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# Aerobic degradation of nonhalogenated organophosphate flame esters (OPEs) by enriched cultures from sludge: Kinetics, pathways, bacterial community evolution, and toxicity evaluation

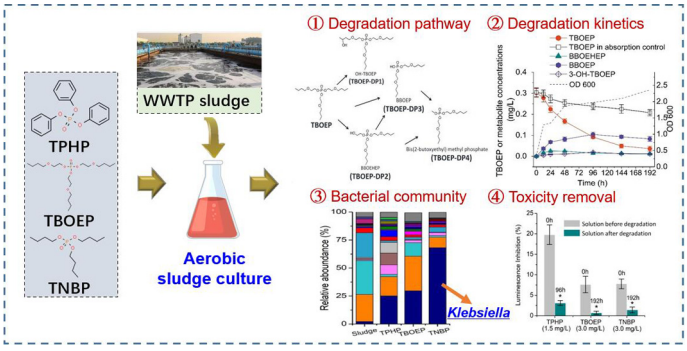
Rui Hou [a,c], Yi Wang [a], Shaofeng Zhou [a], Lihua Zhou [b], Yong Yuan [a,*], Yiping Xu [d]

[a] Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou 510006, China
[b] School of Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, Guangzhou 510006, China
[c] Key Laboratory of Tropical Marine Bio-resources and Ecology, Guangdong Provincial Key Laboratory of Applied Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
[d] Key Laboratory of Drinking Water Science and Technology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

## HIGHLIGHTS

- Aerobic sludge cultures degrade high concentrations of TPHP, TBOEP and TNBP quickly.
- Possible aerobic biotransformation pathways for the three OPEs were proposed.
- Di-alkyl phosphates were quantitated as the most predominant transformation products for OPEs.
- *Klebsiella* involved in the biodegradation of non-halogenated OPEs in sludge culture.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The degradation by bacteria has been considered the main process for eliminating nonhalogenated organophosphate esters (OPEs) from wastewater treatment plants (WWTPs), but limited research has reported the biodegradation processes and clarified the microbial-mediated mechanisms for nonhalogenated OPE degradation in WWTPs. The aim of this study was to monitor the biodegradation of the most common nonhalogenated OPEs, namely, tris(2-butoxyethyl) phosphate (TBOEP), tris (n-butyl) phosphate (TNBP) and trisphenyl phosphate (TPHP), under aerobic conditions by sludge cultures from a conventional sewage plant. The microbial cultures were enriched separately with each OPE from activated sludge cultures, and the presence of glucose significantly enhanced degradation of the OPEs during the enrichment. The removal ratios for the three OPEs reached 29.3–89.9% after 5 cycles (25 days) of cultivation, and the first-order degradation kinetics followed the order of TPHP > TBOEP > TNBP, with their half-lives ranging between 12.8 and 99.0 h. Pathways of hydrolysis, hydroxylation, methoxylation, and substitution were confirmed for the aerobic biodegradation of these nonhalogenated OPEs, but only di-alkyl phosphates (DAPs) largely accumulated in culture medium as the most predominant transformation products. Phylotypes in *Klebsiella* were significantly more abundant during OPE biodegradation than in the initial sludge, which indicated that these microorganisms are associated with the biodegradation of nonhalogenated OPEs in sludge culture. Biodegradation of all investigated nonhalogenated OPEs was associated with a significant reduction in the residual toxicity to *Vibrio fischeri*, indicating a rather positive ecotoxicological outcome of the aerobic biotransformation processes achieved by the enriched sludge culture.



* Corresponding author.
*E-mail address:* yuanyong@soil.gd.cn (Y. Yuan).

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## 1. Introduction

Organophosphate esters (OPEs) are a class of emerging contaminants with increasing use in flame retardants in recent years after some brominated flame retardants (BFRs) were phased out due to their persistence, bioaccumulation and potentially toxic effects (Van der Veen and de Boer, 2012). Based on their different functional groups, OPEs can be further classified as halogenated or nonhalogenated OPEs (Van der Veen and de Boer, 2012). Halogenated OPEs, such as tris(1,3-dichloroisopropyl) phosphate (TDCIPP) and tris(2-chloroethyl) phosphate (TCEP), have been prohibited or restricted in children's products and residential upholstered furniture in the USA (Lalovic et al., 2004). In the EU, restrictions on the use of TDCIPP and tris(chloropropyl) phosphate (TCPP) have also been issued because of their carcinogenic potency (ECHA, 2008a; ECHA, 2008b). The restriction of halogenated OPEs has prompted the development of nonhalogenated OPEs, including tris(2-butoxyethyl) phosphate (TBOEP), tris(n-butyl) phosphate (TNBP) and trisphenyl phosphate (TPHP).

Because nonhalogenated OPEs are not chemically bound to host materials, they can be rapidly released into their surrounding environment (Van der Veen and de Boer, 2012). Considerable levels of nonhalogenated OPEs have been found worldwide in various aquatic environment matrices, such as surface water (Andresen et al., 2004; Cristale et al., 2013; Wang et al., 2015), sediment (Cao et al., 2012; Chung and Ding, 2009; Giulivo et al., 2017), biota (Brandsma et al., 2015; Hou et al., 2017; McGoldrick et al., 2014; Su et al., 2015), and sewage effluents/influents of wastewater treatment plants (WWTPs) (Andresen et al., 2004; Marklund et al., 2005). In WWTP effluent samples from Spain (Rodil et al., 2009), Austria (Martínez-Carballo et al., 2007), Canada (Woudneh et al., 2015), and China (Shi et al., 2016; Xu et al., 2019), TPHP, TBOEP and TNBP were detected as the major OPE components, with concentrations up to 10 μg/L. The treated or untreated wastewater discharges were presumed to be one of the major entry pathways for OPEs into the aquatic environment (Pantelaki and Voutsa, 2019). Additionally, several studies have reported that nonhalogenated OPEs, including TBOEP, TNBP, TPHP and other OPEs, can display reproductive toxicity and endocrine disrupting effects (Liu et al., 2012; Porter et al., 2014; Van der Veen and de Boer, 2012). TNBP and TPHP were also reported to be neurotoxic to animals and humans (WHO, 1991a; WHO, 1991b). Due to their multiple toxicities and widespread distributions, the residue of these OPEs in the aquatic environment poses potential risks to human health and ecological safety.

Nonhalogenated OPEs can be degraded more easily than halogenated OPEs through traditional sewage treatment processes (Reemtsma et al., 2008). The removal rates of nonhalogenated OPEs (i.e., TBOEP, TNBP and TPHP) ranged from 57–86%, 38–68.8%, 27–79% and 20.1–60.7% in typical wastewater treatment plants (WWTPs) in Germany (Meyer and Bester, 2004), USA (Kim et al., 2017), Sweden (Marklund et al., 2005) and China (Fu et al., 2017), respectively. In addition, organophosphate di-esters (di-alkyl phosphates, abbr. DAPs), including di-(n-butyl) phosphate (DNBP) and diphenyl phosphate (DPHP) (metabolites of TNBP and TPHP, respectively), were widely detected in the activated sludge of sewage treatment plants in the USA (Kim et al., 2017; Wang et al., 2019) and China (Fu et al., 2017; Gao et al., 2016). These DAPs were expected to be the degradation products of OPEs based on source analysis (Fu et al., 2017). Therefore, activated sludge is considered a major degradation medium in WWTPs (Fu et al., 2017), but no information is available about the pathways and mechanisms of nonhalogenated OPE degradation.

To date, only limited studies have reported the microbial-mediated biotransformation processes of nonhalogenated OPEs in WWTPs. TBOEP, TNBP and other OPEs were classified as aerobically biodegradable in a study conducted by Kawagoshi et al. (2002) using leachate from a solid waste treatment site, whereas only TPHP showed a rapid degradation rate under anaerobic conditions. Wei et al. (2018) found that *Brevibacillus brevis* could degrade TPHP to DPHP and monophenyl phosphate (MPHP) under aerobic conditions, where cytochrome P450 (CYP) played an important role in the degradation process. However, some human and animal studies have clarified that the hydroxylation and other oxidation biotransformation pathways, as well as the hydrolysis process from OPEs to DAPs, are significant for OPE degradation (Gao et al., 2016; Hou et al., 2018; Hou et al., 2016; Van den Eede et al., 2013). Considering their structural similarities, OPEs may have similar aerobic microbial biodegradation processes as those of organophosphorus pesticides such as chlorpyrifos, parathion and diazinon, including hydrolysis, oxidation, alkylation and dealkylation pathways (Karpouzas and Singh, 2006).

To the best of our knowledge, there is no report about the possible pathways and mechanisms of biodegradation processes of the major nonhalogenated OPEs under aerobic conditions. Thus, the main objectives of this study were to 1) investigate the degradation kinetics of nonhalogenated OPEs (i.e., TBOEP, TNBP and TPHP) in aerobic cultures enriched from sludge in the presence of glucose as a cosubstrate; 2) identify the possible degradation products of OPEs and propose their possible biodegradation pathways in aerobic activated sludge then evaluate the formation rates of known degradation products, including bis(2-butoxyethyl) phosphate (BBOEP), bis(2-butoxyethyl) hydroxyethyl phosphate (BBOEHEP), bis(2-butoxyethyl) hydroxyl-3-butoxyethyl phosphate (3-OH-TBOEP), dibutyl-3-hydroxybutyl phosphate (3-OH-TNBP), and DPHP; 3) characterize the composition of the bacterial community associated with the degradation process using high-throughput sequencing and then propose simple biodegradation mechanisms; and 4) finally, evaluate acute toxicity during the degradation processes. These results will provide a fundamental understanding of the biological removal of nonhalogenated OPEs from wastewater.

