Accelerated biodegradation of BPA in water-sediment microcosms with Bacillus sp. GZB and the associated bacterial community structure

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ABSTRACT

Bisphenol A (BPA) is a synthetic chemical primarily used to produce polycarbonate plastics and epoxy resins. Significant industrial and consumer’s consumption of BPA-containing products has contributed to extensive contamination in different environmental matrices. In this study, microcosms bioaugmented with Bacillus sp. GZB were constructed to investigate BPA biodegradation, identify the main bacterial community, and evaluate bacterial community responses in the microcosms. Under aerobic conditions, BPA was quickly depleted as a result of bioaugmentation with Bacillus sp. GZB in water-sediment contaminated with pollutants. The pollutants used were generally associated with the electronic wastes (mobile phones, computers, televisions) dismantling process. Adding BPA affected the bacterial community composition in the water-sediment. Furthermore, BPA biodegradation was enhanced by adding electron donors/co-substrates: humic acid, NaCl, glucose, and yeast extract. Metagenomic analysis of the total 16S rRNA genes from the BPA-degrading microcosms with bioaugmentation illustrated that the genera Bacillus, Thiobacillus, Phenylobacterium, and Cloacibacterium were dominant after a 7-week incubation period. A consortium of microorganisms from different bacterial genera may be involved in BPA biodegradation in electronic waste contaminated water-sediment. This study provides new insights about BPA bioaugmentation and bacterial ecology in the BPA-degrading environment.

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1. Introduction

Bisphenol A (2,2-bis(4-hydroxyphenyl)propane, BPA, CAS No. 80-05-7) is widely used as an intermediate to produce...
polycarbonate, epoxy resins, flame retardants, and other products (Hoekstra and Simoneau, 2013). Worldwide demand and annual consumption of BPA continues to increase as a result of human activity (Huang et al., 2012). More than one million pounds of BPA are estimated to be released into the environment annually, mainly from the discharged plastic leachate in landfills, processing of BPA in manufacturing, and the combustion of computer printed circuit boards in electronic waste (Erler and Novak, 2010; USEPA, 2010; Huang et al., 2012; Peng et al., 2015). As a result, BPA has been detected in groundwater, industrial water, drinking water, municipal sewage sludge, and surface water (Belfroid et al., 2002; Huang et al., 2012; Careghini et al., 2015; Xiong et al., 2015; Toro-Velez et al., 2016). Further, BPA has been confirmed to be an endocrine-disrupting chemical, and may adversely affect endocrine functions in humans and wildlife. This may cause changes in developmental processes (Hutler Wołkowicz et al., 2016), altered reproductive capacity (Quan et al., 2017), damaged nervous systems (Beronius et al., 2013), increased cancers (Chen et al., 2002), and decreased immunocompetence (Lee et al., 2013). This has led to increased focus on the environmental fate of BPA (Sun et al., 2012; Toro-Velez et al., 2016).

The biological transformation of organics is one of the main transformation pathways in the environment, and microorganisms can effectively remove different environmental pollutants in the natural aquatic environment (Yang et al., 2016). However, most previous studies have focused on isolating different indigenous BPA-degrading bacterial species (Eio et al., 2014) and conducting BPA degradation tests using these bacteria in liquid culture (Wang et al., 2014). For example, BPA-degraders Sphingomonas sp. strain BP-7 (Yang et al., 2015), Sphingomonas sp. strain A01 (Roh et al., 2009), Achromobacter xylosidans strain B1-16 (Zhang et al., 2007), and Bacillus sp. GZB (Li et al., 2012) were isolated from different environments and their degradation efficiencies were studied in the laboratory culture. However, bacteria with strong biodegradation ability in the natural aquatic environments are limited and typically depend on biostimulation and/or bioaugmentation.

Bioaugmentation, using these isolated pollutant-degrading microorganisms, may be an attractive option to quickly remediate contaminated aquatic environments. For example, a tetra-bromobisphenol A (TBBPA) degrader Ochrobactrum sp. T was used to bioaugment TBBPA degradation in river sediment; removal efficiencies were noticeably enhanced by bioaugmentation with this TBBPA-degrader (Li et al., 2016). However, little is known about bioaugmentation using BPA degraders in river sediment. In addition, it is unknown whether a bioaugmentation strategy could significantly change the whole bacterial community structure, such as when a BPA-degrader is added in the BPA-contaminated sediments. In addition, better understanding BPA-degrading bacterial communities can help researchers to better understand BPA bioaugmentation in natural aquatic environments, which can be useful for further practical application. This heightens the importance of studying the accelerated biodegradation of BPA in water-sediment microcosms when treated with Bacillus sp. GZB, and the resulting bacterial community structure.

