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Enhancing tetrabromobisphenol A biodegradation in river sediment microcosms and understanding the corresponding microbial community

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ABSTRACT

In situ remediation of contaminated sediment using microbes is a promising environmental treatment method. This study used bioaugmentation to investigate the biodegradation of tetrabromobisphenol A (TBBPA) in sediment microcosms collected from an electronic-waste recycling site. Treatments included adding possible biodegradation intermediates of TBBPA, including 2,4-dibromophenol (2,4-DBP), 2,4,6-tribromophenol (TBP), and bisphenol A (BPA) as co-substrates. Bioaugmentation was done with *Ochrobactrum* sp. T (TBBPA-degrader) and a mixed culture of *Ochrobactrum* sp. T, *Bacillus* sp. GZT (TBP-degrader) and *Bacillus* sp. GZB (BPA-degrader). Results showed that bioaugmentation with *Ochrobactrum* sp. T significantly improved TBBPA degradation efficiencies in sediment microcosms ($P < 0.01$); aerobic conditions increased the microbes' degradation activities. Co-substrates 2,4-DBP, TBP and BPA inhibited biodegradation of TBBPA. A metagenomic analysis of total 16S rRNA genes from the treated sediment microcosms showed that the following dominant genera: *Ochrobactrum*, *Parasegetibacter*, *Thermithiobacillus*, *Phenylobacterium* and *Sphingomonas*. The genus level of *Ochrobactrum* increased with increased degradation time, within 10-week of incubation. Microbes from genus *Ochrobactrum* are mainly linked to enhance the TBBPA biodegradation.

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1. Introduction

Tetrabromobisphenol A (TBBPA) is a widely used brominated flame retardant, and reduces the flammability of final manufactured products (de Wit, 2002). It is released into the environment during the production, use, and disposal of TBBPA-containing products. TBBPA is very persistent and has been detected in a wide range of environmental media, including air, water, soil and sediment (de Wit et al., 2010; Song et al., 2014; Xiong et al., 2014), and even in human tissues and plasma (Fujii et al., 2014a, b). TBBPA persists due to its high lipophilicity (log $K_{ow} = 5.9$), low volatility (7.0×10^{-11} atm m^3 mol^{-1}), low water solubility (4.16 mg L^{-1} at 25 °C in H_2O) and recalcitrance (McCormick et al., 2010). Unfortunately, the evidence increasingly highlights the possible toxic effects of TBBPA (Decherf et al., 2010; An et al., 2011; Koike et al., 2013; Linhartova et al., 2015). Furthermore, like halogenated polychlorinated biphenyls or dioxins, TBBPA may migrate to sediment due to hydrophobicity. When TBBPA enters the environment, photo-oxidation, chemical oxidation, and biodegradation may transform it (Chang et al., 2012b; Liu et al., 2013; Wang et al., 2015).

The in situ remediation of contaminated sediment using microbes is a promising treatment method (Acosta-González et al., 2013; Tischer et al., 2013; Dalvi et al., 2014; Islam et al., 2015). In a previous study, a novel bacterium *Ochrobactrum* sp. T was isolated and tested for its ability to effectively degrade TBBPA in water; the microorganism successfully degraded TBBPA, and used TBBPA as a sole carbon and energy source under aerobic conditions (An et al., 2011). Little is known, however, about the role of *Ochrobactrum* sp. T in enhancing TBBPA degradation and elimination in sediment. As such, further study is needed to support its practical use as in situ remediation strain.

Adding alternative halogenated compounds (referred to as "haloprimers" agents) can enhance the ability of anaerobic

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microorganisms to dechlorinate polychlorinated biphenyls in sediment (Ahn et al., 2005, 2008; Pöritz et al., 2015). A microbial consortium enriched from sediment using a mixture of 2-, 3-, and 4-bromophenol may improve 1,2,3,4-tetrachlorodibenzo-*p*-dioxin dechlorination (Vargas et al., 2001). Halogenated aromatic compounds (“haloprimers”) with similar analogous structures to 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (TeCDD)/1,2,3,4-tetrachlorodibenzofuran (TeCDF) could also effectively enhance TeCDD/F dechlorination (Ahn et al., 2005). Using halogenated co-substrates as haloprimers could stimulate anaerobic dechlorination, because certain bacteria can use halogenated co-substrate as a terminal electron acceptor during the respiratory reductive dehalogenation or dehalorespiration process (Hiraishi, 2008). This respiratory activity may play an important role in detoxifying organohalides released into the environment (Park et al., 2011). Based on these past studies, this research explored a number of co-substrates to assess their effect on TBBPA biodegradation.

Understanding the biodegradation enhancement of organics in bioaugmentation experiments also requires to understand bacterial community responses. This is because high densities of aboriginal microorganisms existed in sediment, and sediment-based bacterial communities facilitate organic decomposition (Krumins et al., 2009; Lin et al., 2014). Information about the bacterial community composition in sediment is important to understand TBBPA degradation in sediment ecosystems.

This study had four main elements. First, it investigated TBBPA biodegradation in contaminated sediment undergoing bioaugmentation with *Ochrobactrum* sp. T. Next, it compared the influence of co-substrates, by adding 2,4-dibromophenol (2,4-DBP), 2,4,6-tribromophenol (TBP), or bisphenol A (BPA) as co-substrates for TBBPA biodegradation. Third, it investigated the degree of TBBPA biodegradation under aerobic and anaerobic conditions. Finally, it analyzed bacterial community structure responses to bioaugmentation using high-throughput sequencing.

2. Materials and methods

2.1. Chemicals

TBP and TBBPA, with purities exceeding 99%, were purchased from Sigma–Aldrich (St. Louis, MO, USA). N,O-bis(trimethylsilyl) trifluoroacetamide/trimethychlorosilane (BSTFA:TMCS, 99:1, v/v), 2,4-DBP, and BPA, with purities exceeding 99%, were obtained from Acros Organics (New Jersey, USA). Surrogates, including ^{13}C-TBP, ^{13}C-TBBPA, and ^{13}C-BPA, were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Hexane, methanol, and dichloromethane of high-performance liquid chromatography (HPLC) grade came from CNW technologies (Shanghai ANPEL Scientific Instrument Co., Ltd., Shanghai, China). HPLC grade acetone was from J. T. Baker (Center Valley, PA, USA). All other chemicals were of analytical grade and from Guangzhou Chemical Reagent Co., Inc., China (South of Industry AVE, Guangzhou, China). The LC-Florisil cartridge (1 g, 6 cc) was provided by Sigma–Aldrich (St. Louis, MO, USA); silica sorbent (300–400 mesh) was from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). These were used after Soxhlet extraction, activated and deactivated.

