The microbial degradation of 2,4,6-tribromophenol (TBP) in water/sediments interface: Investigating bioaugmentation using Bacillus sp. GZT

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HIGHLIGHTS
• TBP biodegradation in sediment microcosms are conducted.
• TBP degradation was significantly bioaugmented with Bacillus sp. GZT.
• TBP degradation amended with strain GZT was not stimulated by other bromophenols.
• TBP degradation was improved with addition of electron donor and carbon source.
• Microbes from phylum Firmicutes are mainly linked to the enhanced TBP degradation.

GRAPHICAL ABSTRACT

ABSTRACT
The substance 2,4,6-Tribromophenol (TBP) is used as a flame retardant in electronic and electric devices, and is a replacement for pentachlorophenol in wood preservation. TBP is a contaminant in different environmental matrices, at levels where treatment is required. This study examined the relationship between the bioaugmention of TBP degradation and the evolution of the microbial community in river water/sediment microcosms. When compared with unamended controls, bioaugmentation with Bacillus sp. GZT effectively enhanced TBP biodegradation, with approximately 40.7% of the TBP removal after a 7-week incubation period, without a lag phase (p < 0.01). Amendments with 2-bromophenol, 2,6-dibromophenol, and 2,4-dibromophenol did not promote TBP biodegradation in river water/sediments (p > 0.05). However, TBP biodegradation was enhanced by adding other additives, including NaCl, humic acid, sodium lactate, and sodium propionate alone, especially glucose and yeast extract. A metagenomics analysis of the total 16S rRNA genes from the treatment system with bioaugmentation...
1. Introduction

The substance 2,4,6-tribromophenol (TBP) is a bromophenol widely used as a fungicide, brominated flame retardant (BFR), and an intermediate to produce other BFRs (Norman Haldén et al., 2010). TBP production reached approximately 9500 tons per year worldwide in 2001 (Howe et al., 2005). Unfortunately, TBP and TBP-containing substances can be released into a variety of environmental media during their production, use, and disposal. As such, it is present in different environmental matrices, including soils, aquifers, and groundwater (Ronen et al., 2005; Park et al., 2011). Yeast extract, NaCl, cellulose, and co-substrates, may influence the extent that organic pollutants degrade in a given environmental matrix (Fan et al., 2004; Park et al., 2011). Chang et al. (2012) added yeast extract, NaCl, cellulose, and co-substrates, may influence the extent that organic pollutants degrade in a given environmental matrix (Fan et al., 2004; Park et al., 2011). Unfortunately, TBP and TBP-containing substances can be released into a variety of environmental media during their production, use, and disposal. As such, it is present in different environmental matrices, including soils, aquifers, and groundwater (Ronen et al., 2005; Park et al., 2011). Yeast extract, NaCl, cellulose, and co-substrates, may influence the extent that organic pollutants degrade in a given environmental matrix (Fan et al., 2004; Park et al., 2011). Of these, the potential acute toxicity and endocrine-disrupting potency of TBP have attracted widespread and increased attention (Wollenberger et al., 2005; Norman Haldén et al., 2010). Like most BFRs, TBP may cause developmental neurotoxicity, embroyotoxicity, and fetotoxicity (Rios et al., 2003; Hassenklover et al., 2006). It also has estrogen-like properties (Deng et al., 2010; Norman Haldén et al., 2010). All these factors highlight the need to develop cost-effective techniques to purify and eliminate TBP in water and sediment environments.

The use of microorganisms to degrade organic pollutants is a promising strategy for the in-situ remediation of contaminated media (Xu and Lu, 2010; Chang et al., 2012; Zhang et al., 2013; Yang et al., 2014; Li et al., 2015). Previous studies have mainly focused on isolating TBP degradation microbes and applying traditional culture-based approaches to study the diversity of TBP degraders (Ronen et al., 2005; Zhu et al., 2012; Li et al., 2015). However, in the realistic environment, contamination dissipation can be accomplished by a whole microbial community, instead of a single isolated degrader (Zu et al., 2012). For example, the anaerobic mineralization of TBP to CO₂ and H₂O can be achieved with a synthetic anaerobic community that includes *Dehalobacter* sp., *Clostridium* sp., and *Desulfatiglans* sp. strains (Li et al., 2015), rather than by a single bacterial strain. A previous study isolated a *Bacillus* sp. strain GZT; this strain degraded and debrorninated TBP at efficiencies of 93.2% and 89.3%, respectively, within 120 h, under optimal laboratory conditions (Zu et al., 2012). However, here are few studies using this isolated *Bacillus* sp. strain to degrade TBP in real river water/sediments.