Therefore, this study applied bioaugmentation with Bacillus sp. GZB, isolated from an electronic waste dismantling zone. Previous studies have demonstrated that bacteria could effectively degrade BPA in liquid culture. This study first investigated the biodegradation potential of BPA with bioaugmented with Bacillus sp. GZB in simulated water-sediment under aerobic conditions. Second, the study identified the dominant bacterial community responses to bioaugmentation using high-throughput sequencing. Third, we investigated the roles of different specific bacterial communities, based on enrichment during the bioaugmentation process. These results can help optimize current remediation methods.

2. Materials and methods

2.1. Experimental design

Surface sediment samples were collected from a river (0–10 cm depth) in an electronic waste dismantling zone in Guangdong Province, South China. Samples were placed in sterilized glass bottles and stored at −20 °C until they were analyzed. The sampling region was seriously polluted by heavy metals (Wu et al., 2015) and organics, including polycyclic aromatic hydrocarbon (PAHs) (Zhang et al., 2011), brominated flame retardants (BFRs), and BPA (Xiong et al., 2015). This contamination resulted from the primitive techniques used during electronic waste and plastic recycling. The inorganic salt medium used to isolate BPA-degrading bacteria and the growth medium were prepared based on previous research (Li et al., 2012, 2016). The pH value of the inorganic salt medium was adjusted to 7.0 before sterilization.

Microcosms were established using homogenized surface sediment, consisting of 47% (V/V) surface sediment, 53% (V/V) inorganic salt medium, and 10 mg L⁻¹ of BPA (97%, Acros Organics). Each microcosm contained 150 mL homogenized surface sediment in a 250 mL flask. Six microcosms were established, each with a duplicate: (1) unamended controls under aerobic conditions; (2) aerobic conditions + bioaugmentation with Bacillus sp. GZB; (3) yeast extract (5 mg L⁻¹) + bioaugmentation with Bacillus sp. GZB; (4) NaCl (10 ng L⁻¹) + bioaugmentation with Bacillus sp. GZB; (5) humic acid (0.5 g L⁻¹) + bioaugmentation with Bacillus sp. GZB; and (6) glucose (10 mM) + bioaugmentation with Bacillus sp. GZB. Bacillus sp. GZB was placed in sterilized growth medium and cultured at 37 °C in a horizontal shaker at 200 rpm for 15 h. Then, 30 mL of the incubated growth medium was centrifuged and rinsed three times with stroke-physiological saline solution to collect the Bacillus sp. GZB. An identical wet weight (approximately 0.35 g) of Bacillus sp. GZB was added to each microcosm. These microcosms were incubated in a horizontal shaker (150 rpm) in the dark at 25 °C. Previous research showed that the Bacillus sp. GZB isolated from an electronic waste recycling sediment from regions where electronic waste recycling is done could effectively degrade BPA under anaerobic and aerobic conditions (Li et al., 2012). Detailed sample collection methods can be found in previously published research (Li et al., 2016).

2.2. Chemical and molecular analyses

The residual BPA in water-sediment was extracted and measured based on existing references (Xiong et al., 2015, 2016). Genomic DNA of each water-sediment mixed samples was extracted using a E.Z.N.A.™ Soil DNA Kit (Omega Bio-tek, Inc., USA). The bacterial V3–V4 region of the 16S rRNA gene was amplified using the forward primers 341F (5'-CCTACGGGNGGCWCCAA-3') and reverse 805R (5'-GACTGGAGTCTTGGGACCAATTC-3') (Li et al., 2016; Xiong et al., 2017). The PCR programs for bacterial amplicon ran on a Bio-rad T100™ thermal cycler (California, USA) using previously published procedures (Li et al., 2016). PCR amplicons were evaluated using electrophoresis on 2.0% agarose gel and extracted with the SanPrep Column DNA Gel Extraction Kit (Sangon Biotech, China). The amplicons underwent high-throughput sequencing analysis (Illumina MiSeq sequencing) to characterize bacterial communities and examine their relative abundance and diversity.

The generated metagenomic sequences were filtered to check the quality control procedures; low quality sequences were removed based on reference (Li et al., 2016). UCLUST software
Bioaugmentation: addition of Bacillus sp. GZB. The differences between the unamended controls and the bioaugmented microcosms were statistically significant \( (P < 0.01) \).

These results confirmed that the bioaugmentation and bio-stimulation increased BPA removal in the water-sediment system. Furthermore, as expected, the BPA removal efficiency in the microcosms using Bacillus sp. GZB bioaugmentation was higher than the efficiencies in the untreated controls. This suggested that the bioaugmentation with Bacillus sp. GZB could enhance BPA biodegradation in water-sediment \( (P < 0.01) \). Therefore, the enhanced biodegradation efficiency was closely related to the added Bacillus sp. GZB, a facultative anaerobic BPA-degrading bacterium isolated from an electronic waste dismantling zone estuarine \( (\text{Li et al., } 2012) \).

