International Biodeterioration & Biodegradation 134 (2018) 7–15

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Production of more toxic hexa-brominated diphenyl ether from rapid biotransformation of decabromodiphenyl ether in anaerobic granular sludge

Qingxin Zhou[a,b,c,1], Cheng-Hao Ge[a,1], Hafiz Adeel Ahmad[a], Shou-Qing Ni[a,*], Xiaolin Liu[a], Cuina Bu[a], Fengli Wang[c], Baoyu Gao[a,**]

[a] Shandong Provincial Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, Jinan, China

[b] Institute of Agro-Food Science and Technology, Shandong Academy of Agricultural Sciences, Key Laboratory of Agro-Products Processing Technology of Shandong Province, Key Laboratory of Novel Food Resources Processing, Ministry of Agriculture, Jinan, China

[c] College of Life Science, Shandong Normal University, Jinan, China

ARTICLE INFO

Keywords:
Anaerobic granular sludge
Decabromodiphenyl ether
Hexabrominated diphenyl ether
High throughput sequencing
Microbial degradation

ABSTRACT

Decabromodiphenyl ether (BDE-209) is used widely as a flame retardant and deserves more attention due to its potential danger to environment. Previous studies generally considered that BDE-209 degraded slowly in environment. Herein, a rapid reductive debromination of BDE-209 to BDE-154 was demonstrated with simultaneous stoichiometric bromide recovery (45%) in 10 h driven by the anaerobic granules. Debromination occurred in anaerobic granules when they were spiked with BDE-209 dissolved in the solvent dimethyl sulfoxide (DMSO), which was proved a potential cosubstrate for stimulating PBDE debromination. Moreover, the solvent DMSO enhanced the penetration of BDE-209 across cell membranes and forced the dominant phylum in anaerobic granules to change from *Bacteroidetes* to *Euryarchaeota*. Thus, the amended culture could better raise the functional genera *Ralstonia* and *Pseudomonas*, accounting for the rapid degradation of BDE-209. This is the first report that BDE-209 could be quickly debrominated to BDE-154 in anaerobic granules. Given the high cytotoxicity of BDE-154 produced from BDE-209, the fate of BDE-209 in the wastewater treatment plants should be paid more attention.

1. Introduction

Polybrominated diphenyl ethers (PBDEs), a organo-bromine compounds, have been widely used as flame retardants for more than several decades in products such as computers, TV sets, textiles and cars. Due to their wide usage, PBDEs were regarded as ubiquitous contaminants across the world (Stiborova et al., 2015). Structurally related to thyroid hormones, PBDEs have been verified to have potential toxicity on internal visceral organs such as thyroid, liver and developmental systems of living bodies (Chen and Hale, 2010). In May 2009, hexa-, hepta-, tetra- and penta-BDEs were recognized as new Persistent Organic Pollutants (POPs) at the fourth Conference of the Parties in Geneva (Xu et al., 2013). Among three commercial mixtures of deca-, octa-, and penta-PBDE products, decabromodiphenyl ether (Deca-BDE, BDE-209) is the only one currently used in large quantities worldwide, and accounts for approximately 75% of the overall PBDEs (Hooper and Mcdonald, 2000).

It was reported that PBDEs had been found in the biosphere with increasing accumulation of PBDEs in the environment (Wang et al., 2011; Zeng et al., 2013). PBDEs were detected in various environmental media and even in human bodies in Guiyu Town, Guangdong Province, which was famous as one of the largest e-waste recycling centers in China (Jiang et al., 2014). The fate of PBDEs in the environment should be paid attention due to its wide usage, increasing accumulation and biotoxicity. Some chemical and environmental matrixes have been employed to investigate the photolysis and biodegradation of PBDEs, such as hexane (Bezares-Cruz et al., 2004), dimethylsulfoxide (Xie et al., 2009), methanol, methanol/water mixed solvents (Eriksson et al., 2004), soils (Liu et al., 2011), sediments (Qiu et al., 2012), sewage sludge (Shih et al., 2012) and bacteria (He et al., 2006; Shi et al., 2013). Although the fate of PBDEs in the environment has been widely studied, the technology of biotransformation of PBDEs in natural and artificial

