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Influences and mechanisms of surfactants on pyrene biodegradation based on interactions of surfactant with a *Klebsiella oxytoca* strain

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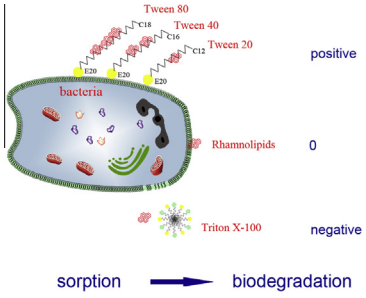
HIGHLIGHTS

- Surfactant-induced sorption was the primary contribution in pyrene biodegradation.
- Highly positive correlation between B_s^*/B_0^* and $K_{d,s}^*/K_{d,0}^*$ was found.
- Alteration of CSH and sorption ability of pyrene depended on surfactants structure.

GRAPHICAL ABSTRACT



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ABSTRACT

Surfactant-enhanced bioremediation has been proposed as a promising technology for the treatment of organic polluted soils; however its application has been hindered by the controversial influences and mechanisms of surfactants on the biodegradation of hydrophobic organic compounds. To address this problem, effects of five surfactants on the sorption and biodegradation of pyrene by *Klebsiella oxytoca* PYR-1, as well as their interactions with bacterial cell surface and membrane lipids were investigated. We found that surfactants enhanced or inhibited pyrene biodegradation depending on their effects on the sorption of pyrene onto bacterial cell, which occurred mainly through modifying cell surface hydrophobicity (such as Tween series surfactants) or disrupting bacterial membrane (such as Triton X-100), respectively. A relatively high positive correlation ($P < 0.0001$) was observed between biodegradation promotion (B_s^*/B_0^*) and enhancement of sorption coefficients ($K_{d,s}^*/K_{d,0}^*$) for pyrene in the presence of surfactant, indicating that surfactant-induced sorption played the dominant role during pyrene biodegradation.

1. Introduction

Surfactant-enhanced bioremediation (SEBR), as a promising treatment of soils polluted with hydrophobic organic compounds (HOCs), has attracted great interest, and numerous studies have been conducted to evaluate the effects and mechanisms of surfactants on the bioavailability and biodegradation of pollutants. Contradictory results have been reported, ranging from stimulation to inhibition of HOCs biodegradation (Laha and Luthy, 1991; Bueno-Montes et al., 2011; Zhang and Zhu, 2012), and several theories such as solubilization (Tiehm et al., 1997; Zhang et al., 1997), low bioavailability of micellar HOCs (Laha and Luthy, 1991), surfactant toxicity (Avramova et al., 2008) or preferential utilization (Kim and Weber, 2003) have been proposed. The uptake of substrate by bacterial cell, including sorption and transmembrane process, is considered as the crucial step for the biodegradation process (Gorna et al., 2011). Since the essential role of surfactants is reduction of interfacial/surface tension (Mulligan et al., 2001),

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the key to surfactant affected biodegradation may be the interactions between surfactant and HOCs as well as surfactant and bacterial cells.

We have proposed that the effects of surfactants on HOCs biodegradation were attributed to surfactant-induced sorption alteration (Zhang and Zhu, 2012), which correlates to bacterial cell surface hydrophobicity (CSH). The modification of CSH occurred via surfactant sorption on cell surface (Zhong et al., 2007; Zeng et al., 2011) or induction of lipopolysaccharides release (Li and Zhu, 2012). The native hydrophobicity of microbial cells has been widely considered as an important parameter determining the influence of surfactants on CSH. Several studies have confirmed that rhamnolipids increased the CSH for relative hydrophilic cells while decrease the CSH for hydrophobic cells (Kaczorek et al., 2008; Zhao et al., 2011). Additionally, surfactant properties are also important parameters affecting the CSH. Brown and colleagues (Brown and Jaffe, 2006) reported a preliminary results on the effects of surfactant polyoxyethylene and alkyl chain length on the change of CSH of a *Sphingomonas* sp. However, little information is available for the influence of surfactant structure on the interaction between surfactant and bacterial cells as well as subsequent sorption and biodegradation of HOCs.

The transmembrane transport of HOCs is the rate-limiting process in their biodegradation. The cytoplasmic membrane lipids of bacterial cells play an essential role in the function of transmembrane processes (Green et al., 1980). Molecules capable of affecting the fluidity of membrane through the interaction with lipids will interfere with biological basic processes. Surfactants molecules or micelles can insert, replace or solubilize the lipid bilayer (Shoji et al., 2012), resulting in membrane area variation or even cell disruption. However, the information of membrane permeability in the presence of surfactant and subsequent effects on biodegradation is not available.

Therefore, the present study investigated the effects of five commonly used surfactants (Tween 80, Tween 40, Tween 20, Triton X-100 and rhamnolipid) on biodegradation of pyrene by a PAH degrader *Klebsiella oxytoca* PYR-1 and their underlying mechanisms based on surfactant–bacteria interactions with the goal to provide recommendation of surfactants choice for enhancing biodegradation. Biosorption of pyrene, bacterial CSH, membrane permeability and phosphatidylcholine liposome experiments were conducted to highlight the different mechanisms based on surfactant–cell surface interactions and surfactant–lipid interactions.