Different environmental factors, including salinity, temperature, nutrients, and co-substrates, may influence the extent that organic pollutants degrade in a given environmental matrix (Fan et al., 2004; Park et al., 2011). Chang et al. (2012) added yeast extract, NaCl, cellulose, humic acid, and surfactin, respectively, to enhance the ability of microbes to degrade tetrabromobisphenol A (TBBPA) in river sediments. In addition, to investigate the ability of indigenous microorganisms in sediment microcosms to dechlorinate polychlorinated biphenyls, halogenated co-substrates have been used as priming compounds to stimulate biodegradation (Krumins et al., 2009; Park et al., 2011). Despite these works, little attention has been paid to the effects of these factors on the bioaugmentation of TBP degradation in river water/sediments as a way to enhance removal efficiency.

Sediments can harbor high densities of diverse microorganisms, which play a crucial role in biodegrading toxic organic chemicals released into the environment (Cheng et al., 2014; Yang et al., 2014; Llado et al., 2015). To better understand TBP biodegradation in river water/sediments which are also contaminated with other organic pollutants, it is important to analyze how the microbial community structure evolution during biodegradation processes. TBP biodegradation has been well documented in culture (Ronen et al., 2005; Yamada et al., 2008; Zu et al., 2012; Li et al., 2015) and in activated sludge (Brenner et al., 2006). However, there are few researches describing phylogenetic information for TBP-degrading microbial communities.

Given this background, this study investigated TBP biodegradation in river water/sediments bioaugmented with *Bacillus* sp. GZT. The effects of different co-substrates, electron donors, and carbon sources on the bioaugmented degradation process were investigated; and the changes of the microbial community were assessed using high-throughput sequencing. The study concluded that identifying TBP-degrading microbial communities in water/sediment microcosms can greatly improve our knowledge about TBP biodegradation in these settings.

2. Materials and methods

2.1. Sampling and medium

Water/sediments samples were collected from the Lianjiang River and the Beigang River in South China (23°32′N, 116°34′E). The two rivers are heavily contaminated with TBBPA, TBP, bisphenol A, polybrominated diphenyl ethers, and heavy metals (Xiong et al., 2015a). The top layer sediment (0–10 cm in depth) samples were collected using a stainless grab sampler, packed into sterile glass jars, sealed, and stored at −20 °C until used. Water sample was collected under 10 cm of the water surface and stored at 4 °C until testing and analysis.

Mineral medium (MM) and growth medium (GM) used to cultivate *Bacillus* sp. GZT were prepared according to reference (An et al., 2011); the pH value of the basal medium was adjusted to 7.0 before autoclaving at 121 °C for 30 min.

2.2. Microcosm setup

Microcosms were created using homogenized water/sediments, containing 47% (v/v) sampling water/sediments, 53% (v/v) MM, and 10 mg L⁻¹ TBP. The TBP was first dissolved in the MM at a fixed concentration (18.87 mg L⁻¹) before the mixture was added to the water/sediments. Co-substrates 2-bromophenol (2-BP, 98% purity, Sigma-Aldrich), 2,6-dibromophenol (2,6-DPB, 99% purity, Sigma-Aldrich), and 2,4-dibromophenol (2,4-DPB, purity > 99%, Acros Organics) were added separately as alternate halogenated electron acceptors into the microcosms, at a final concentration of 10 mg L⁻¹. Sodium lactate or sodium propionate was also added as an electron donor into the microcosms with a final concentration of 10 mM. Yeast extract was added at a final concentration of 5 mg L⁻¹; glucose was added to 10 mM; NaCl was added to 10 ng L⁻¹; and humic acid was added to 0.5 g L⁻¹. Each tested microcosm unit included 150 ml homogenized water/sediments in a 250 ml serum bottle.