* Corresponding author.
** Corresponding author.
E-mail addresses: sqni@sdu.edu.cn (S.-Q. Ni), bygao@sdu.edu.cn (B. Gao).
[1] Qingxin Zhou and Cheng-Hao Ge contributed equally to this work and shared the first authorship.

https://doi.org/10.1016/j.ibiod.2018.07.017
Received 12 May 2018; Received in revised form 30 July 2018; Accepted 30 July 2018

process has not been sufficiently investigated, expecially for the biodegration of PBDEs in anaerobic granules. In addition, biodegradation is considered as a safe and low-cost way to remove PBDEs (Chen et al., 2017a,b).

When exposed in the pure bacteria culture media, highly brominated PBDEs are liable to debrominate to less brominated products which are more bioaccumulative and toxic. It was demonstrated that pure isolates of *Dehalococcoides* species were able to transform an octa-BDE mixture to di-to hepta-BDEs in six months. *Dehalobacter* and *Desulfitobacterium* species were also found to debrominate PBDEs after three months (Robrock et al., 2008). However, the debromination by these pure bacteria only occurred with chloroethenes and chlorophenols as co-metabolism substrates (Lee and He, 2010). In addition, a series of different mixed cultures also have the potential to debrominate PBDEs. It was reported that the di-BDE could be transformed to mono-BDE and diphenyl ether in a fixed-film plug-flow biological reactor (Rayne et al., 2003). About 20% of penta-through hexa-BDEs was degraded by anaerobic bacterial mixed cultures isolated from river sediment after 70 days (Yen et al., 2009). What's more, anaerobic bacteria from soils could debrominate hexa- and nona-BDEs (Lee and He, 2010), serving as further evidence of debromination of PBDEs by biota. In addition, PBDEs can also undergo aerobic debromination by pure bacterial isolates and mixed cultures (Chen et al., 2010; Robrock et al., 2009), which includes adsorption on cell surface, assimilation into the cells, breaking down aromatic ring, and then mineralization (Wang et al., 2016).

Usually, debromination of higher brominated congeners is more difficult compared with less brominated congeners on account of their higher hydrophobicity and lower bioavailability (Zhao et al., 2018), especially for BDE-209. Deca-BDE was degraded to hepta- and octa-BDEs by bacteria *Sulfurospirillum multivorans* after two months cultivation with the addition of trichloroethene (He et al., 2006). Several reports showed that the PBDEs degradation could be increased with the addition of alternative carbon sources (Lu et al., 2013; Shi et al., 2013; Zhang et al., 2013; Stiborova et al., 2015), and this phenomenon is widely recognized as co-metabolism for PBDEs (Gu, 2016). Other works indicated that the absence of electron acceptor was one of the major limiting factors which hindered the biodegradation of refractory compounds under anaerobic conditions, such as nitrate, sulfate and bicarbonate (Huang et al., 2014; Chen et al., 2015). Beyond that, the high hydrophobicity of the PBDEs was another important factor responsible for the limited bioavailability, which could be improved by surfactants like Brij 30 and Brij 35 (Huang et al., 2014). Although the surfactants have the ability to enhance biodegradation of PBDEs, their toxicity and destructive nature hinder the wide applications. Dimethyl sulfoxide (DMSO) was usually uaed as one organic solvent to explore the photolysis of PBDEs (Xie et al., 2009; Sun et al., 2013). The DMSO not only contributes the uniform distribution of PBDEs in the water and enhances their adsorption on cell surface, but also can enhance penetration of other substances across biologic membranes. It seems that the DMSO is the proper solvent to study the fate of PBDEs in anaerobic granules.