## 2. Material and methods

### 2.1. Chemicals and reagents

Standards of TPHP, TBOEP, and TNBP were all purchased from Sigma Chemical (St. Louis, MO, USA). The metabolites of OPEs, including DPHP, BBOEP, BBOEHEP, 3-OH-TBOEP, DNBP, and 3-OH-TNBP, were obtained from Toronto Research Chemicals (Toronto, Canada). All solvents used were of HPLC grade and purchased from Aladdin Industrial Corporation (Shanghai, China). Purified water (18 MΩ) was prepared using a Millipore Milli-Q system (Bedford, USA). The other chemicals used in the biodegradation medium were of analytical grade and purity (J&K Scientific Ltd., Beijing, China).

### 2.2. Enrichment of OPE-degrading sludge culture and biodegradation tests

Aerobic sludge samples were collected from the nitrification (aerobic) tank of a municipal WWTP in Shenzhen, China, in October 2018, where the conventional anoxic and aerobic (AO) process was designed for nitrogen and carbon removal. The mixed sludge samples were collected in amber bottles and transported to the laboratory immediately. The sludge culture medium contained 2.28 g/L $K_2HPO_4$, 0.36 g/L $NaH_2PO_4$, 1.32 g/L $(NH_4)_2SO_4$, and 0.12 g/L $MgSO_4$ (pH = 7) (Terzic et al., 2018). Batch experiments were performed with or without 1 g/L glucose to elucidate the effect of an external carbon supply on the enrichment processes. The sludge cultures were enriched for each OPE by mixing 1 g of wet sewage sludge, individual OPEs (3 mg/L TBOEP, 3 mg/L TNBP and 1.5 mg/L TPHP), and 100 mL of mineral salt medium. The mixed culture media were then incubated at 30 °C on a rotary shaker in the dark during the experimental period. Aerobic conditions were maintained by sealing with a permeable film, which can make sure the concentration of dissolved oxygen (DO) above 1 mg/L during 192 h of incubation (determined by a sterilized DO electrode; HQD40 Hach, USA). Abiotic degradation that spiking of autoclaved biomass (1 g (w/w) of sludge treated at 121 °C for 20 min) was prepared as well in the initial incubation cycle. For the follow-up incubation cycles,

the inoculum was prepared by centrifuging (10,000 ×g, 15 min, 4 °C) the previously enriched fresh cultures and washing with pure water three times (Briones et al., 2018). The final inoculum (1 g wet weight) was added to 100 mL of the culture medium and inoculated with the individual OPE substrates.

After 20 days of cultivation, investigations were performed regarding the biodegradation kinetics and product identification. These experiments were performed in triplicate under the same conditions, and two concentrations were set for the individual OPEs (TBOEP-L: 0.3 mg/L and TBOEP-H: 3 mg/L; TNBP-L: 0.3 mg/L and TNBP-H: 3 mg/L; and TPHP-L: 0.3 mg/L and TPHP-H: 1.5 mg/L). The adsorption experiments were conducted using autoclaved biomass (0.1 g (w/w) of final inoculum treated at 121 °C for 20 min) to test the abiotic absorption of OPEs. Liquid samples (1.5 mL) were collected periodically, extracted using 1:1 (v/v) methanol, immediately passed through a 0.22 μm filter (GHP Acrodisc; PALL, Dreieich, Germany), and stored at −20 °C until determination of the change in OPE concentrations and possible formation of transformation products. During the degradation time, the OD600 and TOC of the medium solution were also monitored. The optical density at 600 nm (OD600) of the culture medium was measured using a Shimadzu UV-2700 spectrophotometer (Shimadzu, Kyoto, Japan) to estimate the bacterial concentration. The total organic carbon (TOC) in the electrolytes was determined using a Shimadzu TOC-L analyzer (Shimadzu, Kyoto, Japan).

### 2.3. Chemical analysis

The concentrations of OPEs and the selected metabolites were determined by a Waters Acquity UPLC coupled to a Waters Quattro Premier XE mass spectrometer (UPLC-MS/MS) equipped with a Waters BEH C18 column (100 mm × 2.1 mm, 3.5 μm particle size). DPHP, DNBP, and BBOEP were quantified using MRM acquisition mode with negative mode electrospray ionization (ESI-), whereas TPHP, TNBP, TBOEP, BBOEHP, 3-OH-TBOEP, and 3-OH-TNBP were quantified using positive mode electrospray ionization (ESI+) (Hou et al., 2017). Detailed information, including elution gradient conditions, multiple reaction monitoring (MRM) parameters and QA/QC results, can be found in SI-1 and Table S2.

The transformation products (TPs) of OPEs were identified using ultra-performance liquid chromatography Q-Exactive Orbitrap mass spectrometry (UPLC-Q-Orbitrap MS, Thermo Fisher Scientific, CA, USA) with a Hypersil GOLD C18 analytical column (100 × 2.1 mm, 3 μm; Thermo Fisher Scientific, CA, USA). Full scan (100–450 Da) data acquisition was run at a resolution of 120,000 (at *m/z* 200), and data-dependent MS/MS scans from 50 Da to the highest peak present in each full scan were implemented. Both ESI+ and ESI− modes were used to detect the possible TPs, and each sample was analyzed three times. The MS parameters were set according to our previous study (Hou et al., 2019). The HRMS data were processed using Xcalibur™ version 2.2.1 and Compound Discoverer 2.0 (Thermo Fisher Scientific, CA, USA). Chromatograms were searched for potential TP peaks based on classical oxidation reactions, including hydroxylation, dealkylation, dehydrogenation, carboxylation, epoxidation and sulfur-oxidation (Choi, 2016; Choi and Lee, 2017; Danzl et al., 2009; Kovačič et al., 2019; Murugananthan et al., 2008; Samet et al., 2010; Yang et al., 2019b). The MS/MS fragments were compared using the m/zCloud database.

### 2.4. DNA extraction and PCR amplification

Microbial community analysis was conducted on the seed sludge sample (0 d) and the enriched sludge culture solutions (25 d) containing the individual OPEs. For each chemical, DNA was extracted from triplicate media with an EZNATM Mag-Bind soil DNA kit (OMEGA, GA, USA) according to the manufacturer's instructions. The total genomic DNA extracts were submitted for high-throughput sequencing of 16S rRNA gene amplicons of the V3-V4 region using the primers 341F and 805R. The amplicons were sequenced on an Illumina MiSeq platform by MajorBio-Pharm Technology Co., Ltd. (Shanghai, China). The sequence data for each operational taxonomic unit (OTU) were assigned using Usearch v5.2.236 at a 97% similarity level. In addition, the richness and diversity of microbial communities (ACE, Chao, Simpson, and Shannon indexes) were calculated and analyzed using Mothur version v.1.30.1. The relative abundances of microbes at the phylum and genus levels were determined based on the taxonomic data. The 16S rRNA functional prediction was conducted depending on the Kyoto Encyclopedia of Genes and Genomes (KEGG) by PICRUSt software (Zhou et al., 2019).

### 2.5. Ecotoxicology evolution

The acute ecotoxicity was assessed for samples collected from solutions before and after the kinetic experiments based on the well-described *Vibrio fischeri* assay (Parvez et al., 2006). The control groups only contained 30 mL of OPE-free culture medium. Tests were carried out in duplicate, and the inhibition of luminescence was recorded after 15 min of incubation at 15 °C using a Microtox® Model 500 toxicity analyzer (Carlsbad, CA, USA).

### 2.6. Statistical analyses

The results are expressed as the mean ± standard deviation (SD) (n = 3). Data were evaluated at a 0.05 level of significance with analysis of variance (ANOVA) by Fisher's protected least significant differences procedure.

## 3. Results and discussion

### 3.1. Adaptation of activated sludge culture and degradation kinetics of OPEs

The degradation ratios of TBEOP, TNBP and TPHP over time in the 25-day enrichment cultures from the same sludge inoculum are shown in Fig. 1. No significance ($P > 0.05$) was found for the degradation ratios of TBEOP, TNBP and TPHP in the initial cycle compared to those of the abiotic degradation samples. These results indicated that the combined effects of abiotic degradation (i.e., volatilization, hydrolysis and photolysis) and sorption to autoclaved biomass were negligible (i.e., 4.9%, 5.1% and 12.6% for TBEOP, TNBP and TPHP, respectively). In contrast to the abiotic groups, the initial activated sludge culture obtained from the WWTP without glucose as a cosubstrate was able to significantly degrade TBEOP, TNBP and TPHP ($P < 0.05$), where their initial 5-day degradation ratios were less than 20.7% for all the OPEs. During the adaptation period of 5 cycles (over 25 days), the 5-day degradation ratios for TBEOP, TNBP and TPHP were increased to 33.8%, 20.8% and 67.4%, respectively, without glucose as a co-substrate. The enhancement of OPE biodegradation could be attributed to the adaptation of the microbial community to the substrates. In addition, we set up other cultivation conditions with glucose as a co-substrate to investigate the possible enhancement of OPE degradation under aerobic conditions. The enhancement of pollutant biodegradation by the microbial consortia using the addition of glucose has been presented in several studies (Luo et al., 2019; Noszczyńska and Piotrowska-Seget, 2018; Xu et al., 2018). In the presence of glucose as an additional carbon source, the 5-day degradation ratio of TPHP was observed to be more than 52.4% at the initial cycle and reached 89.9% after 5 cycles of adaptation. The degradation ratios for TBOEP and TNBP were also significantly higher in the presence of glucose than those not in the presence of glucose (Fig. 1) during 5 cycles of adaptation under aerobic conditions. Our findings that additional carbon supplements can enhance the OPE degradation rate and shorten the domestication period were consistent with previous studies on the biodegradation of other organic pollutants (Briones et al., 2018; Fernando et al., 2014; Schymanski et al., 2014; Su et al., 2019), such as polybrominated diphenyl ethers (PBDEs),