2.2. Sampling and medium

Sediment samples were collected from the Liangjiang River and Beigang River (0–10 cm depth) in South China (23°327′N, 116°342′E). These rivers are heavily contaminated with TBBPA, TBP, BPA, polybrominated diphenyl ethers (PBDEs), and heavy metals (Xiong et al., 2014). Sediment samples were packed into sterile glass jars, sealed, and stored at −20 °C until used.

Preparation of the mineral medium (MM) used in biodegradation and isolation of the TBBPA-degrading bacteria for experiments, and the preparation of the growth medium (GM) were done based on our previously published work (An et al., 2011). The pH value of the basal medium was adjusted to 7.0 before autoclaving at 121 °C for 30 min.

2.3. Microcosm setup

Microcosms were created with homogenized sediment, containing 47% (V/V) sediment, 53% (V/V) MM, and 10 mg L^{-1} of TBBPA. TBBPA was first dissolved in MM at a fixed concentration before being added to the sediment. Each microcosm unit included 150 mL homogenized sediment in a 250 mL serum bottle; bottles with anaerobic microcosms were capped with a silicone stopper.

Eight treatments were created, each with a duplicate: (1) sterile controls (autoclaved at 121 °C for 30 min) under aerobic conditions; (2) unamended controls under aerobic conditions; (3) aerobic conditions plus bioaugmentation with *Ochrobactrum* sp. T; (4) anaerobic conditions plus bioaugmentation with *Ochrobactrum* sp. T; (5) co-substrate TBP plus bioaugmentation with *Ochrobactrum* sp. T under aerobic conditions; (6) co-substrate BPA plus bioaugmentation with *Ochrobactrum* sp. T under aerobic conditions; (7) co-substrate 2,4-DBP plus bioaugmentation with *Ochrobactrum* sp. T under aerobic conditions; and (8) bioaugmentation with a mixed culture containing *Ochrobactrum* sp. T, *Bacillus* sp. GZT and *Bacillus* sp. GZB under aerobic conditions. An equal microbial population was added to each treatment. As such, the microbial population of each bacterium of the mixed culture occupied one-third of the total microbial population.

Previous research demonstrated that *Ochrobactrum* sp. T can degrade and mineralize TBBPA (An et al., 2011); and *Bacillus* sp. GZT can degrade 2,4-DBP and TBP. These latter two substances are also intermediates produced when TBBPA is degraded by *Ochrobactrum* sp. T (Zu et al., 2012); *Bacillus* sp. GZB can degrade BPA, which is also an intermediate produced when TBBPA is degraded by *Ochrobactrum* sp. T (Li et al., 2012).

These bacteria were inoculated in the sterilized GM at 37 °C in a rotary incubator at 200 rpm for 15 h. Then, 30 mL of the incubated GM was centrifuged and rinsed three times with sterilized water to facilitate bacteria collection. The bacteria (0.37 g, wet weight) were then aseptically transferred to the sediment microcosms. All the microcosms were incubated on a horizontal shaker (150 rpm) in the dark at 25 °C. The anaerobic microcosms were obtained by continuously flushing the sealed bottles with N_2 for 1 h.

For the samples to support microbial community analysis, serum bottles were shaken thoroughly and subsamples (5 mL each) were extracted from the main sample with a glass syringe once a week for 10 weeks. At each sampling event, an additional 5 mL sample was collected from the bioaugmentation experiments with a culture containing *Ochrobactrum* sp. T for microbial community analyses.

2.4. Chemical analysis

Sediment samples from microcosms were spiked with 40 ng ^{13}C-TBBPA surrogate standard, freeze-dried, and extracted three times using a 40 kHz ultrasonic processor with 20 mL hexane:acetone (1:1, v/v) for 40 min (Xiong et al., 2014). Elemental sulfur was removed using HCl-activated copper powder. Three extracts were combined and concentrated to 1 mL using ultra-high purity N_2 (99.999%). Cleanup was performed using LC-Florisil cartridges (1 g, 6 cc) (Labadie et al., 2010; Xiong et al., 2014), as follows. First, 0.5 g anhydrous sodium sulfate was packed on the top of the LC-Florisil cartridge, and then sequentially preconditioned with

15 mL of dichloromethane:methanol (95:5, v/v) and 10 mL of hexane. After loading the sample, the cartridge was rinsed with 10 mL of hexane (discarded) and 10 mL of hexane:diethylether (8:2, v/v, discarded). Then, target compounds were eluted with 15 mL of dichloromethane: methanol (95:5, v/v); the eluent was then dried and re-dissolved in 1.5 mL of hexane:diethylether (6:4, v/v). Further cleanup was performed on a 1 g activated silica cartridge, pre-conditioned with 10 mL of hexane:diethylether (6:4, v/v). After sample loading, the target compound TBBPA was eluted with 10 mL of hexane:diethylether (6:4, v/v). Finally, the extracts were dried under a gentle stream of ultra-high purity N_2 and derivatized before the analysis. The derivatized procedure was performed as follows: 50 μL of BSTFA:TMCS (99:1, v/v) and 150 μL of hexane were added to the sample, and the sample then rested at 60 °C for 1 h.

TBBPA concentrations were analyzed using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer, using a HP-5 MS (30 m × 0.32 mm, 0.5 μm film thickness, J & W Scientific, Folsom, Calif.) (Xiong et al., 2014). Surrogate recoveries of all samples were 83.12 ± 15.71%.

2.5. Data analysis

The remaining percentage of TBBPA was calculated as the residue concentration divided by the original concentration, multiplied by 100. The TBBPA biodegradation data collected for this study fit well with first-order kinetic equations: $C = C_0\exp(-kt)$, $t_{1/2} = \ln2/k$, where t is time, C_0 is the initial substrate concentration, C is the substrate concentration at time t, and k the degradation rate constant. Statistically significant differences were set as those with at a p-value of less than 0.01. Statistical analysis was performed using Microsoft Excel 2010 and the Statistical Package for Social Sciences v18.0 software (SPSS Inc., IL, USA). Statistical significance between datasets was tested using analysis of one-way variance (ANOVA).