Eleven experimental treatments in duplicate were designed: (1) unamended control (containing 47% (v/v) sampling water/sediment, 53% (v/v) MM, and 10 mg L⁻¹ TBP) under aerobic condition; (2) bioaugmentation with GZT under aerobic condition; (3) co-substrate 2-BP plus bioaugmentation with GZT under aerobic condition; (4) co-substrate 2,4-DBP plus bioaugmentation with GZT under aerobic condition; (5) co-substrate 2,6-DBP plus bioaugmentation with GZT under aerobic condition; (6) yeast extract plus bioaugmentation with GZT under aerobic condition; (7) yeast extract plus bioaugmentation with GZT under aerobic condition; (8) yeast extract plus bioaugmentation with GZT under aerobic condition; (9) yeast extract plus bioaugmentation with GZT under aerobic condition; (10) yeast extract plus bioaugmentation with GZT under aerobic condition; (11) yeast extract plus bioaugmentation with GZT under aerobic condition.
aerobic condition; (7) glucose plus bioaugmentation with GZT under aerobic condition; (8) NaCl plus bioaugmentation with GZT under aerobic condition; (9) humic acid plus bioaugmentation with GZT under aerobic condition; (10) sodium lactate plus bioaugmentation with GZT under aerobic condition; (11) sodium propionate plus bioaugmentation with GZT under aerobic. The GZT was isolated from the sludge of an e-waste recycling zone (Zu et al., 2012).

Once two inoculating loop of GZT was inoculated as described in a previous publication (Zu et al., 2012), the GZT in the sterilized GM was cultured 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 collect the bacteria. The bacteria (about 0.37 g, wet weight) were then aseptically transferred into the water/sediments microcosms. All microcosms were incubated in a horizontal shaker (150 rpm) in darkness at 25 °C.

To analyze the TBP biodegradation in the water/sediment microcosms, the serum bottles were shaken thoroughly. Once a week for seven weeks, 5 mL water/sediment samples were collected from the water/sediment microcosms with a glass syringes. At each sampling event, an additional 5 mL sample was collected from the water/sediment microcosms amended with GZT bioaugmentation for molecular analyses through 454 high-throughput sequencing.

2.3. Chemical analysis

Water/sediment samples from the microcosms were spiked with 40 ng 13C-TBP surrogate standard (Cambridge Isotope Laboratories, Inc.), 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., 2015a). 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 cm3, Sigma-Aldrich) (Li et al., 2016a). Finally, the extracts were dried under a gentle stream of ultra-high purity N2 and derivatized with N,O-bis(trimethylsilyl) trifluoroacetamide/trimethylchlorosilane (99:1, v/v) (purities > 99%, Acros Organics) before analysis (Xiong et al., 2015b). The method used to analyze TBP concentrations in the samples is provided in supporting information. Surrogate recoveries of all samples were 83.1 ± 16%.

2.4. Data analysis

The remaining percentage was calculated as the TBP residue concentration divided by original TBP concentration, multiplied by 100. TBP biodegradation data collected for this study fit well with first-order kinetic equations:
\[ C = C_0 \exp(-kt), \]
where \( C_0 \) is the initial substrate concentration, \( C \) is substrate concentration at time \( t \), and \( k \) is degradation rate constant. Significant differences were accepted at \( p < 0.05 \). Statistical analysis was also 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 by analysis of one-way variance (ANOVA).

2.5. Microbial community analysis

The method to obtain high quality sequences was provided in Supporting Information. Sequences were clustered into the 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; Haller et al., 2011; Douterele et al., 2013). Representative sequences of OTUs were selected based on the most abundant sequences, and taxonomies were assigned conducted using the Ribosomal Database Project (RDP) classifier (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, abundance-based coverage estimator (ACE)), and the rarefaction curves were generated using Mothur software based on the observed OTUs.