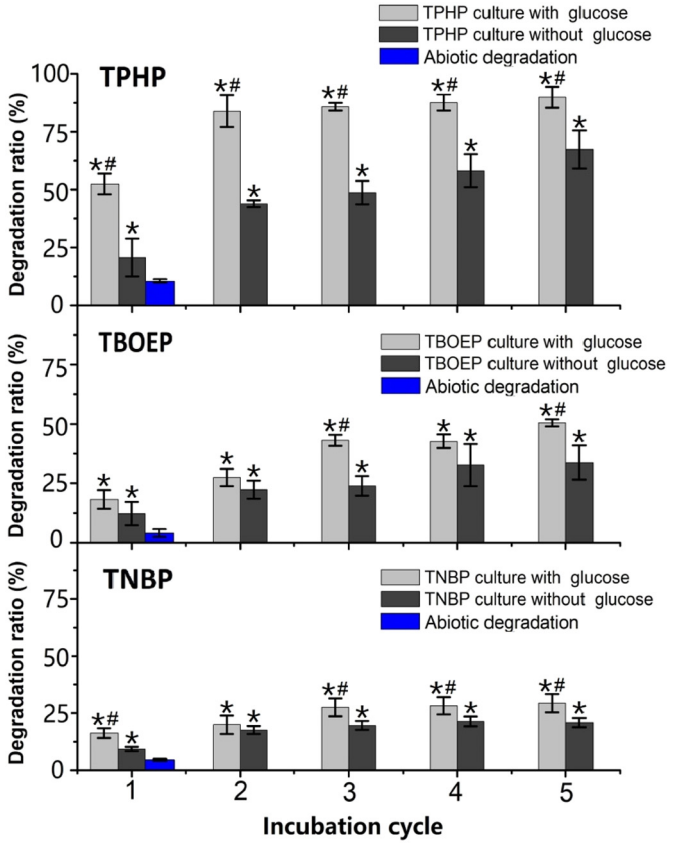



**Fig. 1.** Degradation ratios of TBOEP (A; 3 mg/L), TNBP (B; 3 mg/L) and TPHP (C; 1.5 mg/L) during initial 5 cycles (25 day) of enrichments with individual OPEs and glucose (0 or 1 g/L) as co-substrate (ambient temperature = 28 ± 2 °C, pH = 7.0). Abiotic control was conducted at first incubation cycle in spiking of equivalent autoclaved sludge as inoculum. * indicates significant difference for OPE degradation ratios in aerobic cultures from those in abiotic degradation (one-way ANOVA, $P < 0.05$); and # indicates the significant difference for OPE degradation ratios in aerobic cultures with glucose from those without glucose (one-way ANOVA, $P < 0.05$).

metformin, guanylurea, and chlorophenols. Previous studies also verified that the supplementation of carbon cosubstrates can enhance the degradation efficiency of aromatic compounds in BES (Fan et al., 2017; Luo et al., 2009). The addition of glucose can provide microorganisms with good growth substrates and may increase their potential biodegradation capacity (Milligan and Häggblom, 1998; Zhou et al., 2020).

The enriched sludge cultures containing individual OPEs and incubated for a period of 25 days were used to investigate the biodegradation kinetics of three nonhalogenated OPEs, TBOEP, TNBP and TPHP, in the presence of additional organic carbon sources (Fig. 2). The final elimination efficiency achieved after prolonged incubation of 120 h exceeded 95.4% for both initial TPHP concentrations, and the removal efficiency of TBOEP and TNBP at the low administered concentration (0.3 mg/L) reached more than 67.2% within 196 h of incubation. The enriched microbial culture exhibited a significantly stronger ability to degrade two concentrations of each OPE than the adsorption control. Here, the results confirmed that removal could be attributed primarily to biological degradation by the enriched culture. During the degradation of each OPE, we also found that the microbial biomass sharply increased with an increasing measured optical density from 0.1 to 2.5, whereas TOC measurement confirmed mineralization of the incubation medium with 84.1–92.3% TOC removal after 5 days. The microbial biomass growth indicated that the microbes gradually adjusted to utilize OPEs and glucose as carbon sources for their survival. In a cometabolic system, the easily degradable substrate is preferentially used as an energy and carbon source for microbial growth before the refractory compounds provide sufficient carbon (Uchimiya and Stone, 2006). Accordingly, the co-metabolism of nonhalogenated OPEs and external organic sources could facilitate the degradation of these pollutants, which might be the reason for their lower persistence in bioreaction systems (Reemtsma et al., 2008).

The degradation kinetics of the three OPEs could be best described by a pseudo first-order kinetic model (Table 1). TPHP showed pseudo first-order rate constants ($k$) of 0.023 ± 0.004 $h^{-1}$ and 0.054 ± 0.009 $h^{-1}$ for the TPHP-L and TPHP-H groups, respectively. Remarkably, the degradation rate constants of TPHP are different based on its initial concentration. The big variance in rate constants may be attributed to the limitations of TPHP diffusion into cells and the inhibition of TPHP on bacterial enzymes in TPHP-H group (Wei et al., 2018). The degradation rates for TBOEP and TNBP were much lower than those for TPHP; the degradation rate constants ($k$) were 0.011–0.014 $h^{-1}$ for TBOEP and 0.007–0.009 $h^{-1}$ for TNBP in both concentration groups. The $t_{1/2}$ values of TPHP, TBOEP and TNBP removal were less than 12.8–30.1 h, 49.5–63 h, and 77–99 h, respectively, in which the TPHP degradation kinetics were in the same range as those in several other microbial cultures (Cao et al., 2011b; Chen et al., 2015; Lai et al., 2018; Wei et al., 2018). It is also interesting to note that, at the same administered concentration (0.3 mg/L), the degradation rates ($k$) of the three OPEs followed the order of TPHP > TBOEP > TNBP. This result was in accordance with the reported structure-specific microbial degradability of OPEs by leachate and bioelectrochemical systems (Hou et al., 2019; Kawagoshi et al., 2002). The reported OPE removal efficiencies in WWTPs also showed the trend of aryl-OPEs > alkyl-OPEs > chlorinated OPEs (Fu et al., 2017; Kim et al., 2017; Marklund et al., 2005a; Meyer and Bester, 2004). A previous study showed that the enzymes responsible for TPHP degradation by soil bacterial consortia are intracellular (Chen et al., 2015), and CYP enzymes were confirmed to play an important role in this biodegradation process (Wei et al., 2018). Steric effects that hinder the attack of hydrolases may be responsible for the various biodegradation efficiencies of OPEs with different substituents (Reemtsma et al., 2008).

### 3.2. Biodegradation pathways and transformation product formation

In the case of aerobic incubation, identification of biotransformation for OPEs using UPLC-Q-Orbitrap MS was conducted by matching the observed MS and MS/MS spectra, precursor ion spectra, and accurate mass measurement of the observed transformation products. The corresponding names, molecular ions, retention times, and the proposed structures for the TPs from the degradation of TBOEP, TNBP and TPHP by aerobic cultures are summarized in Table 2. Schymanski et al. proposed levels of certainty in structure confirmation for metabolites ranging from “Level 1” (by reference standard) to “Level 5” (only by exact mass of the candidate) (Wagner et al., 2009). Accordingly, TP names that are bolded represent metabolites that were qualified with reference standards (Level 1 certainty with diagnostic evidence), while those not bolded were tentatively identified with both MS and MS/MS fragmentation data (Level 2 certainty). MS data and MS/MS fragmentation data for all TPs that were confirmed as tentative candidates with Level 2 certainty are provided in Fig. S1.

All OPEs were identified to transform to their respective diesters through hydrolysis, which was reported as the typical pathway for OPE biodegradation (Hou et al., 2019; Van den Eede et al., 2013; Wei et al., 2018). The two hydrolysis TPs of BBOEP and BBOEHEP were degraded via O-dealkylation at the phosphate or butoxyethyl moieties of TBOEP, respectively, and DNBP was identified as the hydrolysis TP of TNBP. For TPHP, except for DPHP, monophenyl phosphate (MPHP) was also detected as an addition TP via hydrolysis of DPHP. In addition, monohydroxylated metabolites were the main stable biodegradation products for the three precursor OPEs. For alkyl-OPEs, hydroxylation was more likely to occur on the third carbon atom of the alkyl substituent, which would be more stable than hydroxylation on other carbon atoms (Van den Eede et al., 2015). In this study, we used standards to