A wastewater treatment plant (WWTP) collecting different types of wastewaters receives many sources of PBDEs, and therefore the discharges from the WWTP can become important contamination sources of PBDEs in receiving environments (Song et al., 2006). It was reorder that BDE-209 was the primary congener distributed in the rivers (Gevao et al., 2014; Salvadó et al., 2012). Anaerobic granular sludge is composed of dense microbial communities that sheltered a mass of organisms, allowing high loading rates in the reactors for the conventional activated sludge processes. Several reports explored the degradation of BDE-209 in the anaerobic sewage sludge (Gerecke et al., 2005; Shih et al., 2012), but except these, little information about the fate of BDE-209 in anaerobic granules can be found and related works are necessary. Whether it can be degraded into more toxic substances in anaerobic granules is still unknown.

The objectives of this study were to: 1) investigate the microbial degradation feasibility of BDE-209 in anaerobic granules; 2) clarify the degradation product of BDE-209 during anaerobic granular sludge treatment process; 3) explore the microbial community changes driven by DMSO and/or BDE-209.

2. Materials and methods

2.1. Materials

A mixture of eight PBDE congeners (Code BDE-CSM) was purchased from Accustandard Inc. (New Haven, CT, USA). The individual congener BDE -209 was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The fresh anaerobic granular sludge used in this study was collected from a UASB reactor (effective volume of ~9.0 L), which was in steady state for more than 6 months at room temperature under an organic loading rate of 1500 mg COD/L/d. The granules had the size distribution of 2–3 mm and were dark black.

2.2. Experimental process

To explore the interaction of anaerobic granular sludge and BDE-209, experiments were conducted in batch mode for a period of 24 h, a typical hydraulic retention time for anaerobic granular reactors. The fresh granular sludge and cultural medium were added to serum bottles at fixed suspended solid (SS) and PBDEs concentrations. All bottles were covered by silver papers to prevent any photolysis of PBDEs. The desired BDE-209 solution (4 mg/L BDE- 209) was diluted from a stock by deionized water, which was made by dissolving BDE-209 (500 mg/L) in DMSO. The DMSO concentration was fixed at 2%. DMSO is a common organic solvent for the biological tests of BDE-209 (Zhao et al., 2011). Sorption experiments were carried out with the inhibition of biological activity via adding sodium azide (0.2%, w/v) into the medium. The controlled trial (CK) was conducted in the same way with no BDE-209 dissolved into DMSO. During the experiments, 8 g SS/L granular sludge was stablely cultured in a rotary shaker (150 rpm, 20 °C). The biodegradation experiments were proceeded with no addition of sodium azide. No sludge was added to the control samples containing 4 mg/L BDE-209 using the same solvent. Argon gas was pumped into the mixed samples before the experiments, to create anaerobic conditions for the experiments.

2.3. Instrumental analysis

To determine bromide concentrations, samples were filtered through 0.45 μm syringe filter and then quantitatively measured by using ion-chromatography (DX 500, Dionex, Sunnyvale, CA, USA). The BDE-209 in samples were monitored by High performance liquid chromatography (HPLC) (model P1201, Elite Analytical Instruments, Dalian, China) with a UV detector (UV 1201) and a Sinochrom ODS-BP C18 column (200 mm × 4.6 mm). Potential biodegradation products were analyzed on gas chromatography-mass spectrometric platform (GC-MS, 7890A-5975C, Agilent Technologies) with a DB-5MS capillary column (15.0m × 250 μm × 0.25 μm). The details were shown in the literature by Ni et al. (2013).

2.4. Microbial community analysis

In order to gain insight into the microbial communities during the treatments, three sludge samples (sample Control, sample DMSO-fed treatment and sample DMSO and BDE-209-dosed treatment) were collected for subsequent analysis. Sample Control, was collected from the seed sludge, sample DMSO-fed treatment was collected from the sludge treated with only DMSO, and sample DMSO and BDE-209-dosed treatment sludge was collected from the end of treatments described in section 2.2. The genomic DNA was extracted according to the