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Spatial distribution and diversity of organohalide-respiring bacteria and their relationships with polybrominated diphenyl ether concentration in Taihu Lake sediments☆

Juan Chen [a, b, c], Pei-Fang Wang [a, b, *], Chao Wang [a, b], Jia-Jia Liu [a, b], Han Gao [a, b], Xun Wang [a, b]

[a] Key Laboratory of Integrated Regulation and Resource Department on Shallow Lakes, Ministry of Education, Hohai University, 1 Xikang Road, Nanjing 210098, PR China
[b] College of Environment, Hohai University, 1 Xikang Road, Nanjing 210098, PR China
[c] The State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, PR China

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ABSTRACT

It is acknowledged that organohalide-respiring bacteria (OHRB) can degrade polybrominated diphenyl ethers (PBDEs); however, very little is known about the distribution of OHRB or their response to PBDE contamination in natural sediments. We collected sediments from 28 sampling sites in Taihu Lake, China, and investigated the spatial distribution and diversity of OHRB, and the relationships between the PBDE contamination levels and the PBDE removal potential. The abundances of five typical OHRB genera, namely *Dehalobacter*, *Dehalococcoides*, *Dehalogenimonas*, *Desulfitobacterium*, and *Geobacter*, ranged from 0.34×10^4 to 19.4×10^7 gene copies g^{-1} dry sediment, and varied significantly among different areas of Taihu Lake. OHRB were more abundant in sediments from Meiliang and Zhushan Bay, where the PBDE concentrations were higher, and the phylotype diversity of the OHRB belonging to the family *Dehalococcoidaceae* was lower, than reported for other areas. While the sulfate concentrations explained much of the spatial distribution of OHRB, PBDE concentrations were also a strong influence on the abundance and diversity of OHRB in the sediments. For *Dehalococcoides*, *Dehalogenimonas* and *Geobacter*, the abundance of each genus was positively related to its own potential to remove PBDEs. The dominant OHRB genus, *Dehalogenimonas*, may contribute most to *in situ* bioremediation of PBDEs in Taihu Lake.

1. Introduction

Polybrominated diphenyl ethers (PBDEs), a class of persistent halogenated organic compounds that are widely used in plastics, textiles, electronics, and construction materials, have become ubiquitous contaminants in the environment, especially in aquatic ecosystems (Binelli et al., 2007; Zhu et al., 2014a). Because of their high sorption capacity, aquatic sediments are important long-term reservoirs of PBDEs, and PBDEs have frequently been detected in aquatic sediments worldwide, even at very high mg g^{-1} dry weight (dw) levels (Wang et al., 2007). For example, the extremely high concentrations of PBDEs, up to 18.7 and 45.9 mg g^{-1} dw, were observed in the sediments of Shihwa Lake in Korea (Moon et al., 2012) and Maozhou River in China (Sun et al., 2013), respectively. From just a few centimeters below the surface, aquatic sediments are oxygen-depleted, and so microbial reductive dehalogenation, which can transform highly halogenated organic pollutants into less toxic and more easily biodegradable products, in anaerobic sediments is a promising method for *in situ* bioremediation of contaminated environments (Zanaroli et al., 2015). Several previous studies have demonstrated the role of microbial reductive dehalogenation in removing PBDEs from contaminated sediments (Song et al., 2015; Pan et al., 2016), while other studies have described how they stimulated these processes by planting and adding nitrogen (N) (Chen et al., 2015; Wang et al., 2015a). However, these previous studies were mostly carried out in well-defined laboratory conditions, much less is known about the occurrence of *in situ*

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* Corresponding author. Key Laboratory of Integrated Regulation and Resource Department on Shallow Lakes, Ministry of Education, Hohai University, 1 Xikang Road, Nanjing 210098, PR China; College of Environment, Hohai University, Xikang Road No.1 Nanjing 210098, Jiangsu Province, PR China.
E-mail address: pfwang2005@hhu.edu.cn (P.-F. Wang).

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reductive dehalogenation for PBDEs and the functional bacterial groups involved.

Organohalide-respiring bacteria (OHRB), the most important functional bacterial group with reductive dehalogenation activity, can effectively use hydrogen or organic acid as electron donor and halogenated organic compounds as terminal electron acceptors, substituting the halogen atoms in these compounds with hydrogen under anaerobic conditions (Zanaroli et al., 2015). Studies have reported that the OHRB species with the ability to couple the reductive dehalogenation with energy conservation and bacterial growth mainly belong to the genera of *Dehalobacter*, *Dehalococcoides*, *Dehalogenimonas*, *Desulfitobacterium*, *Geobacter* and *Sulfurospirillum* (Ding and He, 2012). Isolates belonging to the genera *Desulfitobacterium*, *Geobacter* and *Sulfurospirillum* are more likely to be identified as they are metabolically versatile bacteria and can metabolite a wide range of halogenated organic compounds (Maphosa et al., 2010). However, the *Dehalococcoides*, *Dehalogenimonas* and *Dehalobacter* isolates are difficult to culture due to the fact that they strictly depend on organohalide respiration for growth, in most cases using hydrogen as the sole electron donor (Maphosa et al., 2010). Previous studies of OHRB have mainly examined their ability to degrade or transform halogenated organic pollutants such as dichlorobenzenes (Nelson et al., 2011), trichloroethene (Fung et al., 2007), and polychlorinated biphenyls (PCBs) (Yoshida et al., 2009) by pure or highly enriched cultures in defined media. Anaerobic reductive debromination of PBDEs by OHRB has also been reported by previous studies (He et al., 2006; Wang et al., 2015a; Pan et al., 2016). In particular, the octa-BDE technical mixture was reported to undergo stepwise debromination in *Dehalococcoides*-containing cultures and produce a variety of hepta-through di-BDEs, and debromination of BDE-209 to hepta- and octa-BDEs was also found in *Sulfurospirillum multivorans* culture (He et al., 2006). More recently, some biostimulation treatments like N addition and planting were reported to accelerate the reductive debromination of BDE-47 in mangrove sediments through increasing the abundance of OHRB, especially in genera *Dehalobacter* and *Dehalococcoides* (Chen et al., 2015, 2017). However, even though OHRB are widely distributed in aquatic sediments, very little is known about their natural ecological distribution and diversity in natural sediments (Wasmund et al., 2014a). We are aware of only one study by Pan et al. (2016) who, from their investigation of mangrove sediments, reported information about the vertical distribution of several OHRB genera in the sediments and concluded that the genus *Dehalococcoides* made an important contribution to PBDE removal in the saline habitat.