Previous studies showed that xenobiotic biodegradation can be enhanced by adding electron donors/co-substrates \( (\text{Li et al., } 2016; \text{Xiong et al., } 2017) \). In this study, the data listed in Table 1 show that BPA degradation rates \( (k) \) increased in microcosms where bioaugmentation was supplemented with yeast extract, NaCl, humic acid, and glucose \( (P > 0.05) \). These results illustrated that these additional compounds could promote BPA bioaugmentation in the microcosms; this finding was consistent with existing literature \( (\text{Xiong et al., } 2017) \). Glucose donates H\(_2\) and carbon during bacterial respiration, stimulating the bacteria’s ability to degrade compounds \( (\text{Zu et al., } 2014) \). Yeast extract, a complex mixture of amino acids, peptides, and proteins \( (\text{Fava et al., } 1995) \), is often added as an additional alternative carbon source. It also protects the bacteria, reducing the adverse impact of the degradable compounds, and providing bacteria with good growth and biodegradation capacity. Humic acid effectively improves the sorption and binding of contaminated compounds onto sediment in contaminated aquifers \( (\text{Conte et al., } 2001) \). This facilitates contact between bacteria and BPA, facilitating BPA degradation. Salinity is another environmental factor that impacts the types of bacteria that colonize the sediment and their biodegradation potential \( (\text{Xiong et al., } 2017) \). In this study, BPA biodegradation was enhanced by adding 10 ng L\(^{-1}\) NaCl, suggesting that this salinity benefits BPA degradation.

### 3.2. Diversity of bacterial community

In this study, Illumina MiSeq sequencing analysis was used to investigate the water-sediment bacterial communities in the microcosm, using bioaugmentation at different incubation periods. Approximately 168,664 valid reads of 16S rRNA gene with an average length of approximately 455 bp were obtained after trimming and chimera removal. Using a 97\% sequence similarity cutoff, the number of OTUs in the water-sediment bacterial communities varied from 1147 to 2861 (Table 2). These results indicated that the genera of bacterial community were highly diverse. The rarefaction curve was used to standardize and compare the observed taxon richness between samples, to identify whether the sample was unequally sampled \( (\text{Xiong et al., } 2017) \). In this study, the rarefaction curve for each water-sediment sample did not plateau, indicating a
significantly higher water-sediment bacterial diversity at the sequencing depth used in this study (Fig. S1).

Shannon index values were used to assess bacterial community diversity (Xiong et al., 2017). In this study, the Shannon index values of the water-sediment samples increased from 3.68 to 5.94 during the incubation period. The results indicated a high diversity in bacterial 16S rRNA libraries during the BPA biodegradation process. The species richness indices of the abundance-based coverage estimator (ACE) and Chao1 varied from 5086 to 13,229, and from 3199 to 7831, respectively. These results also highlighted high bacterial species richness. In addition, calculated coverage, ranging from 86.52% to 93.31%, revealed that most bacterial communities were accounted for using 454 pyrosequencing. Principal coordinate analysis (PcoA) (Fig. 2) showed that water-sediment samples occupied the divergent positions. The diversity estimator indexes above suggested that bacterial community diversities did not decline in the microcosm bioaugmented with \textit{Bacillus} sp. GZB during the incubation period. This suggested that bioaugmentation and xenobiotics (BPA) did not change bacterial diversity.

3.3. Composition of bacterial community

First, bacterial community composition was analyzed at the phylum level in the clone library. In this study, there were nine phyla with reads of more than 1.0% in the microcosm samples with bioaugmentation. The nine primary bacterial phyla in the sediment were \textit{Proteobacteria}, \textit{Firmicutes}, \textit{Bacteroidetes}, \textit{Actinobacteria}, \textit{Acidobacteria}, \textit{Verrucomicrobia}, \textit{Planctomycetes}, \textit{Chloroflexi}, and TM7 (Fig. 3).

For the water-sediment in the microcosm, \textit{Proteobacteria} (accounting for 37.5–59.1% of total bacteria) was the first largest phylum group in all samples; it mainly consisted of \textit{Alpha-proteobacteria}, \textit{Beta-proteobacteria}, and \textit{Gamma-proteobacteria} (Fig. 4). Previous studies show that this proteobacteria group is widely distributed in waste water, sediment, and soil, where BPA levels are very high (Conte et al., 2001; Yang et al., 2015; Xiong et al., 2017). For example, Yang et al. found that BPA could be quickly depleted in BPA-spiked sediment; the microbial community structure in river sediment shifted, such that \textit{Gamma-proteobacteria} and \textit{Alpha-proteobacteria} became the predominant bacterial groups during the BPA biodegradation process (Yang et al., 2015). Interestingly, Peng et al.’s research found that both \textit{Beta-proteobacteria} and \textit{Gamma-proteobacteria} were the dominant microorganisms when TBBPA was degraded by sewage sludge, which was from an anaerobic tank of wastewater treatment facilities for TBBPA removal for several years (Wu et al., 2015). Their research concluded that both \textit{Beta-proteobacteria} and \textit{Gamma-proteobacteria} may biodegrade BPA, given that BPA is an important biodegradation intermediate of TBBPA (An et al., 2011).