To compare the community diversity between samples based on phyllogenetic information, the Fast UniFrac online tool (http://unifrac.colorado.edu/) was used to estimate the weighted UniFrac metric (considering the relative proportion of each branch in the community) and to carry out principal coordinate analysis (PCoA). Moreover, heatmaps were developed using the software Heml 1.0.

The 16S rRNA gene sequences were already deposited in the NCBI Sequence Read Archive under accession numbers SRA308130.

3. Results and discussion

3.1. Effects of bioaugmentation on TBP degradation

The water/sediment microcosms containing only indigenous microorganisms were used as unamended controls. As Fig. 1 shows, the TBP concentration in these controls did not significantly decrease. In contrast, approximately 40.7% of the TBP was gradually removed without a lag phase when bioaugmented with GZT after a 7-week incubation period (\( p < 0.01 \)). As Table 1 shows, the biodegradation rate constant (\( k \)) and half-life (\( t_{1/2} \)) of TBP in the water/sediment microcosms with GZT-bioaugmentation were 1.11 × 10^{-2} \text{d}^{-1} and 62 d, respectively.

About mentioned results indicated that indigenous microorganisms in the water/sediment might not significantly contribute to TBP degradation over the experimental incubation period. With strain GZT bioaugmentation, TBP degradation was effectively enhanced, which is also demonstrated by the lack of a lag phase. This is because strain GZT, originally isolated from river sludge near an electronic waste dismantling area, is capable of TBP degradation (Zu et al., 2012). More important, when strain GZT was added into the water/sediment microcosms, it can still effectively degraded TBP in the water/sediment matrix. However, previous studies have not focused on TBP dissipation through bioaugmentation with strain GZT in water/sediment microcosms. In contrast, bioaugmentation has been widely applied to enhance the dechlorination of highly chlorinated environmental pollutants, such as polychlorinated biphenyls (PCBs) (Krumins et al., 2009; Park et al., 2011) and polychlorinated dibenzo-p-dioxins (Ahn et al., 2008). Nevertheless, previous studies also reported that bioaugmentation failed to degrade the pollutants due to ecological considerations, such as the relative spatial and temporal abundance of potential source populations and their ability to tolerate the prevailing conditions in target habitats (Thompson et al., 2005). In all, successful application of bioaugmentation is dependent on the identification and isolation of appropriate bacterial strains, and their subsequent survival and activity.

![Fig. 1. Mole percentage of residual TBP from the unamended controls and from the samples bioaugmented with Bacillus sp. GZT. Data for the bioaugmentation treatment significantly differed from the data for the unamended controls at a \( p < 0.01 \) level.](image-url)
3.2. Effects of halogenated co-substrates on TBP degradation

Bromophenols including 2-BP and 2,4-DBP were often found to be the biodegradation intermediates of TBP according to the previous works (Oshiman et al., 2007; Zu et al., 2012). Therefore, the impact of co-substrates on GZT-bioaugmentation TBP biodegradation in the water/sediment microcosms was compared with and without adding halogenated co-substrates 2-BP, 2,4-DBP or 2,6-DBP. As Fig. 2 shows, TBP was constantly removed as incubation time increased; approximately 40.7% of TBP was removed after the 7-week incubation period in the water/sediment microcosms with bioaugmentation. Comparing the impact of the different halogenated co-substrates revealed that approximately 42.4%, 41.7% and 40.9% of TBP was degraded with bioaugmentation plus 2-BP, 2,4-DBP, and 2,6-DBP, respectively, within the same incubation time. These differences were not statistically significant (p > 0.05), and TBP degradation began with no lag period with all three different co-substrates. The t_{1/2} of TBP in the water/sediment microcosms treated with bioaugmentation plus the halogenated co-substrates 2-BP, 2,4-DBP, and 2,6-DBP were 60, 61, and 59 d, respectively. These half-lives were slightly shorter than the half-life (62 d) of TBP in water/sediment microcosms only treated with bioaugmentation (Table 1).

Previous studies found that halogenated co-substrate compounds enhanced the ability of different bacteria to dechlorinate PCBs and chlorinated dioxins, by affecting both the enzyme regulation and microorganism population (Cho et al., 2002), and did not “prime” a different microbial community capable of degrading TBP in the water/sediment microcosms.