2.6. Microbial community analysis

For the microbial community analysis, total genomic DNA was extracted from sediment using E. Z. N. A.® Soil DNA Kit (Omega Bio-tek, Inc., USA), following the manufacturer's protocol. The V3–V4 region (V3–V4, 460 bps) of the 16S rRNA gene was amplified in a 50 μL reaction mixture containing 5 μL of 10 × PCR buffer, 0.5 μL of 10 mM dNTP each, 10 ng of template genomic DNA, 0.5 μL of 50 μM each primer, 0.5 μL of 5 U μL^{-1} Plantium Taq, and sterilized ddH_2O. The fusion primers included a universal template specific sequence and a recommended barcode sequence by Illumina Miseq 2 × 300 bp sequencing Platform (California, USA). The forward primers contained a universal template specific sequence 341F (CCTACACGACGCTCTTCCGATCTN) (Klindworth et al., 2013) and a recommended barcode sequence (CCTACGGGNGGCWGCAG). Reverse primers contained a universal template specific sequence 805R (GACTGGAGTTCCTTGGCACCCGAGAATTCCA) (Klindworth et al., 2013) and a recommended barcode sequence (GACTACHVGGGTATCTAATCC).

The PCR programs ran on a Bio-rad T 100™ thermal cycler (California, USA) using the following procedures: initial denaturation at 94 °C for 3 min; followed by 5 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 20 s, and elongation at 65 °C for 30 s. This process was followed by another 20 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s, and elongation at 72 °C for 30 s with a final extension at 72 °C for 5 min. PCR amplicons were evaluated using electrophoresis on 1.5% agarose gel and extracted using the SanPrep Column DNA Gel Extraction Kit (Sangon Biotech, Shanghai, China). The resulting material then underwent high-throughput sequencing on the Illumina Miseq 2 × 300 bp sequencing Platform (California, USA) from Sangon Biotech Co., Ltd (Shanghai, China).

The raw sequences from the high-throughput sequencing were checked with quality control procedures to remove low quality sequences containing ambiguous nucleotides and quality values ≤ 20 bp using PRINSEQ-lite software (PRINSEQ-lite 0.19.5, Japan). Clean sequences were then processed to merge the paired-end sequences to form Illumina tags (Zhou et al., 2011) with a length greater than 400 bp using FLASH v1.2.7 software (http://sourceforge.net/projects/flashpage). A python script was used to remove artificial replicates produced by high-throughput sequencing, as these artificial replicates may lead to errors in bioinformatics analysis (Gomez-Alvarez et al., 2009). In addition, chimeras were identified using the Mothur software (http://www.mothur.org) with the "chimera.uchime" command, using the sequences of SILVA database as a reference. Sequencing errors were corrected using the "pre.cluster" command. High quality sequences were normalized to equalize the number of sequences in each sample and to support further bioinformatic analysis.

Sequences were clustered into Operational Taxonomic Units (OTUs) using UCLUST software (http://www.drive5.com/uclust) with a similarity threshold of 97%, based on the Uclust algorithm (Edgar, 2010). Representative sequences of OUTs were selected based on the most abundant sequences, and the Ribosomal Database Project (RDP) classifier was used to assign the taxonomy (Cole et al., 2005) with a threshold of 80%. The sequences were then aligned using the Phyton Nearest Alignment Space Termination (PyNAST) algorithm (Caporaso et al., 2010). Species richness, diversity indices (i.e., Chao1 richness estimator, Shannon diversity index, Simpson diversity index and abundance-based coverage estimator (ACE)), and rarefaction curves were obtained using Mothur software based on the observed OTUs.

The 16S rRNA gene sequences were deposited in the NCBI Sequence Read Archive under accession numbers SRA307262.

3. Results and discussion

3.1. Degradation of TBBPA in sediment microcosms

Sediment microcosms in this study were amended with oxygen (aerobic conditions) and co-substrates (2,4-DBP, TBP or BPA). Extensive TBBPA biodegradation occurred in sediment microcosms, with each treatment showing statistically significant differences when compared to the sterile controls. As Fig. 1a shows, no TBBPA degradation was seen in the sterile controls; and only 3.4% of the TBBPA was degraded in the unamended control after a 10-week incubation period ($P < 0.01$). This suggests that only a few aboriginal TBBPA-degrading bacteria were in the unamended controls.

In contrast, approximately 52.1% of TBBPA was degraded in the samples bioaugmented with *Ochrobactrum* sp. T after a 10-week incubation. The TBBPA degradation rate constant (k) was 1.1×10^{-2} d^{-1} and the half-life ($t_{1/2}$) was 63 d, when bioaugmented with *Ochrobactrum* sp. T (Table 1). These results show that by adding *Ochrobactrum* sp. T into the sediment microcosms could bioaugment TBBPA degradation efficiency (52.1%), compared with the unamended control (3.4%). Thus, TBBPA degradation in sediment microcosms is mainly caused by the *Ochrobactrum* sp. T. This is consistent with previous results (An et al., 2011), although the degradation efficiency in this study (52.1%) was lower. This is explained by the fact that the strong sorption of TBBPA onto sediment may reduce microorganism availability. Together, these results suggest that adding TBBPA-degrading bacterium could enhance TBBPA degradation efficiency in sediment microcosms.

