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Biosorption and biodegradation of BDE-47 by *Pseudomonas stutzier*

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ABSTRACT

The biosorption and biodegradation in the removal process of 2, 2, 4, 4-tetrabromodiphenyl ether (BDE-47) by *Pseudomonas stutzier KS0013* (*Ps*) were investigated to elucidate the bio-dissipation mechanism with the influences of glucose and rhamnolipids. The sorption capacity of live *Ps* (1.163 mg g^{-1}) was significantly larger than that of heat-killed *Ps* (0.845 mg g^{-1}), indicating biosorption of BDE-47 was metabolically mediated. The BDE-47 was rapidly adsorbed by *Ps* at the initial stage, and degradation was observed only after 4 h. Based on the *Kp* values, the BDE-47 was more likely to dissolve in water rather than adsorb on the *Ps*. Furthermore, cell surface hydrophobicity of *Ps* was significantly enhanced with additional rhamnolipids. Meanwhile, rhamnolipids was adsorbed on BDE-47 binding sites and subsequently blocked BDE-47 biosorption, therefore posed an adverse effect on biodegradation.

1. Introduction

Polybrominated diphenyl ethers (PBDEs), a class of ubiquitous environmental contaminants, have been widely used as flame retardants in the past decades. Recently, they have been reported to induce thyroid hormone disruption, neurobehavioral toxicity and potential carcinogenicity via their essential roles on cellular metabolism (Talsness, 2008). PBDEs are lipophilic with low solubility in water and thereby can be easily accumulated in biota through the food chain (Batterman et al., 2007). Among various congeners of PBDEs, a great deal of research efforts have been dedicated to 2, 2, 4, 4-tetrabromodiphenyl ether (BDE-47) mainly due to their wide distribution, detrimental impacts on the reproduction and nervous function of organisms (Liu et al., 2010).

Although a lot of remediation techniques are available, biodegradation still holds a variety of advantages like low cost, few or no by-products, reusability, ecosystem friendly and so on (Kim et al., 2007). According to the previous reports on aerobic biodegradation of PBDEs, *Sphingomonas* sp (Kim et al., 2007), white-hot fungi (Zhou et al., 2011), *Rhodococcus* sp. *RR1*, *Burkholderia xenovorans LB400*, *Pseudonocardia dioxanivorans CB1190* (Robrock et al., 2009), *Bacillus* sp and *Pseudomonas* sp (Huang et al., 2012; Chou et al., 2013; Deng et al., 2011; Lv et al., 2014a,b) were able to catabolize these chemicals by opening loop through oxidization. However, these studies mainly focused on PBDEs biodegradation, while the contributions of biosorption and storage in biomass were ignored in the PBDEs removal process.

The bio-removal process of BDE-47 includes three distinct steps (Li et al., 2013). At the beginning, there is an initial rapid accumulation step in which the BDE-47 binds on the cell wall through sorption. The second metabolism-dependent step is much slower, hydrophobic organic compound BDE-47 transports through the cell membrane due to its hydrophobicity and the cell metabolism, which causes a significant amount intracellular accumulation of BDE-47 by live *Ps*. Hence, the live biomass may show larger adsorption capacity than the dead biomass in the sorption of BDE-47. Finally, the biodegradation of BDE-47 occurred though cellular metabolism.

Biosorption is a physico-chemical process involved in the sorption of organic pollutants in/on a biological matrix/surface (Rouse et al., 1994). Many studies demonstrated that carbon source could stimulate the growth of cell wall and membrane, and surfactants could change the interfacial tension of matrix by increasing the solubility and mobility of organic compounds (Rouse et al., 1994). All these could also modify cell surface hydrophobicity and affect the bioavailability, therefore pose a substantial influence on biosorption and biodegradation. Thus, it is critical to elucidating the relationship between cell properties and the bio-removal of BDE-47 for the better understanding of the fate of PBDEs in biological treatment.

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Pseudomonas stutzier (*Ps*) was reported be able to degrade PBDEs (Zhang et al., 2013). In addition, *Ps* is ubiquitous in nature, which can serve as an option on remediation of PBDEs. The goal of the present study was to investigate the distribution of biosorption and biodegradation in the removal of BDE-47 by *Ps* and the influence of certain environmental factors on the biodegradation efficiencies. Isotherm and adsorption kinetic experiments for BDE-47 were performed using batch sorption experiment. The adsorption amount and portion of degradation with additional carbon sources and rhamnolipids were measured to elucidate the mechanism of biosorption. To the best of our knowledge, this is the first report of taking adsorption into account in the biodegradation of BDE-47. The findings here are fundamentally important for understanding the interaction mechanism on the BDE-47 degradation, which will hopefully facilitate the remediation design in this regime.

2. Materials and methods

2.1. Reagent and bacterial cultures

The reagents used in this work were analytical grade and BDE-47 was purchased from Sigma Aldrich. *P. stutzier KS0013*, kindly provided by State Key Laboratory of Pulp and Paper Engineering, was previously isolated from PBDEs-contaminated site at Shaoguan in Guangdong province.