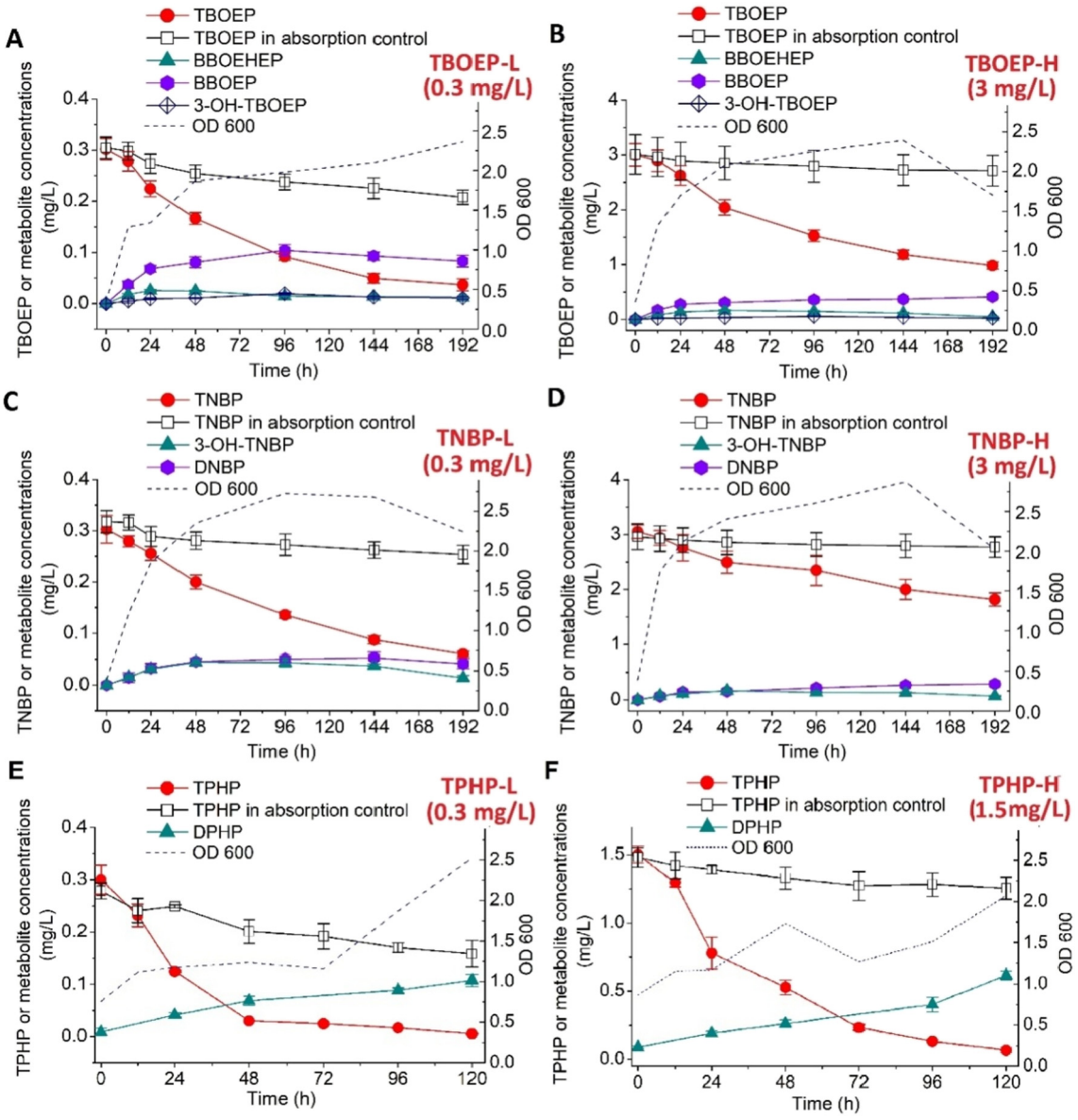


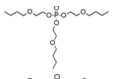
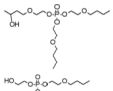
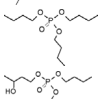
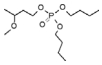
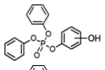

**Fig. 2.** Degradation kinetics of TBOEP (A and B), TNBP (C and D) and TPHP (E and F), and the formation of their most prominent TPs in aerobic biodegradation experiments at low and high initial concentrations, respectively.

confirm the formation of the hydroxylated metabolites of TBOEP and TNBP, namely, 3-OH-TBOEP and 3-OH-TNBP, respectively. Monohydroxylated TPHP (OH-TPHP) was also detected and identified as a product of TPHP, but no information about the exact positions of hydroxylation on benzene was obtained. It is also important to note that the methoxylation metabolites were also detected for TNBP, TBOEP

**Table 1**
The kinetic constant $k$ and $t_{1/2}$ for the OPEs and the conversion percentage of TPs compared to depletion of the respective parent compounds. Data are means ± SD (n = 3).

| OPEs | Metabolites | Low concentration | | | | High concentration | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | OPEs depletion constant $k$ ($h^{-1}$) | $R^2$ | $t_{1/2}$ (h) | Product transformation ratio (%) | OPEs depletion constant $k$ ($h^{-1}$) | $R^2$ | $t_{1/2}$ (h) | Product transformation ratio (%) |
| TBOEP | | 0.014 ± 0.001 | 0.99 | 49.5 | | 0.011 ± 0.001 | 0.99 | 63.0 | |
| | BBOEP | | | | 41.7 | | | | 27.3 |
| | BBOEHEP | | | | 5.4 | | | | 2.7 |
| | 3-OH-TBOEP | | | | 4.1 | | | | 1.1 |
| TNBP | | 0.009 ± 0.001 | 1.0 | 77.0 | | 0.007 ± 0.002 | 0.99 | 99.0 | |
| | DNBP | | | | 21.5 | | | | 29.5 |
| | 3-OH-TNBP | | | | 5.3 | | | | 5.6 |
| TPHP | | 0.054 ± 0.009 | 0.96 | 12.8 | | 0.023 ± 0.004 | 0.99 | 30.1 | |
| | DPHP | | | | 47.8 | | | | 56.4 |

**Table 2**
Summary of the identified transformation products (DPs) of TBOEP, TNBP and TPHP by UPLC-Q-Orbitrap/MS.

| Parent OPEs | RT (min) | Name/abbreviation | ESI | Formula | *m/z* (identified) | *m/z* (theoretical) | Δ *m/z* (ppm) | Proposed structure |
|---|---|---|---|---|---|---|---|---|
| TBOEP | 17.5 | **TBOEP** | + | $C_{18}H_{39}O_7P$ | 399.2511 | 399.2523 | 2.3 |  |
| | 15.9 | **3-OH-TBOEP (TBOEP-TP1)** | + | $C_{18}H_{39}O_8P$ | 415.2460 | 415.2507 | 11.3 |  |
| | 15.1 | **BBOEHEP (TBOEP-TP2)** | + | $C_{14}H_{31}O_7P$ | 343.1885 | 343.1908 | 6.7 |  |
| | 6.8 | **BBOEP (TBOEP-TP3)** | − | $C_{12}H_{27}O_6P$ | 297.1467 | 297.1508 | 13.8 |  |
| | 16.7 | Bis(2-butoxyethyl) methyl phosphate (TBOEP-TP4) | + | $C_{13}H_{27}O_7P$ | 331.1729 | 331.1869 | 42.5 |  |
| TNBP | 17.3 | **TNBP** | + | $C_{12}H_{27}O_4P$ | 267.1725 | 267.1707 | 0.6 |  |
| | 15.0 | **3-OH-TNBP (TNBP-TP1)** | + | $C_{12}H_{27}O_5P$ | 283.1674 | 283.1666 | 2.8 |  |
| | 12.6 | **DNBP (TNBP-TP2)** | − | $C_8H_{19}O_4P$ | 209.0943 | 209.0984 | 19.6 |  |
| | 12.7 | Dibutyl methoxybutyl phosphate (TNBP-TP3) | + | $C_{13}H_{31}O_6P$ | 313.1780 | 313.1814 | 10.8 |  |
| TPHP | 16.6 | **TPHP** | + | $C_{18}H_{15}O_4P$ | 327.0786 | 327.0797 | 3.4 |  |
| | 15.7 | OH-TPHP (TPHP-TP1) | + | $C_{18}H_{15}O_5P$ | 343.0735 | 343.0906 | 49.8 |  |
| | 12.2 | **DPHP (TPHP-TP2)** | − | $C_{12}H_{11}O_4P$ | 249.0317 | 249.0310 | 2.8 |  |
| | 15.2 | Methyl diphenyl phosphate (TPHP-TP3) | + | $C_{12}H_{11}O_7PS$ | 331.0041 | 331.0017 | 7.3 |  |
| | 7.05 | Sulfate conjugate of DPHP (TPHP-TP4) | + | $C_{13}H_{13}O_4P$ | 265.0630 | 265.0708 | 29.4 |  |
| | 10.1 | MPHP (TPHP-TP5) | − | $C_6H_7O_4P$ | 173.0004 | 172.9976 | 16.2 |  |

and TPHP, namely, dibutyl methoxybutyl phosphate (TNBP-TP3), bis (2-butoxyethyl) methyl phosphate (TBOEP-TP4) and methyl diphenyl phosphate (TPHP-TP3), as the important products of biotransformation under aerobic conditions. The methoxylated TPs found were best explained by methylation at the hydroxyl moiety of the OH-OPEs (Lai et al., 2018). In addition, TPHP-TP4 was identified from the pathway of sulfate conjugation of DPHP and was detected for the first time under aerobic conditions in this study.

As evident from the identified TPs, it was suggested that biodegradation of the nonhalogenated OPEs occurred via cleavage of a series of bonds by mixed aerobic microbes, where the prevalent biotransformation pathways were hydrolysis, hydroxylation, methoxylation, and substitution (summarized in Fig. 3). The OPEs could be synchronously converted via hydrolysis and hydroxylation pathways; then, the hydroxylated intermediates might be further converted to products by methyltransferases. The aerobic biodegradation pathways for TBOEP and TNBP were first investigated in the present study, but similar TPHP degradation pathways were previously proposed from confirmed metabolites in studies using different bacterial isolates (Lai et al., 2018; Wei et al., 2018). Our previous study using a bioelectrochemical system also presented the phenol-cleavage and hydroxylation pathways of TPHP degradation (Hou et al., 2019). In that study, further hydroxylation, hydrocarboxylation or benzene cleavage also partially occurred in the former TPs of TPHP (Hou et al., 2019). However, in this study, those further oxidation products were not detected at all, which may be because some degradation steps can be so fast that intermediate metabolite concentrations are too low to observe.

