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, Phenyllobacterium 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^2 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
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 TeCDF 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/trimethylchlorosilane (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 13C-TBP, 13C-TBBPA, and 13C-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 acetonitrile 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°32′7′′N, 116°34′2′′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 75% (v/v) sediment, 25% (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) 2,4-DBP substrate 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 N₂ 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 13C-TBBPA surrogate standard, freeze-dried, and extracted three times using a 40 kHz ultrasonic processor with 20 mL hexane:acetonitrile (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 N₂ (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:dithylether (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:dithylether (6:4, v/v). Further cleanup was performed on a 1 g activated silica cartridge, pre-conditioned with 10 mL of hexane:dithylether (6:4, v/v). After sample loading, the target compound TBBPA was eluted with 10 mL of hexane:dithylether (6:4, v/v). Finally, the extracts were dried under a gentle stream of ultra-high purity N2 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 chromatograph coupled with an Agilent 5975C mass spectrometer, using a HP-5 MS (30 m x 0.25 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 = C0exp(−kt), t1/2 = ln2/k, where t is time, C0 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 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, 450 bp) 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 ddH2O. 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 and a recommended barcode sequence by Illumina Miseq 2 × 300 bp sequencing Platform (California, USA) from Sangon Biotech Co., Ltd (Shanghai, China).

2 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) with UCLUST software (http://www.drive5.com/uclust) with a similarity threshold of 97%, based on the Uclust algorithm (Edgar, 2010). Representative sequences of OTUs 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-DNP, 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 (t1/2) 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 debranched and then mineralized under aerobic conditions by
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 $1.1 \times 10^{-2}$ and $6.7 \times 10^{-3}$ 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 $6.9 \times 10^{-3}$, $6.8 \times 10^{-3}$, and $5.4 \times 10^{-3}$ d$^{-1}$, and the half-lives

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>k (d$^{-1}$)</th>
<th>t$_{1/2}$ (d)</th>
<th>r$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioaugmentation (Ochrobactrum sp. T)</td>
<td>$1.1 \times 10^{-2}$</td>
<td>63</td>
<td>0.992</td>
</tr>
<tr>
<td>Anaerobic bioaugmentation (Ochrobactrum sp. T)</td>
<td>$6.7 \times 10^{-3}$</td>
<td>103</td>
<td>0.993</td>
</tr>
<tr>
<td>TBP plus bioaugmentation (Ochrobactrum sp. T)</td>
<td>$6.8 \times 10^{-3}$</td>
<td>102</td>
<td>0.991</td>
</tr>
<tr>
<td>BPA plus bioaugmentation (Ochrobactrum sp. T)</td>
<td>$5.4 \times 10^{-3}$</td>
<td>128</td>
<td>0.996</td>
</tr>
<tr>
<td>2,4-DBP plus bioaugmentation (Ochrobactrum sp. T)</td>
<td>$6.9 \times 10^{-3}$</td>
<td>100</td>
<td>0.989</td>
</tr>
<tr>
<td>Bioaugmentation (Ochrobactrum sp. T, Bacillus sp. GZT and Bacillus sp. GZB)</td>
<td>$7.6 \times 10^{-3}$</td>
<td>91</td>
<td>0.990</td>
</tr>
</tbody>
</table>

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.
sp. T, when bioaugmented with a mixed culture containing biodegradation intermediates of TBBPA by biodegradation. As such, the co-substrates inhibited TBBPA formation products of TBBPA, they may inhibit the TBBPA transformation reaction. In addition, because the co-substrates are the biotransformation and microbe abundance involved in TBBPA biodegradation in our systems might affect both enzyme regulation and unclassiﬁed phyla were summarized in the group of “other” phyla.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Readsa</th>
<th>OTUsb</th>
<th>Shannon</th>
<th>ACE</th>
<th>Chao1</th>
<th>Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>10,316</td>
<td>1147</td>
<td>3.68</td>
<td>5086</td>
<td>3199</td>
<td>92.81</td>
</tr>
<tr>
<td>Week 1</td>
<td>8295</td>
<td>1219</td>
<td>4.50</td>
<td>4821</td>
<td>3052</td>
<td>90.79</td>
</tr>
<tr>
<td>Week 2</td>
<td>13,133</td>
<td>2665</td>
<td>5.86</td>
<td>12,946</td>
<td>7699</td>
<td>86.52</td>
</tr>
<tr>
<td>Week 3</td>
<td>15,035</td>
<td>2735</td>
<td>5.93</td>
<td>13,660</td>
<td>8192</td>
<td>87.98</td>
</tr>
<tr>
<td>Week 4</td>
<td>16,763</td>
<td>2056</td>
<td>5.88</td>
<td>6269</td>
<td>4282</td>
<td>93.31</td>
</tr>
<tr>
<td>Week 5</td>
<td>8196</td>
<td>1953</td>
<td>5.93</td>
<td>8778</td>
<td>5301</td>
<td>84.33</td>
</tr>
<tr>
<td>Week 6</td>
<td>8039</td>
<td>1684</td>
<td>5.73</td>
<td>6662</td>
<td>4309</td>
<td>86.84</td>
</tr>
<tr>
<td>Week 7</td>
<td>15,246</td>
<td>2861</td>
<td>5.94</td>
<td>13,229</td>
<td>7831</td>
<td>87.88</td>
</tr>
<tr>
<td>Week 8</td>
<td>8392</td>
<td>1974</td>
<td>5.43</td>
<td>5612</td>
<td>3652</td>
<td>92.95</td>
</tr>
<tr>
<td>Week 10</td>
<td>13,382</td>
<td>1674</td>
<td>5.43</td>
<td>5612</td>
<td>3652</td>
<td>92.95</td>
</tr>
</tbody>
</table>

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

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 (t1/2) of 63 and 91 d, respectively (Table 1).

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. 2. Rarefaction curves of OTUs for microbes.
In this study, all rarefaction curves failed to reach saturation (Fig. 2), indicating that the microbial diversity in sediment microcosms 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%), Phenyllobacterium (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 (Ahn et al., 2011). Microbes from genera Ochrobactrum are mainly linked to TBBPA removal. Therefore, the increase of Ochrobactrum genus in the sediment microcosms 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%), Phenyllobacterium (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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