### Table 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>k (d⁻¹)</th>
<th>t_{1/2} (d)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioaugmentation</td>
<td>1.11 × 10⁻²</td>
<td>62</td>
<td>0.996</td>
</tr>
<tr>
<td>2-BP + bioaugmentation</td>
<td>1.15 × 10⁻²</td>
<td>60</td>
<td>0.996</td>
</tr>
<tr>
<td>2,4-DBP + bioaugmentation</td>
<td>1.14 × 10⁻²</td>
<td>61</td>
<td>0.995</td>
</tr>
<tr>
<td>2,6-DBP + bioaugmentation</td>
<td>1.16 × 10⁻²</td>
<td>59</td>
<td>0.996</td>
</tr>
<tr>
<td>Yeast extract + bioaugmentation</td>
<td>1.41 × 10⁻²</td>
<td>49</td>
<td>0.997</td>
</tr>
<tr>
<td>Glucose + bioaugmentation</td>
<td>1.80 × 10⁻²</td>
<td>39</td>
<td>0.995</td>
</tr>
<tr>
<td>NaCl + bioaugmentation</td>
<td>1.33 × 10⁻²</td>
<td>52</td>
<td>0.998</td>
</tr>
<tr>
<td>Humic acid + bioaugmentation</td>
<td>1.31 × 10⁻²</td>
<td>53</td>
<td>0.997</td>
</tr>
<tr>
<td>Sodium lactate + bioaugmentation</td>
<td>1.24 × 10⁻²</td>
<td>50</td>
<td>0.996</td>
</tr>
<tr>
<td>Sodium propionate + bioaugmentation</td>
<td>1.31 × 10⁻²</td>
<td>53</td>
<td>0.998</td>
</tr>
</tbody>
</table>

All degradation rate constants and half-lives reflect the mean of duplicate samples. “Bioaugmentation” indicates that the Bacillus sp. GZT was added to the microcosms. *r: Correlation coefficient. Data for each treatment were significantly different from the data for the unamended controls at a p < 0.01 level.

3.3. Effects of electron donors on TBP biodegradation

This study also investigated the effects of sodium lactate (10 mM) and sodium propionate (10 mM) respectively on TBP biodegradation in the water/sediment microcosms bioaugmented with GZT. As Fig. 3 shows, TBP biodegradation started with no lag period and gradually increased for all the treatment microcosms. Approximately 44.3% and 47.0% of TBP was degraded with bioaugmentation plus sodium lactate and sodium propionate, respectively, after a 7-week incubation period (p < 0.01). As Table 1 shows, the half-lives (t_{1/2}) of TBP with the added sodium lactate and sodium propionate were 56 and 53 d, respectively. These half-lives are significantly shorter than the half-life (62 d) for TBP in water/sediment microcosms only treated with bioaugmentation.

Generally, sodium lactate, sodium propionate, lactate, and propionate can be used as electron donors to create reductive conditions in biodegradation systems (Krumins et al., 2009). In this study, sodium lactate and sodium propionate were selected as individual electron donors. It was found that both could stimulate TBP biodegradation, enhancing the biodegradation efficiency. Sodium lactate and sodium propionate may not on their own effectively enhance the microbial community’s ability to degrade TBP. However, sodium lactate or sodium propionate alone with halogenated co-substrate compounds may effectively enhance TBP biodegradation (Ahn et al., 2005). When sodium lactate or sodium propionate serve as electron donors, they create reductive conditions and adequate reductive equivalents. As known, strain GZT can debrominate TBP to 2-BP, 2,4-DBP, 2,6-DBP and other brominated compounds (Zu et al., 2012). This, in turn, provides halogenated co-substrate compounds that stimulate bioaugmentation with the co-existing electron donors. Thus, this study introduces the possibility that sodium lactate and sodium propionate may be used as electron donors to enhance TBP degradation.

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**Fig. 2.** Mole percentage of residual TBP from the water/sediment microcosms amended with bioaugmentation plus a co-substrate, 2-BP, 2,4-DBP or 2,6-DBP. Data for each treatment with co-substrate did not significantly differ from the data for the treatment samples without co-substrate at a p > 0.05 level.

