Effects of Six Types of Straw Derived Biochar on Anaerobic Biodegradation of Polybrominated Diphenyl Ethers in Mangrove Sediments: A Microcosm Experiment

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Abstract. Biochar has widely applied as sorbent for sequestrating pollutants, however, its effects on biodegradation of organic pollutants and microbial mechanisms behind remains poorly understood. Here, we carried out a microcosm experiment to investigate the effects of six types of straw derived biochar on anaerobic biodegradation of polybrominated diphenyl ethers (PBDEs) in mangrove sediments. The results showed that the reductive debromination was a critical process of PBDE degradation under anaerobic condition. The reductive debromination efficiency was significantly enhanced after the addition of rice straw and corn straw biochar prepared at relatively low temperatures (C300, C400 and R300), but was inhibited by rice straw biochar produced at high temperatures (R500), indicating that the biochar effects on PBDE degradation depended on its pyrolysis temperature and straw type. The stimulatory effects of C300, C400 and R300 may be attributed to the increased abundance of organohaliderespiring bacteria (OHRB), especially genera Dehalogenimonas, and the high diversity of typical OHRB in family Dehalococcoidaceae. In addition, biochar addition significantly altered the bacterial community compositions, in particular, several OHRB genera were enriched by C300, C400 and R300, facilitating the anaerobic biodegradation of BDE-47 in contaminated sediments. These results will help us to understand the potential of biochar amendment for contaminated remediation.

1. Introduction
Polybrominated diphenyl ethers (PBDEs), a group of brominated flame retardants, are ubiquitous and persistent in variable environmental media [1]-[2]. Due to the lipophilic and hydrophobic characteristics of PBDEs, they are easily absorbed on soil/sediment particles [3]. Previous studies have detected high concentrations of PBDEs (up to mg g⁻¹ dry weight) in aquatic sediments around the world, and their contamination levels in the sediments of coastal areas near electronic wastes recycling sites were exceptionally serious [4]-[5]. Mangroves located in the intertidal zones are easily influenced by human activities. Various pollutants including PBDEs have been detected in mangrove sediments [3]-[6]. Most previous studies have focused on the spatial distribution and toxic effects of PBDEs on plants, animals and microbes [7]-[8], researches on the remediation strategy for PBDE-contaminated environment are very limited.

Microbial degradation is a major pathway controlling the dissipation of organic pollutants in contaminated environmental [9]-[10]. Mangrove sediments, especially apart from the surface layer at uppermost few centimeters, are often anaerobic [11]. Under anaerobic conditions, microbial reductive
dehalogenation play an important role in the degradation of halogenated organic pollutants, such as dichlorobenzenes [12], trichloroethene [13], pentachlorophenol (PCP) [14]. It has been demonstrated that organohaliderespiring bacteria (OHRB) can participate in reductive dehalogenation process [11]. Degradation of PBDEs via anaerobic reductive debromination by OHRB in biological reactors [15], or in sediment slurries [16] has been reported previously. However, the degradation efficiency of PBDEs by indigenous microorganism was reported to be very slow, due to its severely low bioavailability and high persistence [17]. Therefore, it is very necessary to explore a remediation strategy to accelerate microbial degradation of PBDEs in contaminated sediments.

Biochar, a carbon-rich material produced under oxygen-limited pyrolysis condition, has been widely used to mitigate climate change, improve soil fertility, increase nutrient availability and remediate contaminated environments [18]. Due to their high aromaticity and large specific surface areas, biochar particles can adsorb organic pollutants to their surfaces and reduce pollutant bioavailability [18]-[19]. To date, the amendments of biochar as an effective sorbent to remove organic pollutants have been extensively reported [20]-[21], but the effect of biochar amendment on degrading organic pollutants is not well understood. Yu et al. [14] found a stimulatory effects of biochar on biodegradation of PCP, while Ren et al. [20] reported an inhibitory effect of biochar on degradation of carbaryl due to its strong sorption and decreased bioavailability. These inconsistent results indicated that the effect of biochar addition on biodegradation of pollutants may depend on its type and amendment content [22]. However, the biochar effects on the biodegradation of PBDEs and the underlying microbial mechanisms are still not clear.

In this study, we carried out a 16 weeks microcosm experiment to (i) investigate the effects of six straw derived biochar amendment on microbial degradation of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in anaerobic mangrove sediments, and (ii) explore the changes in OHRB abundance, diversity and community composition after different biochar amendment. Six types of straw biochar were produced from two different biomass materials (rice and corn) by pyrolyzing at different temperatures (300, 400, and 500°C). BDE-47 is was one of the prevalent and most toxic congeners in aquatic [23], it was therefore selected as the model PBDE congener in this study.