Considering the significance of hydrolysis and hydroxylation pathways for OPE degradation, we also investigated the production kinetics of several of these TPs. The aqueous concentrations of BBOEP, BBOEHEP, 3-OH-TBOEP, DNBP, 3-OH-TNBP, and DPHP in the enriched cultures were continuously monitored during aerobic incubation of their precursor OPEs, as shown in Fig. 2. None of the six TPs were detected in the culture medium at the beginning of incubation. For the low-concentration groups of TBOEP and TNBP, the concentrations of all the TPs rapidly increased within the first 48 h and achieved their maximum values at 96 h, whereas BBOEP and DNBP were observed in the high-concentration groups. However, the increasing concentrations of DPHP did not reach a steady state even at the end of the incubation period (120 h) in the two TPHP groups.

Moreover, product transformation ratios were calculated as the molar ratio of formed TPs to the depletion of their precursor compounds at the end of incubation (Table 1). BBOEP accounted for 21.3–43.4% of depleted TBOEP, while the transformation ratios for BBOEHEP and 3-OH-TBOEP were only 2.7–5.4% and 1.1–4.1%, respectively, in both concentration groups. DNBP was confirmed to be the most prominent TP of TNBP (with a transformation ratio of 21.5–29.5%), whereas a relatively minor contribution (5.3–5.6%) was made by 3-OH-TNBP. These results indicated that di-alkyl phosphates (DAPs) are the predominant TPs in the aerobic degradation of alkyl-OPEs by bacterial consortia. In

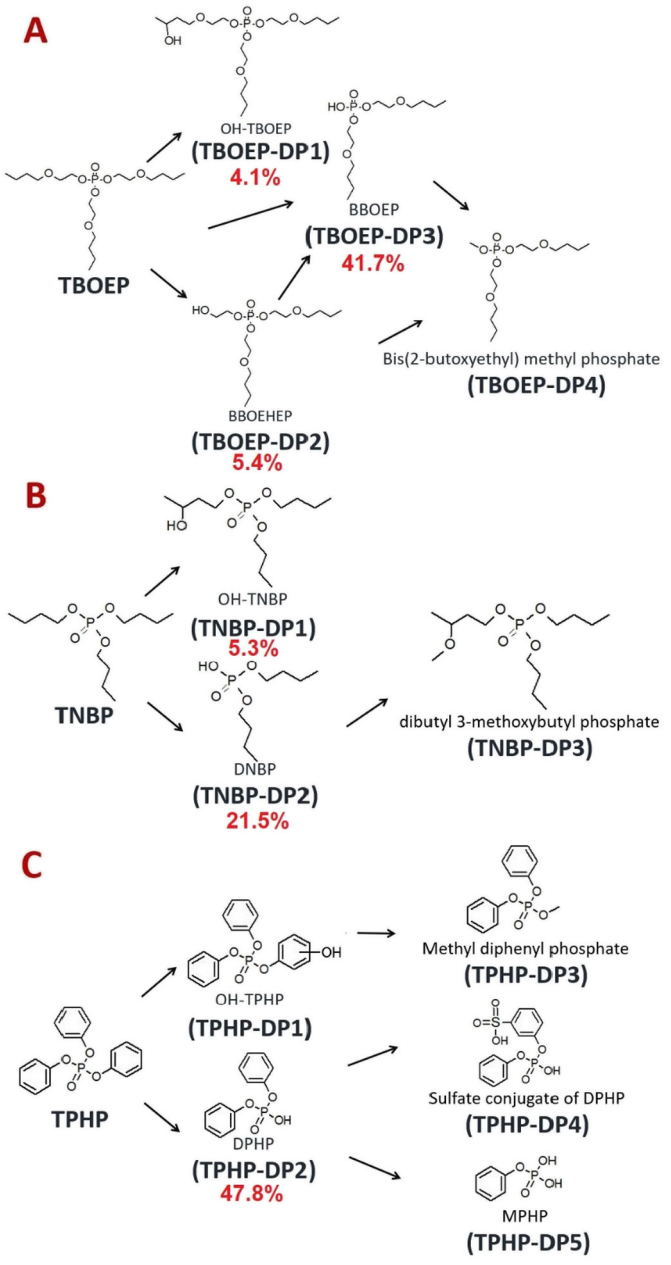



**Fig. 3.** Proposed pathway of the investigated aerobic degradation of TBOEP (A), TNBP (B) and TPHP (C) by sludge cultures in this study. Mean conversion percentages of the metabolites compared to depletion of the respective parent compounds after degradation were added with the structures.

addition, DPHP was also quantified as an important TP of TPHP, which accounted for 47.8–56.4% of the removed TPHP after 96 h of incubation. These data are consistent with the reported relatively larger conversion ratio of DPHP in microbial degradation of TPHP by *B. brevis* under aerobic conditions (Wei et al., 2018). Our findings represent a strong indication that hydrolysis is a critical biodegradation pathway for nonhalogenated OPEs and that di-alkyl phosphates (DAPs) might play an important role in the overall mass balance of these compounds in WWTPs. Studies have reported the occurrence of the DAPs in effluents and sewage sludge from WWTPs from United States and China (Fu et al., 2017; Gao et al., 2016; Kim et al., 2017; Li et al., 2020; Wang et al., 2019). Higher concentrations of several DAPs (i.e., DPHP and DNBP) were found in the effluents compared to in the influents of WWTP from Anhui, China, which confirmed their production from OPEs during wastewater treatment processes (Li et al., 2020). In a nationwide survey of sludge across China, the nonhalogenated DAPs were revealed to be mainly derived from biodegradation processes in WWTPs (Fu et al., 2017). Wang et al. (2019) found that BBOEP was the dominant DAP in sludge from the United States and estimated the annual emissions rates of DAPs to be 663–796 kg/year through wastewater discharges from the United States. Considering the certain formation efficiency for DAPs in WWTPs and their significant discharges, further studies are needed to assess the environmental occurrence and fate of DAPs in the aquatic environment.

### 3.3. Microbial communities in the degradation of OPEs

The initial sludge sample and the enriched sludge cultures containing individual OPEs and cultured for 25 days were collected, and the bacterial diversity was analyzed by Illumina high-throughput sequencing. The raw data generated from these samples contained from 3577 to 4320 OTUs. The average length, coverage index, Chao index, Shannon index, Simpson index, and OTU values are shown in Table S2. The coverage index exceeded 95.1% for all the samples, which represented the coverage degree of the constructed sequence libraries of the microbial diversity. Overall, the Shannon diversity index of the initial sludge sample was higher than that of the three enriched cultures containing individual OPEs. This result indicated that the bacterial community was simplified when acclimated to the different OPEs.

The results of the bacterial community at the phylum, class and genus levels in the aerobic sludge and enriched cultures containing individual OPEs are presented in Fig. 4. The bacterial community structures for all the sludge culture samples at the phylum level were highly similar, and the abundant bacterial phyla (relative abundance >2%) were *Proteobacteria* (45.5–83.8%), *Bacteroidetes* (9.9–36.2%), and *Firmicutes* (4.5–17.4%). At the class level, *Gammaproteobacteria*, *Alphaproteobacteria*, *Bacteroidia*, *Negativicutes*, *Flavobacteriia*, and *Clostridia* were the dominant classes (relative abundance >2%). As the two most abundant bacterial phyla in the initial sludge samples, *Alphaproteobacteria* and *Flavobacteriia* were apparently decreased in the enriched sludge samples containing individual OPEs (from 41.3% to 11.3–25.7% and from 22.1% to 1.7–4.7%, respectively). Furthermore, we observed considerable increases in the relative abundance of *Gammaproteobacteria* in sludge cultures enriched with TBOEP and TNBP and the relative abundance of *Bacteroidia* in sludge cultures enriched with TBOEP and TPHP.

At the genus level, *Azospirillum* and *Chryseobacterium*, which belong to *Alphaproteobacteria* and *Flavobacteriia*, respectively, were the two most abundant genera, accounting for 30.3% and 22.0% in the initial sludge, respectively. *Azospirillum* was reported to be the dominant denitrifier genus in biotreatment processes (Wei et al., 2019), and *Chryseobacterium* is a commonly detected genus in activated sludge (Noda, 2012). For the enriched sludge cultures, the abundance of *Klebsiella* belonging to *Gamaproteobacteria* greatly increased from 2.2% to 25.2%, 29.7% and 68.2% for the sludge containing TPHP, TBOEP or TNBP, respectively, and *Klebsiella* could be responsible for the degradation of these OPEs (Figs. 4 and S2). *Klebsiella* exhibited high activities of organophosphorus hydrolase and ligninolytic enzymes (Sasikala et al., 2012; Yang et al., 2019a), and this genus was reported to participate in the degradation of aromatic compounds, organophosphorus, phthalate esters and many other organic compounds (Comte et al., 2006; Sasikala et al., 2012; Zhang and Fang, 2001; Zhang et al., 1999). In addition, the genera *Paludibacter* (relative abundance of 10.4%) and *Dysgonomonas* (9.6%) were also highly detected in sludge cultures containing TPHP. Based on previous studies, *Paludibacter* plays an important role in denitrification or biodegradation of pyridine and azo dyes in the treatment of industrial wastewater (Cao et al., 2011; Sheng et al., 2010), and *Dysgonomonas* is involved in the degradation of azo dyes and antibiotics (Fernando et al., 2020; Yan et al., 2018). Thus, such various functional microbes in TPHP-enriched culture may have