Previous research demonstrated that TBBPA can be reductively debrominated and then mineralized under aerobic conditions by

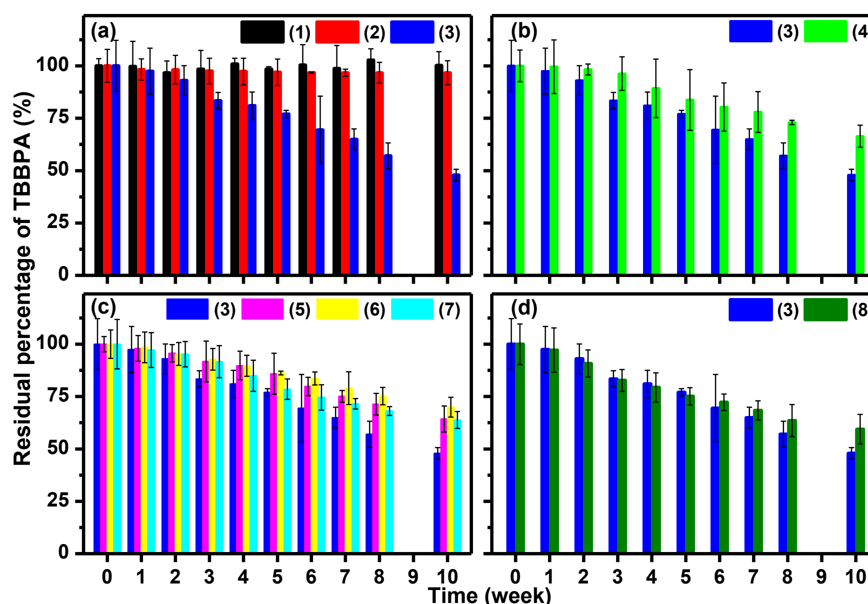

Fig. 1. Percentage of residual TBBPA in the sediment microcosms. (1) sterile controls (autoclaved at 121 °C for 30 min); (2) unamended controls; (3) bioaugmentation with *Ochrobactrum* sp. T; (4) anaerobic condition plus bioaugmentation with *Ochrobactrum* sp. T; (5) co-substrate TBP plus bioaugmentation with *Ochrobactrum* sp. T; (6) co-substrate BPA plus bioaugmentation with *Ochrobactrum* sp. T; (7) co-substrate 2,4-DBP plus bioaugmentation with *Ochrobactrum* sp. T; and (8) bioaugmentation with a mixed culture containing *Ochrobactrum* sp. T, *Bacillus* sp. GZT and *Bacillus* sp. GZB. In (a), data for each treatment were significantly different from that of sterile controls at $P < 0.05$ level; in (b, c, d), data for each treatment were not significantly different from that of bioaugmentation with *Ochrobactrum* sp. T at $P < 0.05$ level.

Table 1
First-order degradation rate constants (k) and half-lives ($t_{1/2}$) of TBBPA in the sediment microcosms.

Treatment	k (d^{-1})	$t_{1/2}$ (d)	r[a]
Bioaugmentation (*Ochrobactrum* sp. T)	1.1×10^{-2}	63	0.992
Anaerobic bioaugmentation (*Ochrobactrum* sp. T)	6.7×10^{-3}	103	0.993
TBP plus bioaugmentation (*Ochrobactrum* sp. T)	6.8×10^{-3}	102	0.991
BPA plus bioaugmentation (*Ochrobactrum* sp. T)	5.4×10^{-3}	128	0.996
2,4-DBP plus bioaugmentation (*Ochrobactrum* sp. T)	6.9×10^{-3}	100	0.989
Bioaugmentation (*Ochrobactrum* sp. T, *Bacillus* sp. GZT and *Bacillus* sp. GZB)	7.6×10^{-3}	91	0.990

All degradation rate constants and half-lives are the mean of duplicate samples. Data for each treatment were significantly different from that of the sterile control at $P < 0.01$ level.
[a] r: correlation coefficient.

Ochrobactrum sp. T (An et al., 2011); however, researchers have not yet explored TBBPA degradation by *Ochrobactrum* sp. T in river sediment under aerobic or anaerobic conditions. In this study, approximately 33.6% of TBBPA was removed under anaerobic conditions with bioaugmentation with *Ochrobactrum* sp. T ($P < 0.01$). Under aerobic conditions, 52.1% of TBBPA was removed through bioaugmentation with *Ochrobactrum* sp. T after the 10-week incubation period under identical conditions (Fig. 1b).

When contrasting aerobic and anaerobic conditions, the TBBPA degradation rate constants (k) were 1.1×10^{-2} and 6.7×10^{-3} d^{-1}, and the biodegradation half-lives ($t_{1/2}$) were 63 and 103 d (aerobic and anaerobic, respectively) (Table 1). These results indicate that TBBPA is more easily removed in aerobic than anaerobic sediment when amended with *Ochrobactrum* sp. T. Other research has suggested that TBBPA is a recalcitrant organic compound when degraded by microbes under aerobic conditions (Ronen and Abeliovich, 2000). However, in this study, higher degradation efficiency with *Ochrobactrum* sp. T under aerobic condition is due to the initial reductive debromination of TBBPA, followed by aerobic mineralization with the same *Ochrobactrum* sp. T strain (An et al., 2011). Under anaerobic condition, TBBPA can be only reductively debrominated by *Ochrobactrum* sp. T, without the subsequent aeration. Several other studies have shown that TBBPA can be reductively debrominated by the microbial communities in oxic or anoxic soil slurry, river sediment, and sewage sludge (Chang et al., 2012a; Potvin et al., 2012; Liu et al., 2013; Li et al., 2014).

TBBPA is often present in the environment with BPA and other phenolic brominated flame retardants, such as 2,4-DBP and TBP. This is because they are TBBPA biodegradation intermediates (Eriksson et al., 2004; An et al., 2011), and are also independently released to the environment as manufacturing co-additives (de Wit, 2002). These compounds are also terminal electron acceptors, competing to inhibit TBBPA reductive debromination or impacting *Ochrobactrum* sp. T in aerobic water (Zu et al., 2014). Despite these interactions, no studies have specifically investigated TBBPA biodegradation by *Ochrobactrum* sp. T in river sediment with the addition of 2,4-DBP, TBP, or BPA as co-substrates.

In this study, the effect of 2,4-DBP, TBP, and BPA on TBBPA biodegradation with *Ochrobactrum* sp. T was evident within a 10-week aerobic incubation period. As Fig. 1c shows, approximately 52.1% of TBBPA was degraded in sediment microcosms with the bioaugmentation with *Ochrobactrum* sp. T after 10 weeks of incubation. Comparatively, only 36.2%, 35.7%, and 30.1% of TBBPA could be removed in sediment microcosms using 2,4-DBP, TBP, or BPA as a co-substrate, respectively. TBBPA degradation rate constants (k) were 6.9×10^{-3}, 6.8×10^{-3}, and 5.4×10^{-3} d^{-1}, and the half-lives

Table 2
Comparison of phylotype coverage and the diversity estimators of bacteria.

