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Effects of root exudates on gel-beads/reeds combination remediation of high molecular weight polycyclic aromatic hydrocarbons

Weijun Tian[a,b,*], Jing Zhao[a], Yuhang Zhou[a], Kaili Qiao[a], Xin Jin[a], Qing Liu[a]

[a] College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, PR China
[b] Key Laboratory of Marine Environment and Ecology, Ministry of Education, Qingdao 266100, PR China

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ABSTRACT

Changes in root exudates, including low molecular weight organic acids (LMWOAs), amino acids and sugars, in rhizosphere soils during the gel-beads/reeds combination remediation for high molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs) and the degree of the effects on HMW-PAH biodegradation were evaluated in this study. The results showed that the gel-beads/reeds combination remediation notably increased the removal rates of pyrene, benzo(a)pyrene and indeno(1,2,3-cd)pyrene (65.0–68.9%, 60.0–68.5% and 85.2–85.9%, respectively). During the removal of HMW-PAHs, the LMWOAs, particularly maleic acid, enhanced the biodegradation of HMW-PAHs. Arginine and trehalose monitored in reed root exudates promoted the growth of plants and microorganisms and then improved the removal of HMW-PAHs, especially pyrene. However, the contribution of reed root exudates on degradation of 5- and 6-ring PAHs was minor. These results indicated that the utilization of root exudates was certainly not the only important trait for the removal of HMW-PAHs.

1. Introduction

Estuarine wetlands are an important transition zone between land and sea ecological systems. They have abundant biodiversity and high biological productivity, as well as valuable ecological functions in improving climatic conditions and recharging groundwater (Yang et al., 2011; Cheng and Zhou, 2012). Most pollutants from land can be purified in estuarine wetlands, and they are often considered 'a last barrier' to protect sea environments from being contaminated by pollutants from land.

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds polymerized by two or more benzene rings in different ways, many of which have been identified to have potential adverse health effects (Khalil, et al., 2006; Tsai et al., 2007; Balachandran et al., 2012). There is serious concern about the presence of PAHs in the environment, especially their tendency for bioaccumulation in food chains (Zhai et al., 2011). Certain PAHs have been listed as priority pollutants by the Environmental Protection Agencies of the United States, Europe and China (USEPA, 1989). PAHs are strongly hydrophobic and poorly water-soluble (Balachandran et al., 2012), resulting in their long-term sequestration in various organic domains of the soil matrix and reduced mass transfer into passing groundwater (Chan et al., 2006). In recent years, different strategies have been considered to remediate PAH-contaminated soils, including physical, chemical and biological techniques (Biache et al., 2008; Gan et al., 2009; Usman et al., 2012). Phytoremediation and microbial remediation of PAHs are considered cost-effective and environmentally friendly technologies compared to physicochemical treatments (François et al., 2016). Phytoremediation of PAHs occurs via three pathways: (1) PAHs can be directly taken up into vegetative tissues, resulting in transformation by plant enzymes, sequestration within the plant, or transpiration through leaves (Schnoor et al., 1995). (2) The release of oxygen by plant roots promotes PAH biodegradation in the rhizosphere (Jouanneau et al., 2005). (3) The plant rhizosphere exudates provide nutrients to stimulate microbial biodegradation of PAHs (Muratova et al., 2009), which is one of the major pathways for the removal of PAHs from contaminated soil (Sun et al., 2010). Root exudates are a range of organic compounds actively or passively released by roots during plant growth (Phillips et al., 2003). Major exudates include organic acids, amino acids and sugars (Gao et al., 2011). Several studies have reported that the degradation of PAHs increased when organic acids were added to the soil (Reilley et al., 1996; Ling et al., 2009). Previous researchers have shown that the bioactivity of the degraders of PAHs was weak in natural environments, especially toward high molecular weight PAHs (more than four benzene rings, HMW-PAHs), although the plant rhizosphere exudates appeared to favor microbial activity (Quantin et al., 2005; Hale et al., 2010; Meng and Zhu, 2010; Xie et al., 2012). Microbial remediation means using certain microbes isolated

* Corresponding author at: College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, PR China.
E-mail address: weijunas@ouc.edu.cn (W. Tian).