**Fig. 3.** Mole percentage of residual TBP from the water/sediment microcosms amended with bioaugmentation. Data for each treatment did not significantly differ from the data for the bioaugmented samples at a p > 0.05 level. (SL: sodium lactate; SP: sodium propionate).
3.4. Effects of other additives on TBP biodegradation

Previous studies have found that the degradation of some xenobiotics can be enhanced by adding readily metabolized organic substrates (Krumins et al., 2009; Chang et al., 2012). This study examined the effects of other additives on TBP biodegradation in water/sediment microcosms amended with GZT-bioaugmentation (Fig. S1). When water/sediment microcosms were amended with bioaugmentation plus yeast extract (5 mg L\(^{-1}\)), glucose (10 mM), NaCl (10 ng L\(^{-1}\)), and humic acid (0.5 g L\(^{-1}\)), approximately 54.0%, 59.0%, 46.6%, and 46.0% of the TBP was degraded after a 7-week incubation period, respectively (p > 0.01). As Table 1 shows, when yeast extract, glucose, NaCl, and humic acid were added as xenobiotics, the corresponding degradation rate constants (k) of TBP were 1.41 \times 10^{-2}, 1.80 \times 10^{-2}, 1.33 \times 10^{-2}, and 1.31 \times 10^{-2} d\(^{-1}\), with half-lives of 49, 39, 52, and 53 d, respectively.

Comparing the TBP biodegradation efficiency in bioaugmented water/sediment microcosms with and without additives reveals that the additives significantly impacted biodegradation. Highest TBP biodegradation was obtained by adding glucose (p < 0.05), followed by the yeast extract (p < 0.05), NaCl, and humic acid (p > 0.05). Glucose may provide H\(_2\) as an electron donor during the respiration of dehalorespiring bacteria. This finding is consistent with our previous finding that when glucose is added as an electron donor and carbon source, it can stimulate the ability of *Ochrobactrum* sp. T to degrade TBPBA (Zu et al., 2014).

Adding yeast extract (5 mg L\(^{-1}\)) also enhances TBP biodegradation. This is because yeast extract, a complex mixture of amino acids, peptides, and proteins (Fava et al., 1995), adds an additional alternative carbon source. It also has a protective effect, reducing the toxic effect of the degradable compounds and providing microorganisms with good growth and biodegradation capacity. A similar result was seen in a different study examining the ability of microbes in river sediments to aerobically degrade TBPBA in the presence of yeast extract (Chang et al., 2012).

TBP biodegradation was also slightly enhanced by adding NaCl (10 ng L\(^{-1}\)) because salinity is one of the environmental factors that varies the most in the sediments. Salinity affects the types of microorganisms that colonize in sediments and their biodegradation potential. Tam et al. (2002) reported that the optimum salinity was 10–20 ng L\(^{-1}\) for indigenous phenanthrene-degrading bacterial consortia, while high salinity (35 ng L\(^{-1}\)) inhibited phenanthrene biodegradation. In this study, TBP biodegradation was enhanced by adding 10 ng L\(^{-1}\) NaCl. This suggests that the microbial species that effectively degrade TBP may be well adapted to this salinity.

This study also investigated the effect of humic acid on TBP biodegradation (Table 1 and Fig. S1), finding that adding humic acid enhanced TBP biodegradation by the microorganisms. When humic acid is added to contaminated aquifers, it can effectively improve the sorption and binding of contaminated compounds onto sediments. This is due to the addition of hydrophobic sites, altering pollutant adsorption kinetics (Conte et al., 2001). Higher TBP concentrations in sediments may provide higher substrate concentrations, supporting indigenous microorganism growth. This facilitates microbial contacting with pollutants, and therefore, pollutants could be degraded by microbes.

These results suggest that altering the nutritional composition of the water/sediment microcosms, particularly by adding glucose and yeast extract, can improve the biodegradation efficiency of xenobiotics and optimize the treatment processes using these microorganisms.