Sample	Reads[a]	OTUs[b]	Shannon	ACE	Chao1	Coverage
Week 0	10,316	1147	3.68	5086	3199	92.81
Week 1	8295	1219	4.50	4821	3052	90.79
Week 2	13,133	2665	5.86	12,946	7699	86.52
Week 3	15,035	2735	5.93	13,660	8192	87.98
Week 4	16,763	2056	5.88	6269	4282	93.31
Week 5	8196	1953	5.93	8778	5301	84.33
Week 6	8039	1684	5.73	6662	4309	86.84
Week 7	15,246	2861	5.94	13,229	7831	87.88
Week 8	13,382	1674	5.43	5612	3652	92.95
Week 10	11,541	2317	5.64	11,366	6533	86.59

[a] Reads after filtering, trimming and normalizing.
[b] The operational taxonomic units (OTUs) were defined with 97% similarity.

($t_{1/2}$) were 100, 102, and 128 d for 2,4-DBP, TBP, and BPA added systems, respectively (Table 1).

TBBPA biodegradation efficiencies without co-substrates were much larger than with them. These results are inconsistent with other related studies. For instance, Ahn et al. found that the reductive dechlorination of TeCDD in sediment microcosms were stimulated by adding halogenated priming compounds (haloprimers) (Ahn et al., 2008). Further, haloprimers with a more analogous structure to the target compound (TeCDD) were found to be more effective in enhancing dechlorination (Ahn et al., 2005). However, in this study, TBBPA biodegradation in sediment microcosms amended with bioaugmentation with *Ochrobactrum* sp. T decreased when co-substrates were present.

This finding can be explained as follows. First, the system for TBBPA biodegradation by *Ochrobactrum* sp. T contains spontaneous reductive debromination and aerobic mineralization processes. This is a more complicated processes than other systems only containing reductive dechlorination (An et al., 2011). Second, the co-substrates in this system might affect both enzyme regulation and microbe abundance involved in TBBPA biodegradation in our system. In addition, because the co-substrates are the biotransformation products of TBBPA, they may inhibit the TBBPA transformation reaction. As such, the co-substrates inhibited TBBPA biodegradation.

Previous studies showed that 2,4-DBP, TBP, and BPA were biodegradation intermediates of TBBPA by *Ochrobactrum* sp. T (An et al., 2011). Therefore, we also considered TBBPA biodegradation when bioaugmented with a mixed culture containing *Ochrobactrum* sp. T, *Bacillus* sp. GZT and *Bacillus* sp. GZB. As Fig. 1 d shows, approximately 52.1% of TBBPA was degraded in sediment microcosms with the bioaugmentation with *Ochrobactrum* sp. T after 10 weeks incubation under aerobic conditions. A slightly lower biodegradation efficiency (40.6%) was seen in sediment microcosms when bioaugmented with a mixed culture containing the three bacterial strains alone, with TBBPA biodegradation rate constants (k) of 1.1×10^{-2} and 7.6×10^{-3} d^{-1}, and half-lives ($t_{1/2}$) of 63 and 91 d, respectively (Table 1).

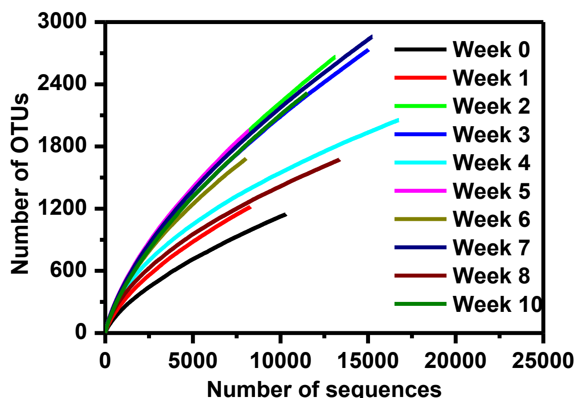

Fig. 2. Rarefaction curves of OTUs for microbes.

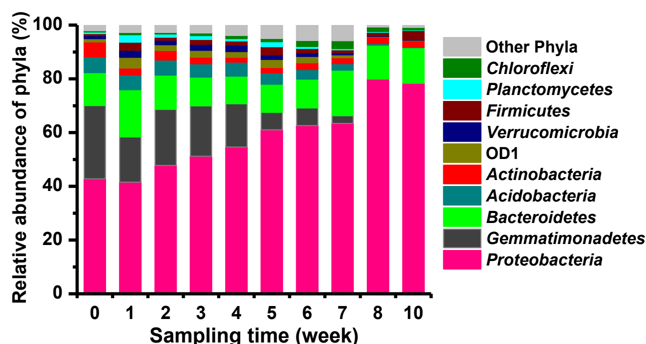

Fig. 3. Phylogenetic classification of the microbial communities for pyrosequencing at the phylum level obtained from Ribosomal Database Project (RDP) classifier analysis. Sequences classified as *Crenarchaeota*, *Chlamydiae*, *Spirochaetes*, *BRC1*, *Armatimonadetes*, *Nitrospira*, *TM7*, *Tenericutes*, *Synergistetes*, *WS3*, *Caldiserica*, *Cyanobacteria*, *Euryarchaeota*, *Elusimicrobia*, *Fusobacteria*, *Thermodesulfobacteria*, *Chlorobi*, *Deinococcus-Thermus* and unclassified phyla were summarized in the group of "other" phyla.

These results suggested that the bioaugmentation with *Ochrobactrum* sp. T led to a larger TBBPA biodegradation efficiency than when bioaugmentation was done with a mixed culture of the three bacterial strains alone. The same total microbial population was used across the degradation systems; however, the microbial population used for TBBPA degradation differed in diverse systems. This suggests that TBBPA biodegradation efficiency in sediment microcosms relates to microbial population size. Cho et al. observed the same phenomenon (Cho et al., 2002).

3.2. Diversity of microbial community

The high-throughput sequencing of the sediment microcosm amended with *Ochrobactrum* sp. T generated approximately 167,322 sequence reads of 16S rRNA gene, with an average length of approximately 453 bp. After low quality reads were filtered using PRINSEQ-lite 0.19.5 software; trimming the chimera, barcodes and primers; and normalizing sequence reads, 119,946 high-quality reads remained. A 97% similarity cut-off was used to group OTUs for downstream analyses.