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from PAH-polluted soils to remove PAHs. Most of these microbes belong to the genera *Mycobacterium* sp., *Rhodococcus* sp., *Sphingomonas* sp., *Pseudomonas* sp. and *Achromobacter* sp. (Daane et al., 2001; Dean-Ross et al., 2002; Yuan et al., 2002; Leys et al., 2004; Miller et al., 2004; Moody et al., 2004; Cunliffe and Kertesz, 2006; Hennessee et al., 2009; Tiwari et al., 2010; Zhong et al., 2011). These chains can effectively degrade low molecular weight PAHs (LMW-PAHs) with less than four benzene rings, but their function for HMW-PAHs is limited because their high hydrophobicity results in their partitioning into clay and organic matter and concomitant low bioavailability (Bence et al., 1996). Therefore, there is an urgent need for the establishment of adequate HMW-PAH remediation technologies to reduce their continual accumulation in estuarine wetlands.

The plant/microbial combination remediation technology is very promising for removal of HMW-PAHs because it combines phytoremediation with microbial remediation. Plants release oxygen and exudates to support and promote microbial degradation in rhizosphere soil, and functional microbes stimulate the growth of plants (Toyama et al., 2011). Previously, our research group reported on the application of the cinder gel-beads/reeds combination strategy for bioremediation of pyrene and indeno(1,2,3-cd)pyrene-contaminated estuarine wetlands and found that 69.2% pyrene and 89.8% indeno(1,2,3-cd)pyrene were removed (Tian et al., 2016). Previous studies have examined the association of ryegrass with the microbial remediation of HMW-PAH-contaminated soils (Xu et al., 2014). However, there is little information on the potential of root exudates to promote the biodegradation of HMW-PAHs in sediments. Therefore, further investigation detailing which exudate components play a role in the removal of HMW-PAHs would be beneficial for the understanding of the synergy mechanism between plants and microbes.

In this study, pyrene, Benzo(a)pyrene and indeno(1,2,3-cd)pyrene were selected as representative HMW-PAHs to investigate the change in components and to identify the main functional exudate components during the plant/microbial combination remediation technology for HMW-PAH removal. The results of this research will provide important information on the synergy mechanism between root exudates and microbes for HMW-PAH removal in estuary wetlands.

2. Materials and methods

2.1. Chemicals and media

All solvents and chemicals used in this study were of an analytical grade or better. Pyrene, Benzo(a)pyrene and indeno(1,2,3-cd)pyrene standard solutions with a purity > 98% were purchased from Accustandard, Inc. (New Haven, Connecticut, USA).

Basal salt medium (BSM) contained (per liter) 1.0 g KH_2PO_4, 0.5 g NaH_2PO_4, 1.0 g NH_4Cl, 0.2 g $MgSO_4{\cdot}7H_2O$, 5 mg $FeSO_4{\cdot}7H_2O$, 10 mg $CaCl_2{\cdot}2H_2O$, and 1 mL trace element solution ($(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 35 mg L^{-1}, $MnSO_4{\cdot}H_2O$ 40 mg L^{-1}, $ZnSO_4{\cdot}H_2O$ 43 mg L^{-1}) at pH 7.2. For the preparation of BSM plates, 2% agar was added.

2.2. Soils

Soils from the Liaohe estuarine wetland, Panjin, China, without PAH contamination, were collected. The pH of the soil sample was 8.6, the total organic carbon was 0.170%, the total nitrogen was 159 mg/kg, the total phosphorus was 386 mg/kg and the salinity was 5.5‰.