3.5. Diversity and response of degrading bacteria

Analysis using 454 pyrosequencing allowed for the determination of bacterial species diversity. In total, high-throughput sequencing of the water/sediment microcosm samples bioaugmented with GZT generated approximately 123,340 sequence reads of 16S rRNA gene, with an average length of approximately 454 bp. After filtering the low quality reads using PRINSEQ-lite 0.19.5 software; trimming the chimera, barcodes and primers; and normalizing sequence reads, 89,825 high-quality reads remained. A 97% similarity cut-off was then used to group OTUs for the downstream analyses.

Table S1 summarizes corresponding numbers of OTUs, the Shannon diversity Index (H'), and the Chao1, which indicate a high level of bacterial diversity in the water/sediment microcosms. During the 7-week incubation period, there were 1320 to 2208 OTUs in these samples at a 3% cut-off level; Chao1 values varied from 2766 to 6046. The H' values varied from 4.94 to 5.45, indicating a high diversity in the bacterial 16S rRNA libraries from these samples. These results point to high bacterial species richness; this conclusion is also supported by high ACE diversity indices.

The coverage was used to assess whether the library was sufficiently large to obtain meaningful, stable richness estimates. The calculated coverage ranged from 90.00% to 93.60%. This indicated that the microbial species contained in the clone library were accounted for the high-throughput sequencing, and the library sufficiently yielded stable phylogenotype richness estimates. Rarefaction analysis was also used to standardize and compare observed taxon richness between samples, to identify whether the sample was unequally sampled. Generally, a rarefaction curve with no plateau indicates the need for additional sampling to assess ecosystem diversity (Roh et al., 2010).

In this study, all rarefaction curves failed to reach saturation (Fig. S2), indicating that the microbial diversity in water/sediment microcosms was much larger than expected. PcoA (Fig. 4) analysis demonstrated that the samples occupied divergent positions, whereas the samples collected from the water/sediment microcosm samples bioaugmented with GZT showed a different cluster, and therefore, a different species composition.

This study also examined changes of the microbial community in response to GZT-bioaugmented TBP degradation in water/sediments microcosms. Fig. 5 shows that >30 microbial phyla were recovered from the samples, and the microbial community changed during the biodegradation processes. There were eight different phylogenetic groups at the phylum taxonomic rank: *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Acidobacteria*, *Chloroflexi*, *TM7*, and the unclassified phyla. The dominant microbial phyla were *Proteobacteria* (52.08–66.22%), *Actinobacteria* (20.03–5.47%), *Bacteroidetes* (6.68–13.68%), and *Firmicutes* (4.53–20.83%), *Acidobacteria*, *Chloroflexi*, *TM7*, and the unclassified phyla were found to be minor groups during the 7-week TBP degradation process.

The heatmap (Fig. 6) shows the top 33 most abundant genera in each sample. Some genera, such as *Bacillus*, *Novosphingobium*, *Thiobacillus*, *Flavibacterium*, *Gemmatimonas*, *Phenyllobacterium*, and *Dyella*, dominated the water/sediment microcosms amended with GZT-bioaugmentation. The other 26 genera were considered to be minor groups.

![Fig. 4. Principal coordinate analysis (PCoA) of the samples using Weighted-UniFrac from pyrosequencing.](image-url)
The strain GZT used for bioaugmentation was affiliated with the *Bacillus* genus of the *Firmicutes* phylum, which continuously increased from 4.53% to 20.83% over the 7-week incubation. Our previous study found that strain GZT is capable of debrominating and mineralizing TBP, by using TBP as a sole carbon and energy source (Zu et al., 2012). This suggests that the microbes from the *Bacillus* genus of *Firmicutes* phylum may be primarily linked to TBP removal. This makes the increase of *Firmicutes* phylum over the 7-week incubation period seem more reasonable, because the microbial community continuously acclimated to the TBP substrate.