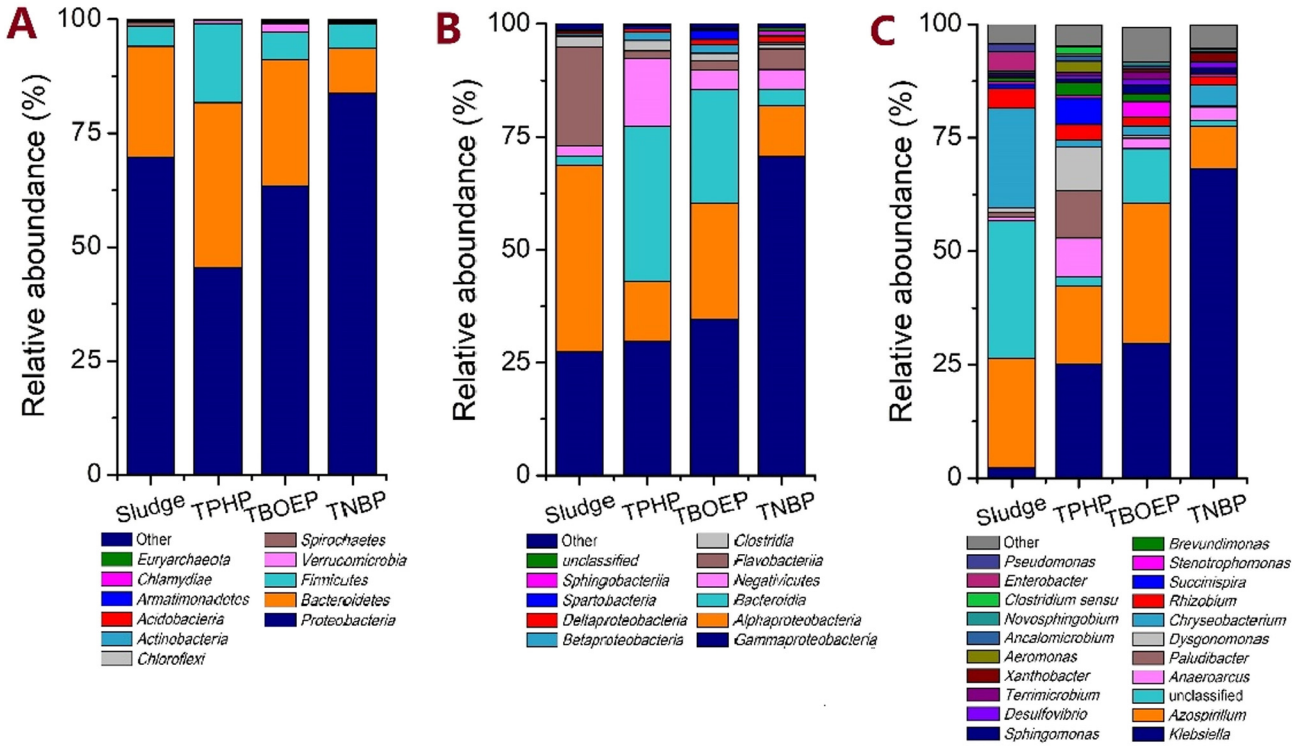



**Fig. 4.** Changes of bacterial structure at the phylum (A), class (B), and genus (C) levels in the aerobic sludge and enriched cultures by individual OPEs.

resulted in its relatively high degradation efficiency in the present study.

### 3.4. Ecotoxicology evaluation

Luminescence by *Vibrio fischeri* is a sensitive and easy-to-use endpoint for identifying ecotoxicity patterns in various types of modified water bodies (Bhatia et al., 2013). In our experiments, luminescence inhibition of *Vibrio Fischeri* was evaluated by culture medium before and after the aerobic degradation processes of TPHP, TBOEP, and TNBP (Fig. 5). The initial culture medium of 1.5 mg/L TPHP showed the highest luminescence inhibition of 19.6%, while 3 mg/L TBOEP or TNB showed luminescence inhibition percentages of 7.4% and 7.5%,

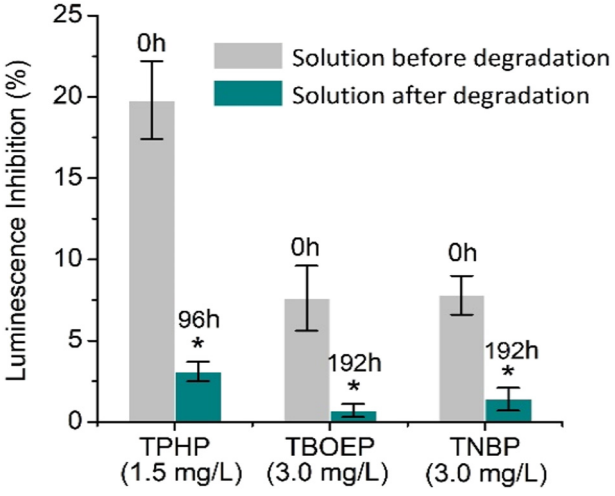



**Fig. 5.** Ecotoxicological evaluation of biotransformation of TBOEP, TNBP, and TPHP as reflected by luminescence inhibition ratios of *Vibrio fischeri* bacteria.

respectively. These results were within the same ranges of the values assessed using *Vibrio qinghaiensis* sp. -Q67 (Kang et al., 2014). After incubation, a marked decrease in acute toxicity (with inhibition less than 2.9%) coincided with the removal of the precursor OPEs, which indicated that the formed TPs were less toxic than the precursor compounds.

Current information on the toxicity of OPE transformation products is relatively rare. A recent study stated that no significant cytotoxic effects for chicken embryonic hepatocytes were observed for DPHP concentrations up to 1000 μM (Su et al., 2014). Kojima et al. also reported that DPHP may have limited nuclear receptor activity compared to that of its precursor triesters, and neither DNBP nor BBOEP exhibit any nuclear receptor activity, although the precursor compounds showed androgen receptor (AR) and glucocorticoid receptor (GR) antagonistic activity (Kojima et al., 2016). The lower toxicity of the degradation products than of the precursor compounds can be attributed to their low Log octanol-water partition coefficient (Log $K_{OW}$) (Su et al., 2014). The Log $K_{OW}$ values as well as other physicochemical properties were also predicted for all the TPs using USEPA EPI suit v4.1. The predicted Log $K_{OW}$ values for the evaluated substances were ≤4.22 (Table S3), which indicated their comparably limited potential for bioaccumulation. All the surveyed metabolites had lower Log $K_{OW}$ values but higher water solubility than those of their precursor chemicals. This finding indicated that the aerobic degradation of alkyl-OPEs resulted in more polar products (Table S3), which led to less preferential accumulation in aquatic animals and humans (Hou et al., 2019b; Wang et al., 2017). However, recent studies have identified that hydroxylated TPs, such as OH-TPHP, BBOEHEP and 3-OH-TBOEP, act as strong endocrine disruptors via nuclear receptors (Kojima et al., 2016). Therefore, further investigation of the formation of these intermediate products of OPE degradation and their ecotoxicological risks is definitely worthwhile.

## 4. Conclusion

Our study demonstrated that microbial cultures could readily degrade TBOEP, TNBP and TPHP under aerobic conditions after long-

term pollution as substrates in conventional activated sludge from WWTPs. It was evident that the addition of easily degradable carbon (glucose) shortened the acclimatization periods of the microbial cultures and enhanced the degradation performance of the three nonhalogenated OPEs. The degradation rates ($k$) of the three OPEs followed the order of TPHP > TBOEP > TNBP, but their relatively low half-lives ($t_{1/2}$) demonstrated that these nonhalogenated OPEs were easily degradable by sludge microbes under aerobic conditions. The aerobic biodegradation pathways were elucidated for the nonhalogenated OPEs and involved hydrolysis, hydroxylation, methoxylation, and substitution. For the first time, we quantitatively confirmed hydrolysis as the primary degradation pathway of nonhalogenated OPEs in activated sludge. The microbial community composition of sludge cultures was altered after acclimatization, and *Klebsiella* was found to be responsible for the degradation of all the nonhalogenated OPEs. Even though the corresponding degradation DAP products largely accumulated in the culture medium after incubation, a significant reduction in their harmful effects relative to those of the precursor OPEs occurred in the biotransformation process based on their toxicity to *Vibrio fischeri* antibiotic activity. Such results revealed aerobic biodegradation by active sludge as an ecotoxicologically favorable process for nonhalogenated OPE degradation.

## CRediT authorship contribution statement

**Rui Hou:** Investigation, Conceptualization, Methodology, Formal analysis, Validation, Writing - original draft, Funding acquisition. **Yi Wang:** Resources, Validation. **Shaofeng Zhou:** Resources, Visualization. **Lihua Zhou:** Data curation, Funding acquisition, Project administration. **Yong Yuan:** Conceptualization, Data curation, Writing - review & editing, Supervision, Funding acquisition. **Yiping Xu:** Resources, Methodology, Supervision.

## Declaration of competing interest

The authors declared that they have no conflicts of interest to this work.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.143385.

## References

Andresen, J.A., Grundmann, A., Bester, K., 2004. Organophosphorus flame retardants and plasticisers in surface waters. Sci. Total Environ. 332, 155–166.

Bhatia, D., Bourven, I., Simon, S., Bordas, F., van Hullebusch, E.D., Rossano, S., et al., 2013. Fluorescence detection to determine proteins and humic-like substances fingerprints of exopolymeric substances (EPS) from biological sludges performed by size exclusion chromatography (SEC). Bioresour. Technol. 131, 159–165.

Brandsma, S.H., Leonards, P.E.G., Leslie, H.A., de Boer, J., 2015. Tracing organophosphorus and brominated flame retardants and plasticizers in an estuarine food web. Sci. Total Environ. 505, 22–31.

Briones, R.M., Zhuang, W.-Q., Sarmah, A.K., 2018. Biodegradation of metformin and guanylurea by aerobic cultures enriched from sludge. Environ. Pollut. 243, 255–262.