2.3. Coal cinder gel-beads preparation

The two strains (*Pseudomonas putida* PYR1 and *Acinetobacter baumannii* INP1) for pyrene and indeno(1,2,3-cd)pyrene biodegradation used in this study were prepared in previous research (Huang et al., 2016). The strain BAP1 was isolated from PAH-contaminated Liaohe estuarine wetlands in Panjin, Liaoning Province, China. The strain BAP1 was cultured and harvested as described by Huang et al. (2016). 10 g of the soil sample were put into a 250 mL sterile flask containing 100 mL of sterile saline solution. Then, the flask was placed in a laboratory shaker at 150 rpm at 30 °C for 4 h. The flask was allowed to settle for 2 h, and then, benzo(a)pyrene (4 mg/L) dissolved in acetones was added into the three flasks. After the acetone volatilized entirely, 2 mL of the supernatant and 18 mL of Basal salt medium (BSM) were transferred to these flasks to culture at 150 rpm at 30 °C for one week. This process was repeated using the higher concentrations of Benzo(a)pyrene (50, 75,100 mg/L), until pure strains were obtained. Pure strains were routinely subcultivated on BSM plates containing 100 mg/L benzo(a)pyrene. The 16S rDNA sequence data of strain BAP1 was submitted to the GenBank database under accession number KU199711. The strain BAP1 was identified as a strain of *Pseudomonas veronii* (97.9% similarity).

Three strains were immobilized in coal cinder powder using the method of entrapping described by Huang et al. (2016) and made into coal cinder gel-beads for HMW-PAHs biodegradation.

2.4. Experimental design

2.4.1. Batch experiment

Fifteen plexiglass barrels were prepared and filled with 8 kg of HMW-PAH-contaminated soils. The concentrations of pyrene, benzo(a)pyrene and indeno(1,2,3-cd)pyrene in the soils were kept at 168–175, 41–43 and 68–74 ng/g by adding diesel oils according to the actual PAH-contamination characteristics in the Liaohe coastal wetlands. The barrels were then divided into five plots (Plots A, B, C, D, E) with different treatments as described below:

Plot A: Served as the control without planting reeds or embedding cinder gel-beads.
Plot B: Only planting four reeds with 60 cm height.
Plot C: Planting four reeds with 60 cm height, and at the same time embedding 150 g of the cinder gel-beads entrapping strain PYR1 (containing 0.3×10^9–0.6×10^9 cells) in 10 cm deep rhizosphere soils.
Plot D: Planting four reeds with 60 cm height, and at the same time embedding 150 g of cinder gel-beads entrapping strain BAP1 (containing 0.3×10^9–0.6×10^9 cells) in 10 cm deep rhizosphere soils.
Plot E: Planting four reeds with 60 cm height, and at the same time embedding 150 g of the cinder gel-beads entrapping strain INP1 (containing 0.3×10^9–0.6×10^9 cells) in 10 cm deep rhizosphere soils.

The water-holding capacity of the soil in all of the plots was maintained at 30% throughout the period of the experiment by sprinkling water periodically when required.

In each plot, at a given sampling time, 2 g of the soil samples were collected at 0, 2, 4, 6, 10, 14, 20, 30 and 40 days from a depth of 10 cm using a soil collecting pipe from three different random positions for pyrene, benzo(a)pyrene and indeno(1,2,3-cd)pyrene analysis. Root exudates in 10 cm deep rhizosphere soils were examined at a given sampling time in this research.

2.4.2. Artificial root exudates adding experiment

Soil samples were naturally dried at room temperature and sieved by 1-mm mesh. Pyrene, benzo(a)pyrene and indeno(1,2,3-cd)pyrene dissolved in acetone were added to the soil samples to a final concentration of 150 ng/g, 35 ng/g and 55 ng/g dry soil, respectively. After the acetone evaporated, pyrene-, benzo(a)pyrene- and indeno(1,2,3-cd)pyrene-spiked soils samples were progressively mixed with unspiked soils and sieved again to ensure the homogeneity of samples.

Three types of sterilized soil samples were packed into 234 amber glass jars, and each jar was filled with 200 g of the soils and 5 g of the cinder gel-beads entrapping strains PYR1, BAP1 and INP1, respectively (containing 0.1×10^8–0.2×10^8 cells). Maleic, succinic and acetic acids, arginine and trehalose solutions were added, the adding original