Table 2 summarizes corresponding numbers of OTUs, the Shannon Index (H′), and the Chao1. These results show a rich diversity of bacteria in the sediment microcosm samples amended with *Ochrobactrum* sp. T. There were 1147–2861 OTUs in these samples at a 3% cut-off level; Chao1 values varied from 3052 to 8192. The H' values varied from 3.68 to 5.94, indicating high diversity in the bacterial 16S rRNA libraries. This suggests that the sediment microcosms contained high bacterial species richness. Similar results were found for the ACE diversity indices.

This coverage was used to assess whether the library was sufficiently large to obtain meaningful and stable richness estimates. The calculated coverage, ranging from 84.33% to 93.31%, indicated that the microbial species contained in the clone library were accounted for using high-throughput sequencing, and the library yielded sufficiently stable phylotype richness estimates. Rarefaction analysis was also used to standardize and compare the observed taxon richness to identify whether unequal sampling occurred. A rarefaction curve with no plateau indicated the need for additional sampling to reveal the extent of ecosystem diversity

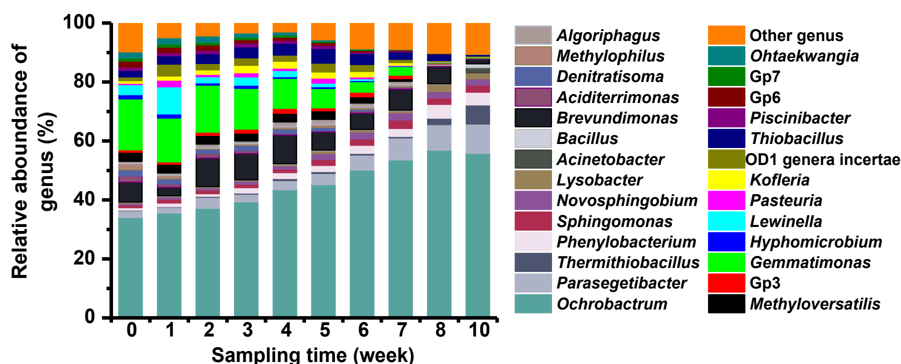

Fig. 4. Phylogenetic classification of the microbial communities for pyrosequencing at the genera level obtained from Ribosomal Database Project (RDP) classifier analysis. The genera whose reads were less than 1.0% and unclassified genera were summarized in the group of "other" genera.

(Roh et al., 2010). In this study, all rarefaction curves failed to reach saturation (Fig. 2), indicating that the microbial diversity in sediment microcosm samples was much greater than expected.

3.3. Composition evolution of microbial community

This study also investigated changes in the microbial community response to the bioaugmentation with *Ochrobactrum* sp. T. As Fig. 3 shows, the microbial communities changed with the experimental processes. There were 11 different phylogenetic groups at the phylum taxonomic rank, including *Proteobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, OD1, *Verrucomicrobia*, *Firmicutes*, *Planctomycetes*, *Chloroflexi* and the unclassified phyla. Notably, the dominant bacterial phyla were *Proteobacteria* (42.79–79.80%), *Gemmatimonadetes* (27.27–0.23%), *Bacteroidetes* (10.27–17.60%), *Acidobacteria* (5.88–0.25%) and *Actinobacteria* (5.52–2.36%); the reads belonging to OD1, *Verrucomicrobia*, *Firmicutes*, *Planctomycetes*, *Chloroflexi* and the unclassified phyla were the minor groups during the 10-week TBBPA biodegradation process. The reads of *Proteobacteria* were 42.79% at the beginning of the incubation period; decreased to 41.52% after one week, and then increased to 79.80% (week 8). The reads of *Gemmatimonadetes*, *Acidobacteria* and *Actinobacteria* decreased from 27.27% to 0.23%, 5.88%–0.25%, and 5.52%–2.36%, between week 0 and 10, respectively. The reads of *Bacteroidetes* (12.28–13.09%) remained relatively constant during the 10-week incubation. These results indicated that *Proteobacteria* was the predominant bacterial phylum, and represents the microbial phylum response to TBBPA biodegradation.

More specifically, the Ribosomal Database Project (RDP) classifier analysis was used to classify phyla of the microbial communities for pyrosequencing at the genera level. As Fig. 4 shows, 27 main genera were detected in the total bacterial population in the sediment microcosms amended with *Ochrobactrum* sp. T during the TBBPA biodegradation process. The most dominant bacterial genera were *Ochrobactrum* (34.08–55.80%), *Parasegetibacter* (2.28–9.92%), *Thermithiobacillus* (0.20–6.49%), *Phenylobacterium* (0.82–4.33%) and *Sphingomonas* (0.90–2.39%) during the 10-week incubation period.

The *Ochrobactrum* genus (*Proteobacteria*) was the dominant genus, gradually increasing with incubation time. *Ochrobactrum* sp. T is affiliated with the *Ochrobactrum* genus and is capable of simultaneously reducing debromination and aerobically mineralizing TBBPA (An et al., 2011). Microbes from genera *Ochrobactrum* are mainly linked to TBBPA removal. Therefore, the increase of *Ochrobactrum* genus in the sediment microcosm samples within a 10-week incubation period is reasonable, because the bacterial community underwent continuous acclimation by TBBPA, the sole carbon source of *Ochrobactrum*. In addition, many previously documented TBBPA-degrading microbes, including the members of genera *Comamonas*, *Achromobacter*, *Sphingomonas*, *Flavobacterium* (Penget al., 2012; Li et al., 2014), *Dehalobacter* (Zhang et al., 2013), *Bacillus* and *Rhodococcus* (Chang et al., 2012a) were either minor genera or were not detected in the sediment microcosm bioaugmented with *Ochrobactrum* sp. T. These results demonstrated that *Ochrobactrum* sp. T in the sediment microcosms could greatly enhance TBBPA biodegradation efficiency.