2. Methods

2.1. Chemicals

Pyrene was selected as representative polycyclic aromatic hydrocarbons (PAHs) to model HOCs, and was purchased from Sigma–Aldrich Chemical Co. with a purity >98%. Nonionic surfactant Tween 80, Tween 20 and Tween 40, Triton X-100 were purchased from Acros Organics, Aladdin Reagent Co. and Sigma–Aldrich Chemical Co., respectively and used without further purification. Anionic biosurfactant rhamnolipids was purchased from Huzhou Gemking Biotechnology Co., Ltd. with a purity >90%. The properties of surfactants were listed in Supplementary Table S1. The critical micelle concentrations (CMC) were 13.1, 15.8, 19.0, 189, 10.86 mg/L, respectively. Fluorometric reagent *N*-phenyl-1-naphthylamine (NPN) and substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG) were purchased from Aladdin Reagent Co., Shanghai with purities of 98% and 99%, respectively. The inorganic reagents were of analytical grade (Sinopharm Chemical Reagent Co., Ltd., Shanghai).

2.2. Strain, medium and culture condition

The test bacterial strain *K. oxytoca* PYR-1 was isolated in our previous work (Zhang and Zhu, 2012), deposited at China Center for Type Culture Collection (accession number CCTCC AB 2010358). The mineral salt media (MSM) used in the bacterial growth and biodegradation assay was prepared as described by Yu et al. (2007). MSM excluded $FeSO_4{\cdot}7H_2O$ were autoclaved before used. Pyrene, $FeSO_4{\cdot}7H_2O$ and surfactant solutions were sterilized by vacuum filtration to avoid any transformation of these compounds.

2.3. Biodegradation and sorption of pyrene by live bacterial strain

Effects of surfactants on the sorption and biodegradation of pyrene by *K. oxytoca* PYR-1 were carried out in 500 mL autoclaved Erlenmeyer flasks as described in our previous work (Zhang and Zhu, 2012). Surfactants concentrations were used as followed: 0, 0.2, 0.4, 0.6, 0.8, 1, 2, 4, 8 times of each CMC. MSM (350 mL) containing 0.12 mg/L pyrene and 5×10^6 CFU/mL *K. oxytoca* PYR-1 with and without surfactants were incubated at 28 °C on constant temperature shaker at 150 rpm. Flasks were prepared in triplicate. Samples were taken every three days. The apparent removal, sorption and biodegradation of pyrene was measured by Waters UPLC system and calculated as described in our previous work (Zhang and Zhu, 2012) by the Eqs. (1)-(3).

$$\mathrm{ARR} = (C_0 - C_t - C_{\mathrm{ABL}})/C_0 \times 100\% \quad (1)$$

$$\mathrm{SR} = \frac{C_s V_{\mathrm{ex}} V_0 / V_{\mathrm{spl}}}{C_0 V_0} \times 100\% = \frac{C_s V_{\mathrm{ex}} / V_{\mathrm{spl}}}{C_0} \times 100\% \quad (2)$$

$$\mathrm{DR} = \mathrm{ARR} - \mathrm{SR} \quad (3)$$

where ARR, SR, DR are apparent removal ratio, sorption ratio and biodegradation ratio, respectively. C_0 is the initial concentration of pyrene; C_t is the concentration of pyrene in each interval sample, and C_{ABL} is the concentration of solute abiotic loss measured in the abiotic control. C_s is the extraction concentration of pyrene; V_{ex} is the volume of extraction methanol, 5 ml; V_{spl} is the sample volume, 5 ml; V_0 is the initial volume of solution.

2.4. Biosorption of pyrene in the presence of surfactant by inactive biomass

Inactive biomass of *K. oxytoca* PYR-1 was obtained by cryodesiccation followed by mechanical grinding. Two milligrams of inactive biomass was weighed into 22 mL centrifuge tubes with 20 mL MSM and surfactant solution containing 0.12 mg/L pyrene and 0.02% NaN_3 to avoid any possible biodegradation. The concentrations of surfactants were as followed: 0, 0.2, 0.4, 0.6, 0.8, 1, 2, 4, 8 times of each CMC. Tubes were then put in a shaker at 150 rpm at 28 °C for 20 h and were prepared in duplicate. Control treatments without biomass were prepared to account for the possible solute loss by handling and volatilization. After centrifuged for 20 min at 4000*g*, the supernatants were sampled to calculate the biosorption of pyrene.

2.5. Biomass and cell surface hydrophobicity

Biomass was measured by UV-spectrophotometer at 600 nm (UV-2401, Shimadzu, Japan). Bacterial cell surface hydrophobicity (CSH) was assessed by the bacterial adherence to hydrocarbons (BATH) method modified from Rosenberg et al. (1980). The CSH was calculated by the Eq. (4).