Cao, B., Ahmed, B., Kennedy, D.W., Wang, Z., Shi, L., Marshall, M.J., et al., 2011. Contribution of extracellular polymeric substances from Shewanella sp. HRCR-1 biofilms to U(VI) immobilization. Environ. Sci. Technol. 45 (13), 5483–5490.

Cao, B., Shi, L., Brown, R.N., Xiong, Y., Fredrickson, J.K., Romine, M.F., et al., 2011b. Extracellular polymeric substances from Shewanella sp. HRCR-1 biofilms: characterization by infrared spectroscopy and proteomics. Environ. Microbiol. 13, 1018–1031.

Cao, S., Zeng, X., Song, H., Li, H., Yu, Z., Sheng, G., et al., 2012. Levels and distributions of organophosphate flame retardants and plasticizers in sediment from Taihu Lake, China. Environ. Toxicol. Chem. 31, 1478–1484.

Chen, W., Habibul, N., Liu, X.-Y., Sheng, G.-P., Yu, H.-Q., 2015. FTIR and synchronous fluorescence heterospectral two-dimensional correlation analyses on the binding characteristics of copper onto dissolved organic matter. Environ. Sci. Technol. 49 (4), 2052–2058.

Choi, Y., 2016. Distribution and Degradation of Bisphenol A (BPA) Substitutes BPAF and BPS Compared to BPA in Aerobic Soil and Anaerobic. Purdue University.

Choi, Y., Lee, L., 2017. Aerobic soil biodegradation of bisphenol (BPA) alternatives bisphenol S and bisphenol AF compared to BPA. Environ. Sci. Technol. 51 (23), 13698–13704.

Chung, H.-W., Ding, W.-H., 2009. Determination of organophosphate flame retardants in sediments by microwave-assisted extraction and gas chromatography–mass spectrometry with electron impact and chemical ionization. Anal. Bioanal. Chem. 395, 2325–2334.

Comte, S., Guibaud, G., Baudu, M., 2006. Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties: part I. Comparison of the efficiency of eight EPS extraction methods. Enzym. Microb. Technol. 38, 237–245.

Cristale, J., Katsoyiannis, A., Sweetman, A.J., Jones, K.C., Lacorte, S., 2013. Occurrence and risk assessment of organophosphorus and brominated flame retardants in the River Aire (UK). Environ. Pollut. 179, 194–200.

Danzl, E., Sei, K., Soda, S., Ike, M., Fujita, M., 2009. Biodegradation of bisphenol A, bisphenol F and bisphenol S in seawater. Int. J. Environ. Res. Public Health 6 (4), 1472–1484.

ECHA, 2008a. EU RISK ASSESSMENT-tris(1,3-dichloro-2-propyl)phosphate TCPP CAS 13674-84-5. URL. https://echa.europa.eu/documents/10162/13630/trd_rar_ireland_tccp_en.pdf.

ECHA, 2008b. EU RISK ASSESSMENT-tris(2-chloro-1-(chloromethyl)ethyl) phosphate TDCP CAS 13674-87-8. URL. https://echa.europa.eu/documents/10162/13630/trd_rar_ireland_tccp_en.pdf.

Fan, M., Zhou, N., Li, P., Chen, L., Chen, Y., Shen, S., et al., 2017. Anaerobic co-metabolic biodegradation of tetrabromobisphenol A using a bioelectrochemical system. J. Hazard. Mater. 321, 791–800.

Fernando, E., Keshavarz, T., Kyazze, G., 2014. Complete degradation of the azo dye Acid Orange-7 and bioelectricity generation in an integrated microbial fuel cell, aerobic two-stage bioreactor system in continuous flow mode at ambient temperature. Bioresour. Technol. 156, 155–162.

Fernando, I., Lu, D., Zhou, Y., 2020. Interactive influence of extracellular polymeric substances (EPS) and electrolytes on the colloidal stability of silver nanoparticles. Environ. Sci. Nano 7, 186–197.

Fu, L., Du, B., Wang, F., Lam, J.C.W., Zeng, L., Zeng, E.Y., 2017. Organophosphate triesters and diester degradation products in municipal sludge from wastewater treatment plants in China: spatial patterns and ecological implications. Environ. Sci. Technol. 51 (23), 13614–13623.

Gao, L., Shi, Y., Li, W., Liu, J., Cai, Y., 2016. Occurrence and distribution of organophosphate triesters and diesters in sludge from sewage treatment plants of Beijing, China. Sci. Total Environ. 544, 143–149.

Giulivo, M., Capri, E., Kalogianni, E., Milacic, R., Majone, B., Ferrari, F., et al., 2017. Occurrence of halogenated and organophosphate flame retardants in sediment and fish samples from three European river basins. Sci. Total Environ. 586, 782–791.

Hou, R., Luo, X., Liu, C., Zhou, L., Wen, J., Yuan, Y., 2019. Enhanced degradation of triphenyl phosphate (TPHP) in bioelectrochemical systems: kinetics, pathway and degradation mechanisms. Environ. Pollut. 254, 1–8.

Hou, R., Xu, Y., Wang, Z., 2016. Review of OPFRs in animals and humans: absorption, bioaccumulation, metabolism, and internal exposure research. Chemosphere 153, 78–90.

Hou, R., Liu, C., Gao, X., Xu, Y., Zha, J., Wang, Z., 2017. Accumulation and distribution of organophosphate flame retardants (PFRs) and their di-alkyl phosphates (DAPs) metabolites in different freshwater fish from locations around Beijing, China. Environ. Pollut. 229, 548–556.

Hou, R., Huang, C., Rao, K., Xu, Y., Wang, Z., 2018. Characterized in vitro metabolism kinetics of alkyl organophosphate esters in fish liver and intestinal microsomes. Environ. Sci. Technol. 52 (2), 3202–3210.

Hou, R., Yuan, S., Feng, C., Xu, Y., Rao, K., Wang, Z., 2019b. Toxicokinetic patterns, metabolites formation and distribution in various tissues of the Chinese rare minnow (Gobiocypris rarus) exposed to tri(2-butoxyethyl) phosphate (TBOEP) and tri-n-butyl phosphate (TNBP). Sci. Total Environ. 668, 806–814.

Kang, F., Alvarez, P.J., Zhu, D., 2014. Microbial extracellular polymeric substances reduce Ag+ to silver nanoparticles and antagonize bactericidal activity. Environ. Sci. Technol. 48 (1), 316–322.

Karpouzas, D.G., Singh, B.K., 2006. Microbial degradation of organophosphorus xenobiotics: metabolic pathways and molecular basis. In: Poole, R.K. (Ed.), Advances in Microbial Physiology. 51. Academic Press, pp. 119–225.

Kawagoshi, Y., Nakamura, S., Fukunaga, I., 2002. Degradation of organophosphoric esters in leachate from a sea-based solid waste disposal site. Chemosphere 48, 219–225.

Kim, U.-J., Oh, J.K., Kannan, K., 2017. Occurrence, removal, and environmental emission of organophosphate flame retardants/plasticizers in a wastewater treatment plant in New York State. Environ. Sci. Technol. 51 (14), 7872–7880.

Kojima, H., Takeuchi, S., Van den Eede, N., Covaci, A., 2016. Effects of primary metabolites of organophosphate flame retardants on transcriptional activity via human nuclear receptors. Toxicol. Lett. 245, 31–39.

Kovačič, A., Gys, C., Kosjek, T., Covaci, A., Heath, E., 2019. Photochemical degradation of BPF, BPS and BPZ in aqueous solution: identification of transformation products and degradation kinetics. Sci. Total Environ. 664, 595–604.