Many previously documented TBP degraders either evolved as minor genera or disappeared in the sediment microcosms during the TBP degradation process. A different study found that the *Desulfovibrio* sp. TBP-1, which is capable of growth through TBP halorespiration, was affiliated with the *Proteobacteria* phylum (Boyle et al., 1999). The *Ochrobactrum* sp. TB01, affiliated with the *Proteobacteria* phylum, also uses TBP as sole carbon and energy source (Yamada et al., 2008). An *Achromobacter piechaudii* TBPZ, affiliated with the *Proteobacteria* phylum, was isolated from desert soil contaminated with chemical industry waste, and was found to reductively dehalogenate TBP into phenol (Ronen et al., 2000). However, in this study, TBP degradation was not significant in the unamended controls. This indicates that strain GZT played a major role in degrading TBP and enhancing TBP biodegradation efficiency.

The abundant genus *Novosphingobium* in in the water/sediment microcosm samples is affiliated with the *Proteobacteria* phylum. Other studies have shown that this group can degrade aromatic compounds, such as polycyclic aromatic hydrocarbons (PAHs), aniline, nitrobenzene, and phenanthrene (Liu et al., 2005). The water/sediments collected from the two rivers in this study had significant PAH contamination (Guo et al., 2012), possibly explaining why *Novosphingobium* were abundant in the samples. The abundant genus of *Thiocapsa* is also affiliated with the *Proteobacteria* phylum; this genus may be abundant because of the presence of nitrate, which *Thiocapsa* uses to conduct denitrifying activity (Li et al., 2016b).

*Flavibacterium*, another abundant group, is affiliated to the new *Bacteroidetes* phylum. This genus can use aromatic compounds as a sole carbon source, supporting its use in environmental bioremediation (Koma et al., 2012). The abundant genus of *Gemmatimonas* in the water/sediment microcosm samples is affiliated with the *Gemmatimonadetes* phylum, which is a poorly understood bacterial phylum with a small number of cultured species. This phylum originates in different habitats, including river water/sediments, estuarine water, lake water, soils and wastewater treatment plants (Zeng et al., 2016).

![Fig. 5. Phylogenetic classification of the microbial communities for pyrosequencing at the phylum level obtained from Ribosomal Database Project (RDP) classifier analysis. Sequences classified as other phyla and unclassified phyla are included in the group of “other” phyla.](image)

![Fig. 6. Heatmap showing the 33 most abundant genera of microbial communities for each sample. The relative frequencies are indicated by color intensity; the legend is at the bottom right corner.](image)
The genus of *Phenylobacterium* has been previously seen in petroleum reservoirs and polychlorinated biphenvyl-contaminated soils, making up the majority of anthraquinone degraders in the untreated soil (Rodgers-Vieira et al., 2015). For the genus *Dyella*, Li et al. (2009) reported that *Dyellaginsengsilis* LA-4 can use aromatic compounds, such as benzoic acid, naphthalene, and toluene, as sole carbon and energy sources. Muangchinda et al. (2013) also reported that the genus *Dyella* could degrade phenanthrene.

In summary, the dominant genera found in this study mainly degraded other organic compounds, whereas the *Baillus* genus mainly degraded TBP. As such, this study validated the effective use of strain GZT, associated with *Baillus*, to enhance TBP degradation in water/sediments.

### 4. Conclusions

In this study, bioaugmentation with *Baillus* sp. GZT significantly enhanced TBP biodegradation in water/sediment microcosms. TBP biodegradation was not stimulated by the co-substrates 2-BrP, 2,4-DBP, or 2,6-DBP (p < 0.05); however, sodium lactate or sodium propionate could stimulate the ability of the strain GZT to degrade TBP. Adding glucose stimulated TBP degradation the most, followed by yeast extract, NaCl, and humic acid.

In response to TBP degradation, the microbial community was dominated by eight different phylogenetic groups at the phylum taxonomic rank: *Proteobacteria* (52.08–62.22%), *Actinobacteria* (20.03–5.47%), *Bacteroidetes* (6.68–13.68%), *Firmicutes* (4.53–20.83%), *Acidobacteria* (1.30–4.31%), *Chloroflexi* (1.87–0.97%), *TM7 (3.19–0.87%), and the unclassified phyla (3.50–0.68%). The study's findings have practical and ecological significance, and may be useful for improving the efficiency and the stability of biological treatment processes.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.09.017.

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