles of 30 s denaturation step at 95 °C, 30 s annealing step at 53 °C and 1 min elongation step at 72 °C; and a final extension step at 72 °C for 10 min. After electrophoresis detection PCR results, the PCR product obtained was sequenced by BGI (Beijing Genomics Technology Co., Ltd.) using 3730 DNA sequencers (ABI, USA). The similarity rank from FASTA Nucleotide Database Queries was used to estimate the degree of similarity to other 16S rDNA gene sequences by the NCBI website (http://www.ncbi.nlm.nih.gov) and structure phylogenetic trees. The sequence alignment was carried