4. Conclusion

In conclusion, this study showed that bioaugmenting TBBPA-contaminated sediment microcosms with *Ochrobactrum* sp. T prominently enhanced TBBPA biodegradation, demonstrating its bioremediation capabilities. Adding extra co-substrates inhibited the effect. In addition, this study investigated how oxygen affects bioaugmentation with *Ochrobactrum* sp. T, finding that aerobic conditions enhance the biodegradation efficiency of TBBPA by *Ochrobactrum* sp. T. Furthermore, the changing diversity of microbial communities in the sediment microcosms amended with *Ochrobactrum* sp. T bioaugmentation showed that the *Ochrobactrum* genus level increased gradually, with an important increasing role as incubation continued over the 10-week TBBPA treatment period. Other minor genera, such as *Parasegetibacter* (2.28–9.92%), *Thermithiobacillus* (0.20–6.49%), *Phenylobacterium* (0.82–4.33%), and *Sphingomonas* (0.90–2.39%) were also observed. This study confirms that *Ochrobactrum* genus plays a dominant role in TBBPA biodegradation in the sediment microcosms amended with bioaugmentation using *Ochrobactrum* sp. T. As such, the *Ochrobactrum* sp. T strain might be practically used in the in situ remediation of TBBPA-contaminated sediments.

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References

Acosta-González, A., Rosselló-Móra, R., Marqués, S., 2013. Characterization of the anaerobic microbial community in oil-polluted subtidal sediments: aromatic biodegradation potential after the Prestige oil spill. Environ. Microbiol. 15, 77–92.

Ahn, Y.B., Liu, F., Fennell, D.E., Häggblom, M.M., 2008. Biostimulation and bioaugmentation to enhance dechlorination of polychlorinated dibenzo-p-dioxins