2. Materials and Methods

2.1 Sediment collection and biochar preparation
Subsurface sediment (5-15 cm) was freshly collected from a National Nature Reserve for Mangroves in Zhongzhou City, Fujian Province, China (23°55’N, 117°26’E). The sediment preparation and its characteristics have been described elsewhere [24]. The biochars were produced by charring rice straw and corn straw, respectively, at three temperature conditions (300, 400, and 500°C) for 4 h using nitrogen gas (N2) as the medium gas. A total of six biochar materials, that is rice straw at 300°C (R300), rice straw at 400°C (R400), rice straw at 500°C (R500), corn straw at 300°C (C300), corn straw at 400°C (C400), corn straw at 500°C (C500) were obtained. Detailed methods for biochar pretreatment was the same procedure as described previously [25].

2.2 PBDE degradation experiment
The microcosm experiment was conducted using a 250 mL Quick-fit conical flask containing 100 mL of mineral salt medium and 100 g of fresh sediment, as described previously [6]. BDE-47 was added to the flask at a final concentration of 1 mg kg⁻¹ dw. Then, six kinds of biochar were separately added to obtain the biochar-to-sediment (dw) ratios of 2% (w/w). The headspace of each flask was vacuumed and then refilled with high purity N2. The sterilized negative controls were set up using autoclaved sediments, following the method of Chen et al. [9]. The sediment slurry was sampled at weeks 0, 2, 4, 8 and 16 for the following analysis.

2.3 Sediment chemical analysis
PBDE concentrations in the sediments were analyzed according to the previous method [16]. The detail
methods for the quality control and the recovery are shown elsewhere [3]. Dissolved organic carbon (DOC), iron, sulfate concentration and other chemical properties of sediment were measured and analyzed as described previously [26].

2.4 Determination of total bacterial and OHRB abundances
Total DNA in 0.5 g of sediment was extracted using a FastDNA SPIN Kit for soil (MP Biomedicals, Irvine, CA, USA). The abundances of total bacteria and five OHRB genera (Geobacter, Dehalobacter, Dehalococcoides, Dehalogenimonas and Desulfotubacterium) were determined by the quantitative real-time PCR (qPCR) analysis. Primers used for each reaction, PCR amplification mixture constituent, thermal cycling conditions of qPCR and standard curve creation have been provided elsewhere [26].

2.5 Barcode pyrosequencing and bioinformation analysis
The V4 hypervariable region of bacterial 16S rRNA gene was amplified from genomic DNA and purified PCR products were then sequenced on an Illumina HiSeq platform [16]. The data processing program and bioinformation analysis methods have been described in detailed by Chen et al. [26]. To analyze the diversity and community composition of typical OHRB, we selected the Operational Taxonomic Units (OTUs) in the family Dehalococcoidaceae. Non-metric multidimensional scaling (NMDS) and canonical correspondence analysis (CCA) were conducted in R using the “vegan” package. The “pheatmap” package in R was used for hierarchical cluster analysis.

3. Results and Discussion
3.1 PBDE concentration in the sediments

Figure 1. Changes in the percentages of residual BDE-47 to initial spiked concentration (a, b), the concentration of total debromination products (∑de-PBDEs) (c) and the percentage of each de-PBDEs to the total (d) in the sediment slurries during the anaerobic microcosm experiment.

The BDE-47 concentrations significantly decrease in all biotic groups (Figure 1a, b), but no significantly changes were observed among different treatments in the abiotic groups (data not shown). Three debromination products (de-PBDEs), including BDE-7, -17, and -28, were identified in the sediments of all biotic groups at week 16 (Figure 1d), following the concentration order that BDE-28<BDE-7<BDE-17, which is consistent with the results of Zhu et al. [23]. This result indicates the critical role of the indigenous microorganisms in reductive debromination of PBDEs in anaerobic sediments. Compared with the control, the sediment with C300, C400 and R300 addition had lower BDE-47 residual percentages at week 16, and the C300 group has the lowest value of BDE-47 residual percentages (only 58.6%) (Figure 1a). The concentration of the de-PBDEs was higher in C300, C400 and R300-amended groups, suggesting the enhancement effects of these biochar types on biodegradation of BDE-47. Similarly, Tong et al. [25] reported that rape-straw derived biochar enhanced microbial reductive dechlorination of PCP. On the contrary, a significant inhibitory effect of R500 on BDE-47 degradation was observed, with higher BDE-47 residual and lower de-PBDE concentrations in the anaerobic microcosms (Figure 1c). Investigations have revealed that sorption affinity of pollutions
increased with the pyrolytic temperature of biochars [20], which in turn reduced the bioavailability and biodegradation rate of organic compounds [18]. Consistent with our results, Ren et al. [20] also reported that the effect of biochar amendment on the degradation of carbaryl varied among different biochar types.