The geochemical characteristics of sediment in aquatic ecosystems, such as the pH and organic carbon contents, show considerable spatial variations that may contribute to the formation and distribution of specific bacterial communities (Bates et al., 2011; Shen et al., 2013). The bacterial abundances and community compositions can also be modified considerably by environmental pollution that results in enrichment of tolerant or degrading bacteria (Liu et al., 2015). Researchers have reported that sulfate and biogenic organohalogen compounds are an important influence on, and help shape, indigenous OHRB communities in marine sediments (Krzmarzick et al., 2013; Zanaroli et al., 2015). To date, however, there have been few studies of OHRB in freshwater habitats. Taihu Lake, the third largest freshwater lake in China, is one of the most polluted lakes in its basin (Xu et al., 2016), and various organic pollutants like PCBs and organochlorine pesticides have been detected in Taihu Lake sediments (Yin et al., 2017; Zhao et al., 2017). In particular, several previous studies reported that the PBDE contamination level in Taihu Lake sediments varied spatially, and that the concentrations were highest in Meiliang Bay (347 ng g^{-1} dw) (Wang et al., 2015a; Zhou et al., 2012a). A rich source of halogenated organic compounds in contaminated sediments has been reported to be able to favor the selection of native OHRB and to decrease microbial diversity *via* the process of environmental filtering (Liang et al., 2014; Liu et al., 2015). Therefore, it is hypothesized that the spatial distribution and diversity of OHRB may be correlated with PBDE concentration in Taihu Lake sediments. However, various other questions, relating to the abundance and distribution of OHRB in Taihu Lake sediments, the influence of PBDE contamination on the diversity of the indigenous OHRB community, and the potential relationships between indigenous OHRB and PBDE removal efficiency, remain unanswered.

The aims of the present study were to (i) investigate the spatial distribution of OHRB in the sediments from different areas of Taihu Lake and the factors that influenced their distribution, (ii) determine the phylotype diversity of the family *Dehalococcoidaceae* in the sediments using high-throughput sequencing technology, (iii) determine the intrinsic potential of Taihu Lake sediments to remove PBDEs, and (iv) explore the possible contribution of OHRB to PBDE removal from the contaminated sediments. The family *Dehalococcoidaceae* belonging to phylum *Chloroflexi* made an important contribution to reductive dehalogenation (Wasmund et al., 2014b). As one of the most common toxic congeners in Taihu Lake, we used BDE-47 generated through BDE-209 debromination as the model congener in our PBDE removal potential experiments.

2. Materials and methods

2.1. Site description and sediment sampling

Taihu Lake (30°56′–31°33′ N, 119°53′–120°36′E) to the south of the Yangtze Delta in China, has a mean depth of 1.9 m, covers an area of 2338.1 km^2, and serves as an important source of drinking water for the more than 34 million residents of the lake watershed. We collected surface sediments (from 0 to 15 cm depth) from 28 sampling sites in Taihu Lake in July 2014, including 5 from Meiliang Bay (ML1–ML5), 4 from Zhushan Bay (ZS1–ZS4), 6 from Gonghu Bay (GH1–GH6), 3 from the southern coastal area (SA1–SA3), 3 from the western coastal area (WA1–WA3), 4 from the eastern coast area (EA1–EA4), and 3 from the central area (CA1–CA3). The locations of the sampling sites were recorded with a GPS receiver and mapped, as shown in Fig. 1. At each site, surface sediment samples were collected from 5 random points within a given area of 10 m^2 with a grab sampler and composited into a single sediment sample. Visible stones and plant residues were removed before the sediment sample was homogenized. Fresh sediment samples were passed through an 8-mm sieve and subdivided into three portions for different analyses. The chemical properties and the PBDE removal potential were determined on one part; another part was freeze-dried before PBDE analysis, and the third part was stored at −20 °C until DNA extraction.

2.2. Determination of sediment chemical properties

The sediments were mixed with distilled and deionized water (ddH_2O) at a ratio of 1:2.5 (*w/v*) before the pH was measured using a pH meter (Mettler Toledo FE20, Switzerland). The total organic carbon concentrations were measured by high temperature oxidation with an automatic analyzer (LiquiTOC, Elementar, Germany). The total nitrogen (TN) concentrations were determined colorimetrically in a flow injection analyzer (FIA) (Lachat QuikChem Method 8000, USA) after digestion with concentrated sulphuric acid. Ammonia (NH_4^+) and nitrate (NO_3^-) concentrations in sediment were also determined colorimetrically by FIA after extraction with 2 M potassium chloride. Sulfate (SO_4^{2-})

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