Lai, C.-Y., Dong, Q.-Y., Chen, J.-X., Zhu, Q.-S., Yang, X., Chen, W.-D., et al., 2018. Role of extracellular polymeric substances in a methane based membrane biofilm reactor reducing vanadate. Environ. Sci. Technol. 52 (18), 10680–10688.
Lalovic, B., Phillips, B., Risler, L.L., Howald, W., Shen, D.D., 2004. Quantitative contribution of CYP2D6 and CYP3A to oxycodone metabolism in human liver and intestinal microsomes. Drug Metab. Dispos. 32, 447–454.
Li, Y., Yao, C., Zheng, Q., Yang, W., Niu, X., Zhang, Y., et al., 2020. Occurrence and ecological implications of organophosphate triesters and diester degradation products in wastewater, river water, and tap water. Environ. Pollut. 259, 1–11.
Liu, X., Ji, K., Choi, K., 2012. Endocrine disruption potentials of organophosphate flame retardants and related mechanisms in H295R and MVLN cell lines and in zebrafish. Aquat. Toxicol. 114–115, 173–181.
Luo, H., Liu, G., Zhang, R., Jin, S., 2009. Phenol degradation in microbial fuel cells. Chem. Eng. J. 147, 259–264.
Luo, C., Hou, R., Chen, G., Liu, C., Zhou, L., Yuan, Y., 2019. UVC-assisted electrochemical degradation of novel bisphenol analogues with boron-doped diamond electrodes: kinetics, pathways and eco-toxicity removal. Sci. Total Environ. 711, 1–11.
Marklund, A., Andersson, B., Haglund, P., 2005a. Organophosphorus flame retardants and plasticizers in air from various indoor environments. J. Environ. Monit. 7, 814–819.
Marklund, A., Andersson, B., Haglund, P., 2005. Organophosphorus flame retardants and plasticizers in Swedish sewage treatment plants. Environ. Sci. Technol. 39 (19), 7423–9429.
Martínez-Carballo, E., González-Barreiro, C., Sitka, A., Scharf, S., Gans, O., 2007. Determination of selected organophosphate esters in the aquatic environment of Austria. Sci. Total Environ. 388, 290–299.
McGoldrick, D.J., Letcher, R.J., Barresi, E., Keir, M.J., Small, J., Clark, M.G., et al., 2014. Organophosphate flame retardants and organosiloxanes in predatory freshwater fish from locations across Canada. Environ. Pollut. 193, 254–261.
Meyer, J., Bester, K., 2004. Organophosphate flame retardants and plasticisers in wastewater treatment plants. J. Environ. Monit. 6, 599–605.
Milligan, P.W., Häggblom, M.M., 1998. Biodegradation of resorcinol and catechol by denitrifying enrichment cultures. Environ. Toxicol. Chem. 17, 1456–1461.
Murugananthan, M., Yoshihara, S., Rakuma, T., Shirakashi, T., 2008. Mineralization of bisphenol A (BPA) by anodic oxidation with boron-doped diamond (BDD) electrode. J. Hazard. Mater. 154, 213–220.
Noda, I., 2012. Close-up view on the inner workings of two-dimensional correlation spectroscopy. Vib. Spectrosc. 60, 146–153.
Noszczyńska, M., Piotrowska-Seget, Z., 2018. Bisphenols: application, occurrence, safety, and biodegradation mediated by bacterial communities in wastewater treatment plants and rivers. Chemosphere 201, 214–223.
Pantelaki, I., Voutsa, D., 2019. Organophosphate flame retardants (OPFRs): a review on analytical methods and occurrence in wastewater and aquatic environment. Sci. Total Environ. 649, 247–263.
Parvez, S., Venkataraman, C., Mukherji, S., 2006. A review on advantages of implementing luminescence inhibition test (Vibrio fischeri) for acute toxicity prediction of chemicals. Environ. Int. 32, 265–268.
Porter, E., Crump, D., Egloff, C., Chiu, S., Kennedy, S.W., 2014. Use of an avian hepatocyte assay and the avian Toxchip Polymerse chain reaction array for testing prioritization of 16 organic flame retardants. Environ. Toxicol. Chem. 33, 573–582.
Reemtsma, T., Quintana, J.B., Rodil, R., Garcia Lopez, M., Rodriguez, I., 2008. Organophosphorus flame retardants and plasticizers in water and air I. occurrence and fate. TrAC Trends Anal. Chem. 27, 727–737.
Rodil, R., Quintana, J.B., López-Mahía, P., Muniategui-Lorenzo, S., Prada-Rodríguez, D., 2009. Multi-residue analytical method for the determination of emerging pollutants in water by solid-phase extraction and liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1216, 2958–2969.
Samet, Y., Agengui, L., Abdelhédi, R., 2010. Electrochemical degradation of chlorpyrifos pesticide in aqueous solutions by anodic oxidation at boron-doped diamond electrodes. Chem. Eng. J. 161, 167–172.
Sasikala, C., Jiwal, S., Rout, P., Ramya, M., 2012. Biodegradation of chlorpyrifos by bacterial consortium isolated from agriculture soil. World J. Microbiol. Biotechnol. 28, 1301–1308.
Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., et al., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ. Sci. Technol. 48 (4), 2097–2098.
Sheng, G.-P., Yu, H.-Q., Li, X.-Y., 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechnol. Adv. 28, 882–894.
Shi, Y., Gao, L., Li, W., Wang, Y., Liu, J., Cai, Y., 2016. Occurrence, distribution and seasonal variation of organophosphate flame retardants and plasticizers in urban surface water in Beijing, China. Environ. Pollut. 209, 1–10.
Su, G., Crump, D., Letcher, R.J., Kennedy, S.W., 2014. Rapid in vitro metabolism of the flame retardant triphenyl phosphate and effects on cytotoxicity and mRNA expression in chicken embryonic hepatocytes. Environ. Sci. Technol. 48 (22), 13511–13519.
Su, G., Letcher, R.J., Moore, J.N., Williams, L.L., Martin, P.A., de Solla, S.R., et al., 2015. Spatial and temporal comparisons of legacy and emerging flame retardants in herring gull eggs from colonies spanning the Laurentian Great Lakes of Canada and United States. Environ. Res. 142, 720–730.
Su, C., Lu, Y., Deng, Q., Chen, S., Pang, G., Chen, W., et al., 2019. Performance of a novel ABR-bioelectricity-Fenton coupling reactor for treating traditional Chinese medicine wastewater containing catechol. Ecotoxicol. Environ. Saf. 177, 39–46.
Terzic, S., Udikovic-Kolic, N., Jurina, T., Krizman-Matasic, I., Senta, I., Mihaljevic, I., et al., 2018. Biotransformation of macrolide antibiotics using enriched activated sludge culture: kinetics, transformation routes and ecotoxicological evaluation. J. Hazard. Mater. 349, 143–152.
Uchimiya, M., Stone, A.T., 2006. Aqueous oxidation of substituted dihydroxybenzenes by substituted benzoquinones. Environ. Sci. Technol. 40 (11), 3515–3521.
Van den Eede, N., Maho, W., Erratico, C., Neels, H., Covaci, A., 2013. First insights in the metabolism of phosphate flame retardants and plasticizers using human liver fractions. Toxicol. Lett. 223, 9–15.
Van den Eede, N., Erratico, C., Exarchou, V., Maho, W., Neels, H., Covaci, A., 2015. In vitro biotransformation of tris(2-butoxyethyl) phosphate (TBOEP) in human liver and serum. Toxicol. Appl. Pharmacol. 284, 246–253.
Van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88, 1119–1153.
Wagner, M., Ivleva, N.P., Haisch, C., Niessner, R., Horn, H., 2009. Combined use of confocal laser scanning microscopy (CLSM) and Raman microscopy (RM): investigations on EPS – matrix. Water Res. 43, 63–76.
Wang, R., Tang, J., Xie, Z., Mi, W., Chen, Y., Wolschke, H., et al., 2015. Occurrence and spatial distribution of organophosphate ester flame retardants and plasticizers in 40 rivers draining into the Bohai Sea, north China. Environ. Pollut. 198, 172–178.
Wang, G., Chen, H., Du, Z., Li, J., Wang, Z., Gao, S., 2017. In vivo metabolism of organophosphate flame retardants and distribution of their main metabolites in adult zebrafish. Sci. Total Environ. 590, 50–59.
Wang, Y., Kannan, P., Halden, R.U., Kannan, K., 2019. A nationwide survey of 31 organophosphate esters in sewage sludge from the United States. Sci. Total Environ. 655, 446–453.
Wei, K., Yin, H., Peng, H., Lu, G., Dang, Z., 2018. Bioremediation of triphenyl phosphate by Brevibacillus brevis: degradation characteristics and role of cytochrome P450 monooxygenase. Sci. Total Environ. 627, 1389–1395.
Wei, L., Li, J., Xue, M., Wang, S., Li, Q., Qin, K., et al., 2019. Adsorption behaviors of Cu2+, Zn2+ and Cd2+ onto proteins, humic acid, and polysaccharides extracted from sludge EPS: sorption properties and mechanisms. Bioresour. Technol. 291, 1–9.
WHO, 1991a. Tributyl phosphate. Environmental Health Criteria 112 [R]. World Health Organization, Geneva.
WHO, 1991b. Triphenyl phosphate. Environmental Health Criteria 111 [R]. World Health Organization, Geneva.
Woudneh, M.B., Benskin, J.P., Wang, G., Grace, R., Hamilton, M.C., Cosgrove, J.R., 2015. Quantitative determination of 13 organophosphorous flame retardants and plasticizers in a wastewater treatment system by high performance liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1400, 149–155.
Xu, H., Tong, N., Huang, S., Zhou, S., Li, S., Li, J., et al., 2018. Degradation of 2,4,6-trichlorophenol and determination of bacterial community structure by microelectrical stimulation with or without external organic carbon source. Bioresour. Technol. 263, 266–272.
Xu, L., Hu, Q., Liu, J., Liu, S., Liu, C., Deng, Q., et al., 2019. Occurrence of organophosphate esters and their diesters degradation products in industrial wastewater treatment plants in China: implication for the usage and potential degradation during production processing. Environ. Pollut. 250, 559–566.
Yan, W., Sun, F., Liu, J., Zhou, Y., 2018. Enhanced anaerobic phenol degradation by conductive materials via EPS and microbial community alteration. Chem. Eng. J. 352, 1–9.
Yang, G., Huang, L., Yu, Z., Liu, X., Chen, S., Zeng, J., et al., 2019a. Anode potentials regulate Geobacter biofilms: new insights from the composition and spatial structure of extracellular polymeric substances. Water Res. 159, 294–301.
Yang, T., Wang, L., Liu, Y., Huang, Z., He, H., Wang, X., et al., 2019b. Comparative study on ferrate oxidation of BPS and BPAF: kinetics, reaction mechanism, and the improvement on their biodegradability. Water Res. 148, 115–125.
Zhang, T., Fang, H.H.P., 2001. Quantification of extracellular polymeric substances in biofilms by confocal laser scanning microscopy. Biotechnol. Lett. 23, 405–409.
Zhang, X., Bishop, P.L., Kinkle, B.K., 1999. Comparison of extraction methods for quantifying extracellular polymers in biofilms. Water Sci. Technol. 39, 211–218.
Zhou, H., Zhang, D., Jiang, Z., Sun, P., Xiao, H., Yuxin, W., et al., 2019. Changes in the soil microbial communities of alpine steppe at Qinghai-Tibetan Plateau under different degradation levels. Sci. Total Environ. 651, 2281–2291.
Zhou, Y., Zou, Q., Fan, M., Xu, Y., Chen, Y., 2020. Highly efficient anaerobic co-degradation of complex persistent polycyclic aromatic hydrocarbons by a bioelectrochemical system. J. Hazard. Mater. 381, 1–10.