3.2 Abundances of total bacteria and OHRB in the sediments
To reveal the microbial mechanisms behinds the different effects of six types of straw derived biochars on the BDE-47 degradation, we measured the copy numbers of total bacterial and OHRB 16S rRNA gene in the sediments at week 16 using qPCR. Some OHRB species have been identified in mangrove sediments, which can degrade PBDEs by reductive dehalogenation process [26]. As shown in Figure 2, the copy numbers of total bacterial 16S rRNA gene were higher in the groups with biochar addition, irrespective of biochar type, possibly due to the increased carbon nutrient level provided by biochar, which can promote microbial growth and activity [22]. No significant differences in total bacteria were found among three types of biochars produced under different pyrolytic temperature. For OHRB, the abundance of Geobacter spp. were higher in C300 and C400, and that of Dehalobacter spp. were comparable between the control and the biochar produced under 300 and 400℃. The abundances of three genera including Dehalococcoides, Dehalogenimonas and Desulfotobacterium were higher in C300, C400 and R300 groups, and those were lowest in R500 group, which was in line with the pattern of BDE-47 degradation rate in the sediments. This result implied that these OHRB were involved in the biodegradation process of BDE-47 in anaerobic mangrove sediments.

![Figure 2](image-url)

**Figure 2.** The 16S rRNA gene copies of total bacteria (a), Geobacter spp. (b), Dehalobacter spp. (c), Dehalococcoides spp. (d), Dehalogenimonas spp. (e) and Desulfotobacterium spp. (f) in the sediments at the end of the experiment.

Compared with other four OHRB genera, Dehalogenimonas with up to 10^6 copies g^-1 dw had higher abundances and had the strongest effect on the removal of BDE-47 based on the results of stepwise multiple linear regression analysis, which is in line with a recent study on Taihu Lake sediment [9]. Dehalogenimonas has been reported to sustain its syntrophic growth with other bacterial species, contributing greatly to reductive debromination process [27]-[28]. The stronger stimulatory effects of C300, C400 and R300 on OHRB may be attributed to the relatively higher DOC in the sediment slurries (data not shown), which may act as electron donors for enhancing the growth of OHRB [14]. Tong et al. [25] reported that the biochar addition, due to the increased DOC, enhanced the OHRB growth and the biodegradation of PCPs by reductive dechlorination.

3.3 Bacterial diversity and community composition
To further investigate the role of OHRB in BDE-47 degradation, the OTUs belonging to Dehalococcoidaceae family, a typical OHRB taxa [28], were selected from the whole sequencing database for the OHRB diversity and community composition analysis. The OTU numbers (phytotype diversity) of OHRB belonging to the family Dehalococcoidaceae was highest in the C300 group, but lowest in the R500 groups (Figure 3), similar to the results of OHRB abundances (Figure 2). Differently, Chen et al. [26] found that the alpha diversity indices of total bacteria were comparable between the control and the biochar addition groups. The significant variations among different biochar groups in
this study indicated a specific response of degrading microbes to organic pollutants [25]. A negative relationship was observed between BDE-47 concentration and OHRB diversity (Figure 3). This result could be explained by “the environmental filtering” of pollutants [29], that is, the high PBDE contamination exerted a strong selective pressure on some OHRB species having strong tolerance and had toxic effect on some sensitive species.

Based on the NMDS analysis, the OHRB communities in the sediment without biochar addition were clearly separately from those with, and the biochar produced under 500°C were significantly separated from those under lower temperature (300°C and 400°C) (Figure 4). This result indicated that the biochar addition significantly altered the OHRB community composition and such effects were dependent on the pyrolysis temperature and straw type. The relative abundances of several OHRB genera including Geobacter, Dehalobacterium, Dehalogenimonas, Desulfovibrio, Dehalococcoides and Sulfurospirillum, which are known to participate in reductive dehalogenation process [11], were higher in the C300, R300 and C400 groups, but lower in C500 and R500 groups (Figure 5). The positive effects of C300, R300 and C400 on these microbial communities may result in the stronger reductive debromination of BDE-47 in sediments, as reported previously [26]. The decreased relative abundance on the OHRB genera by C500 and R500 might be related to the low bioavailability and high residual concentration of BDE-47, which became toxic to these bacteria species [25]. We used CCA, based the OTUs detected by amplicon sequencing, to identify the main factors driving the variations in community composition of OHRB in family Dehalococcoidaceae. As shown in Figure 6, Fe(II)/Fe(III) and DOC contributed positively, while the residual BDE-47 concentration and NO₃ contributed positively to separating groups with the biochar produced under low temperature (C300 and R300) and that under high temperature (C500 and R500). Fe(III) reduction mediated by iron-reducing bacteria (Geobacter) has been reported to affect the efficiency of anaerobic reductive dehalogenation [30]. The positive correlation of Fe(II)/Fe(III) and
bacterial community composition in this study indicated an important role of anaerobic Fe(III) reduction in affecting BDE-47 degradation [26]. The C300 and R300 groups had high DOC concentration compared with other groups (data not shown). Organic C is known to be a critical factor for OHRB growth because it can directly provide C source or electron donors like hydrogen and acetate [25]. Previous studies have reported that biochar addition increased the sediment DOC concentrations and stimulated the OHRB growth, which in turn accelerated the biodegradation of PBDEs and PCP [25]-[26].

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