Minimal salt medium (MSM) (Chen et al., 2009) was prepared by combing 5.2 g K_2HPO_4, 3.7 g KH_2PO_4, 1.0 g Na_2SO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, and 2.0 g NH_4Cl in 1 l deionized water. The enrichment culture medium contained 3 g l^{-1} beef extract, 10 g l^{-1} peptone, 5 g l^{-1} NaCl and 10 mg l^{-1} $MgSO_4$. The stain was inoculated in the enrichment culture medium and agitated at 100 r min^{-1} in the dark at 30 °C. After 72 h incubation, the stain was harvested by centrifugation and washed three times with sterile phosphate buffer solution. The heat-killed *Ps* was obtained after 15 min preservation in high-pressure steam sterilization pot at 120 °C.

2.2. Adsorption experiment

Adsorption experiments were firstly carried out in 40 ml brown serum vial containing 20 ml of BDE-47 solution. The quantified BDE-47 dichloromethane solution was transferred to the 40 ml brown serum vial, and evaporated dry. Live or killed *Ps* were added to MSM solutions to make the initial OD_{600} 0.2 (the mass of *Ps* in vial is approximately 2.78 mg). After that, the vials were sealed with membrane to prevent evaporation, and agitated at 100 r min^{-1} in the dark at 30 °C. Note that the pH of the solution was adjusted to 7.0 by either hydrochloric acid or sodium hydroxide.

2.3. Adsorption isotherm

Langmuir and Freundlich isotherms were adopted to describe the adsorption process. The Langmuir isotherm is:

$$Q_e = Q_{\max}\frac{K_L C_e}{1 + K_L C_e} \tag{1}$$

The Freundlich isotherm is:

$$Q_e = K_F C_e^{1/n} \tag{2}$$

where *Ce* is equilibrium concentration, *Qe* (mg g^{-1}) and Q_{max} (mg g^{-1}) are equilibrium adsorption capacity and the maximum adsorption capacity, K_L (L g^{-1}) is the affinity constant, K_F (mg g^{-1} (mg $l^{-1})^{1/n}$) and n are the Freundlich constants related to adsorption capacity and adsorption intensity (Fierro et al., 2008).

A modification of the fixed solids concentration procedure described by Dobbs (1987) was employed to determine the equilibrium partition coefficient (K_p) for the sorption of BDE-47 by bacterial biomass. Sorption of BDE-47 by biomass in the removal process was fitted to a linear sorption model given as follows:

$$K_p = P_x/P_w \tag{3}$$

where P_x is the mass of BDE-47 sorbed per gram of bacterial dry weight and P_w is the mass of dissolved BDE-47 per liter solution. This approach allows the quantification of sorption coefficients (K_p) and has a 95% confidence intervals by using a common spreadsheet program.

2.4. Adsorption kinetics

The pseudo-first-order and the pseudo-second-order equations were employed to fit the adsorption dynamic data (Xu et al., 2008).

The pseudo-first-order equation is:

$$dQ_t/dt = k_1(Q_e - Q_t) \tag{4}$$

where k_1 is the rate constant of pseudo-first-order adsorption, Q_t is the adsorption amount at contact time t and Q_e presents the amount of adsorption at equilibrium. To obtain the Q_e from the model, Eq. (4) becomes

$$Q_t = Q_e\left(1 - e^{-k_1 t}\right) \tag{5}$$

The pseudo-second-order equation based on adsorption capacity may be expressed in the form:

$$dQ_t/dt = k_2(Q_e - Q_t)^2 \tag{6}$$

where k_2 is the rate constant of pseudo-second-order adsorption. Integrating Eq. (6) and applying the initial conditions, we have

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{1}{Q_e}t \tag{7}$$

where, k_2 and Q_e in Eq. (6) can be received from the intercept and slope of the plot of (t/Q_t) vs *t*.

The Weber and Morris model (Sulaymon et al., 2013) was used to express the intracellular metastasis and expressed in the form:

$$Q_t = K_{W-M}\sqrt{t} + C \tag{8}$$

K_{W-M} is the Weber–Morris intraparticle diffusion rate constant (mg g^{-1} $t^{1/2}$) and *C* is the thickness of the boundary layer.

2.5. Biosorption and biodegradation experiment

Biodegradation were conducted the same way as adsorption, additional five group experiments were performed to investigate the contributions of biosorption and biodegradation to the removal of BDE-47 under different nutrient conditions. The minimal salt medium (MSM) was used as background solution. Five groups were designed as follows: (A) MSM was used as control; (B) heat-killed *Ps* + MSM; (C) live *Ps* + MSM; (D) live *Ps* + MSM with 0.2 g l^{-1} glucose; and (E) live *Ps* + MSM with 0.5 g l^{-1} glucose.

The quantified BDE-47 dissolved in dichloromethane was transferred in the 40-ml brown serum vial, and was evaporated dry to make the 20-ml solution of 0.16 mg l^{-1}. The vials were sealed