For this study, Fig. 4 shows that there was a large variation in the proportion of \textit{Alpha-proteobacteria} (from 56.9% to 33.3%) and \textit{Beta-proteobacteria} (from 8.0% to 38.0%) in the microcosms during the 7-week incubation period. Other studies have reported that the genera of \textit{Alpha-proteobacteria} can produce extracellular polymeric substances (EPS) or are motile using polar flagella, providing a protective environment for cells to grow and persist (Pang and Liu, 2007). Therefore, \textit{Alpha-proteobacteria} appeared to be one of the major divisions in the microcosm. The ecologically diverse \textit{Beta-
proteobacteria were the most frequent bacteria in the clone library, consistent with published literature (Wu et al., 2015). Beta-proteobacteria can attach more easily to surfaces and dominated the sediment (Douterelo et al., 2013). Furthermore, this group contained many heterotrophic, chemoorganotrophic, and facultative anaerobic respiring bacteria (Haller et al., 2011). For example, members of this group, including Dechloromonas aromatica, were found in sediment habitats and could oxidize aromatic compounds (Coates et al., 2001). In addition, other species of this group can grow using other carbon compounds or reduced sulphur compounds (Coates et al., 2001). The sediment in microcosm was obtained many heterotrophic, chemoorganotrophic, and facultative anaerobic respiring bacteria (Haller et al., 2011). For example, the most frequent bacteria in the clone library was the second largest phylum group in the water-sediment from the microcosm (Fig. 3). Firmicutes was present at 21.3% during Week 0; levels slightly decreased during Week 2, and gradually increased to 34.0% during Week 7. These changes may be that the genus Bacillus sp., which can degrade BPA (Li et al., 2012), is affiliated with the Firmicutes phylum. First, Bacillus sp. GZB experienced a lag phase in adapting to the water-sediment environment; levels increased as incubation continued. As such, Firmicutes phylum levels slightly decreased during Week 2, and then increased. Bacteroidetes levels increased from 8.6% to 18.6% during the incubation period, while Actinobacteria decreased during the incubation period.

These results demonstrated that both Bacteroidetes and Bacteroidetes may be able to degrade BPA, supporting previous studies. For example, both Bacteroidetes and Actinobacteria phyla were also present in sediment bacterial communities associated with BPA biodegradation under anaerobic conditions (Yang et al., 2015).

Fig. 5. Heatmap showing the top 18 most abundant genera of bacterial communities for each sample. The relative frequencies are indicated by color intensity, coded in the legend in the bottom right corner. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Bacillus (33.4%), Thiobacillus (11.3%, Proteobacteria), Phenyl-
obacterium (14.2%, Proteobacteria), Oscillibacter (2.7%), and Cloa-
cibacterium (5.5%), became the dominant genera by Week 7. There was a significant increase of Bacillus (from 13.4% to 33.4%) during the incubation period. There were also increases in Thiobacillus (from 0.7% to 11.3%) and Phenyl
obacterium (from 0.9% to 14.2%). In summary, there was a shift in major bacterial group composition in the microcosms that were bioaugmented with Bacillus sp. GZB after 7 weeks of incubation.

With respect to the decrease of Ochrobactrum genus, previous research suggested that Ochrobactrum can effectively debrrominate and mineralize TBBPA (An et al., 2011), and members of the genus Ochrobactrum sp. can degrade BPA. Therefore, the prominent increase of Bacillus sp. GZB may explain the rapid attenuation of BPA biodegradation in water-sediment.

4. Conclusions

Large amounts of BPA could be biodegraded in water-sediment microcosms bioaugmented with Bacillus sp. GZB under aerobic conditions. Bacillus sp. GZB was found to play a key role in BPA biodegradation. Furthermore, a variety of relative abundant bacterial species may help increase BPA biodegradation in water-sediment microcosms with bioaugmentation. Bacterial community structure varied as incubation continued. Bioaugmentation or xenobiotics (BPA) impacted the water-sediment bacterial community structure. Finally, supplemental yeast extract, humic acid, or glucose act as electron donors/co-substrates to improve BPA biodegradation. The study might give a light to the future applications of strategy to remediate water-sediment contaminated with organics.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.05.163.

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