in contaminated sediments. FEMS Microbiol. Ecol. 66, 271–281.
Ahn, Y.B., Haggblom, M.M., Fennell, D.E., 2005. Co-amendment with halogenated compounds enhances anaerobic microbial dechlorination of 1,2,3,4-tetrachlorodibenzo-p-dioxin and 1,2,3,4-tetrachlorodibenzofuran in estuarine sediments. Environ. Toxicol. Chem. 24, 2775–2784.
An, T.C., Zu, L., Li, G.Y., Wan, S.G., Mai, B.X., Wong, P.K., 2011. One-step process for debromination and aerobic mineralization of tetrabromobisphenol-A by a novel *Ochrobactrum* sp T isolated from an e-waste recycling site. Bioresour. Technol. 102, 9148–9154.
Caporaso, J.G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L., Knight, R., 2010. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26, 266–267.
Chang, B.V., Yuan, S.Y., Ren, Y.L., 2012a. Anaerobic degradation of tetrabromobisphenol-A in river sediment. Ecol. Eng. 49, 73–76.
Chang, B.V., Yuan, S.Y., Ren, Y.L., 2012b. Aerobic degradation of tetrabromobisphenol-A by microbes in river sediment. Chemosphere 87, 535–541.
Cho, Y.C., Ostrofsky, E.B., Sokol, R.C., Frohnhoefer, R.C., Rhee, G.Y., 2002. Enhancement of microbial PCB dechlorination by chlorobenzoates, chlorophenols and chlorobenzenes. FEMS Microbiol. Ecol. 42, 51–58.
Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam, S.A., McGarrell, D.M., Garrity, G.M., Tiedje, J.M., 2005. The ribosomal database project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucleic Acids Res. 33, D294–D296.
Dalvi, S., Nicholson, C., Najar, F., Roe, B.A., Canaan, P., Hartson, S.D., Fathepure, B.Z., 2014. *Arhodomonas* sp. strain seminole and its genetic potential to degrade aromatic compounds under high-salinity conditions. Appl. Environ. Microbiol. 80, 6664–6676.
de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46, 583–624.
de Wit, C.A., Herzke, D., Vorkamp, K., 2010. Brominated flame retardants in the Arctic environment – trends and new candidates. Sci. Total Environ. 408, 2885–2918.
Decherf, S., Seugnet, I., Fini, J.B., Clerget-Froidevaux, M.S., Demeneix, B.A., 2010. Disruption of thyroid hormone-dependent hypothalamic set-points by environmental contaminants. Mol. Cell. Endocrinol. 323, 172–182.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.
Eriksson, J., Rahm, S., Green, N., Bergman, A., Jakobsson, E., 2004. Photochemical transformations of tetrabromobisphenol A and related phenols in water. Chemosphere 54, 117–126.
Fujii, Y., Harada, K.H., Hitomi, T., Kobayashi, H., Koizumi, A., Haraguchi, K., 2014a. Temporal trend and age-dependent serum concentration of phenolic organohalogen contaminants in Japanese men during 1989–2010. Environ. Pollut. 185, 228–233.
Fujii, Y., Nishimura, E., Kato, Y., Harada, K.H., Koizumi, A., Haraguchi, K., 2014b. Dietary exposure to phenolic and methoxylated organohalogen contaminants in relation to their concentrations in breast milk and serum in Japan. Environ. Int. 63, 19–25.
Gomez-Alvarez, V., Teal, T.K., Schmidt, T.M., 2009. Systematic artifacts in metagenomes from complex microbial communities. ISME J. 3, 1314–1317.
Hiraishi, A., 2008. Biodiversity of dehalorespiring bacteria with special emphasis on polychlorinated biphenyl/dioxin dechlorinators. Microbes Environ. 23, 1–12.
Islam, M.S., Zhang, Y., McPhedran, K.N., Liu, Y., Gamal El-Din, M., 2015. Next-generation pyrosequencing analysis of microbial biofilm communities on granular activated carbon in treatment of oil sands process-affected water. Appl. Environ. Microbiol. 81, 4037–4048.
Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Gloeckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41 http://dx.doi.org/10.1093/nar/gks808.
Koike, E., Yanagisawa, R., Takigami, H., Takano, H., 2013. Brominated flame retardants stimulate mouse immune cells in vitro. J. Appl. Toxicol. 33, 1451–1459.
Krumins, V., Park, J.W., Son, E.K., Rodenburg, L.A., Kerkhof, L.J., Häggblom, M.M., Fennell, D.E., 2009. PCB dechlorination enhancement in Anacostia River sediment microcosms. Water Res. 43, 4549–4558.
Labadie, P., Tlili, K., Alliot, F., Bourges, C., Desportes, A., Chevreuil, M., 2010. Development of analytical procedures for trace-level determination of polybrominated diphenyl ethers and tetrabromobisphenol A in river water and sediment. Anal. Bioanal. Chem. 396, 865–875.
Li, F., Wang, J., Nastold, P., Jiang, B., Sun, F., Zenker, A., Kolvenbach, B.A., Ji, R., François-Xavier Corvini, P., 2014. Fate and metabolism of tetrabromobisphenol A in soil slurries without and with the amendment with the alkylphenol degrading bacterium *Sphingomonas* sp. strain TTNP3. Environ. Pollut. 193, 181–188.
Li, G.Y., Zu, L., Wong, P.K., Hui, X.P., Lu, Y., Xiong, J.K., An, T.C., 2012. Biodegradation and detoxification of bisphenol A with one newly-isolated strain *Bacillus* sp GZB: kinetics, mechanism and estrogenic transition. Bioresour. Technol. 114, 224–230.
Lin, W., Yu, Z., Zhang, H., Thompson, I.P., 2014. Diversity and dynamics of microbial communities at each step of treatment plant for potable water generation. Water Res. 52, 218–230.
Linhartova, P., Gazo, I., Shaliutina-Kolesova, A., Hulak, M., Kaspar, V., 2015. Effects of tetrabrombisphenol A on DNA integrity, oxidative stress, and sterlet (*Acipenser ruthenus*) spermatozoa quality variables. Environ. Toxicol. 30, 735–745.
Liu, J., Wang, Y., Jiang, B., Wang, L., Chen, J., Guo, H., Ji, R., 2013. Degradation, metabolism, and bound-residue formation and release of tetrabromobisphenol A in soil during sequential anoxic-oxic incubation. Environ. Sci. Technol. 47, 8348–8354.
McCormick, J.M., Paiva, M.S., Häggblom, M.M., Cooper, K.R., White, L.A., 2010. Embryonic exposure to tetrabromobisphenol A and its metabolites, bisphenol A and tetrabromobisphenol A dimethyl ether disrupts normal zebrafish (Danio rerio) development and matrix metalloproteinase expression. Aquat. Toxicol. 100, 255–262.
Pöritz, M., Schiffmann, C.L., Hause, G., Heinemann, U., Seifert, J., Jehmlich, N., von Bergen, M., Nijenhuis, I., Lechner, U., 2015. Dehalococcoides mccartyi strain DCMB5 respires a broad spectrum of chlorinated aromatic compounds. Appl. Environ. Microbiol. 81, 587–596.
Park, J.W., Krumins, V., Kjellerup, B.V., Fennell, D.E., Rodenburg, L.A., Sowers, K.R., Kerkhof, L.J., Häggblom, M.M., 2011. The effect of co-substrate activation on indigenous and bioaugmented PCB dechlorinating bacterial communities in sediment microcosms. Appl. Microbiol. Biotechnol. 89, 2005–2017.
Peng, X., Zhang, Z., Zhao, Z., Jia, X., 2012. 16S ribosomal DNA clone libraries to reveal bacterial diversity in anaerobic reactor-degraded tetrabromobisphenol A. Bioresour. Technol. 112, 75–82.
Potvin, C.M., Long, Z., Zhou, H., 2012. Removal of tetrabromobisphenol A by conventional activated sludge, submerged membrane and membrane aerated biofilm reactors. Chemosphere 89, 1183–1188.
Roh, S.W., Kim, K.H., Nam, Y.D., Chang, H.W., Park, E.J., Bae, J.W., 2010. Investigation of archaeal and bacterial diversity in fermented seafood using barcoded pyrosequencing. ISME J. 4, 1–16.
Ronen, Z., Abeliovich, A., 2000. Anaerobic–aerobic process for microbial degradation of tetrabromobisphenol A. Appl. Environ. Microbiol. 66, 2372–2377.
Song, S., Song, M., Zeng, L., Wang, T., Liu, R., Ruan, T., Jiang, G., 2014. Occurrence and profiles of bisphenol analogues in municipal sewage sludge in China. Environ. Pollut. 186, 14–19.
Tischer, K., Kleinsteuber, S., Schleinitz, K.M., Fetzer, I., Spott, O., Stange, F., Lohse, U., Franz, J., Neumann, F., Gerling, S., Schmidt, C., Hasselwander, E., Harms, H., Wendeberg, A., 2013. Microbial communities along biogeochemical gradients in a hydrocarbon-contaminated aquifer. Environ. Microbiol. 15, 2603–2615.
Vargas, C., Fennell, D.E., Haggblom, M.M., 2001. Anaerobic reductive dechlorination of chlorinated dioxins in estuarine sediments. Appl. Microbiol. Biotechnol. 57, 786–790.
Wang, X., Hu, X., Zhang, H., Chang, F., Luo, Y., 2015. Photolysis kinetics, mechanisms, and pathways of tetrabromobisphenol A in water under simulated solar light irradiation. Environ. Sci. Technol. 49, 6683–6690.
Xiong, J.K., An, T.C., Zhang, C.S., Li, G.Y., 2014. Pollution profiles and risk assessment of PBDEs and phenolic brominated flame retardants in water environments within a typical electronic waste dismantling region. Environ. Geochem. Health 1–17.
Zhang, C., Li, Z., Suzuki, D., Ye, L., Yoshida, N., Katayama, A., 2013. A humin-dependent Dehalobacter species is involved in reductive debromination of tetrabromobisphenol A. Chemosphere 92, 1343–1348.
Zhou, H.W., Li, D.F., Tam, N.F.Y., Jiang, X.T., Zhang, H., Sheng, H.F., Qin, J., Liu, X., Zou, F., 2011. BIPES, a cost-effective high-throughput method for assessing microbial diversity. ISME J. 5, 741–749.
Zu, L., Li, G.Y., An, T.C., Wong, P.K., 2012. Biodegradation kinetics and mechanism of 2,4,6-tribromophenol by *Bacillus* sp GZT: a phenomenon of xenobiotic methylation during debromination. Bioresour. Technol. 110, 153–159.
Zu, L., Xiong, J.K., Li, G.Y., Fang, Y.J., An, T.C., 2014. Concurrent degradation of tetrabromobisphenol A by *Ochrobactrum* sp T under aerobic condition and estrogenic transition during these processes. Ecotox. Environ. Safe 104, 220–225.