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Research Paper

Removal pathway quantification and co-metabolic mechanism evaluation of alkylphenols from synthetic wastewater by phenolic root exudates in the rhizosphere of *Phragmites australis*

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ABSTRACT

Phenolic root exudates (PREs) released from wetland plants are potentially effective for accelerating the biodegradation of alkylphenols, yet the inherent behavior is still unclear. In this study, two representative root exudates (REs), namely *p*-coumaric acid (PREs) and oxalic acid (non-PREs) were exogenously added as specific and non-specific co-metabolic substrates, respectively, to elucidate the quantification of each removal pathway and degradation mechanism of co-metabolism for alkylphenols (i.e. *p-tert*-butylphenol (PTBP)) from synthetic wastewater. The results showed that soil adsorption (31–37%), microbial degradation (27–37%), and plant uptake (16–41%) are the main removal pathways of PTBP by PREs in the *Phragmites australis* rhizosphere. Both REs enriched anaerobic functional community (anaerobic ammonium oxidation bacteria and denitrifying bacteria) and promoted the usage of PTBP as carbon source and/or electron donor. The activity of non-specific enzyme (polyphenol oxidase) was enhanced by RE which owning a significant positive correlation with bacterial abundance, whereas only PREs strengthened the activity of specific enzyme (monophenol oxidase) catalyzing the phenolic ring hydroxylation of PTBP followed by a dehydrogenation route. Moreover, exogenous PREs significantly improved the growth of degrading-related bacteria (*Sphingomonas* and *Gemmatimonas*), especially in unplanted soils with high activity of dioxygenase catalyzing the cleavage pathway of PTBP, instead of plant presence.

1. Introduction

Alkylphenols are important industrial materials that are widely used in the production of synthetic resins and surfactants. Because of the extensive application, the wastewater of Alkylphenols is directly discharged through manufacturing process and wastewater effluent into aquatic environment (Ko et al., 2007), which has been frequently detected in seawater, river water, and sewage treatment plant (Koh et al., 2006; Ogata et al., 2013; Xu et al., 2016). As typical environmental endocrine disruptors, Alkylphenols have estrogen activity, bioaccumulation, and biological toxicity (Ismail et al., 2018; Klein et al., 2005; Kühn et al., 1989), posing a serious threat to ecological stability, food safety, and even human health (Sun et al., 2008). However, such persistent organic pollutants cost a long period of time on natural decomposition. The efficient degradation of those compounds depends on microbial co-metabolism (Nsenga Kumwimba and Meng, 2019).

Phenolic root exudates (PREs) secreted by wetland plants are potentially effective for the biodegradation of aromatic compounds (Donnelly et al., 1994; Leigh et al., 2002). Toyama et al. (2011) found that the root exudate (REs) of *Phragmites australis* containing phenolic compounds supported growth as a carbon source for the *Mycobacterium* strain tested, and induced benzo[α]pyrene-degrading activity of the strain. Hoang et al. (2010) observed that *Spirodela polyrrhiza* has a great ability to release phenolic compound-rich REs, which seem to stimulate

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bacterial degradation of aromatic compounds. Our previous studies (A et al., 2020, 2017) also demonstrated that PREs may accelerate the biodegradation of Alkylphenols in the *Phragmites australis* rhizosphere and eventually enhance their removal, especially *p*-coumaric acid (PCA), whereas the inherent behavior is still unclear. Hence, PCA was selected as a specific substrate in this study to investigate the removal pathway and co-metabolic mechanism of Alkylphenols by PREs.

Additionally, oxalic acid (OA) released from plants is a typical low-molecular-weight organic acid, which cultivated with microorganism in a 60 mL-brown bottle at 25 °C for 21 days to effectively reduce phenanthrene pollution in the soil by positively mediating shifts in microbial communities (Li et al., 2019). Therefore, OA was chosen as a non-specific substrate in this study to compare the influence of Alkylphenols reduction with specific substrates (PREs).

In this study, a series of experiments were carried out by using *p-tert*-butylphenol (PTBP) as the representative Alkylphenols pollutant and using different representative REs as the co-metabolic substrates. PCA is one of representative PREs produced by reed, which has similar structure with target pollutant/ intermediate metabolite; OA is a widely common organic acid which easy to use by microorganisms, PCA (PREs) and OA (non-PREs) were as the specific and non-specific co-metabolic substrates, respectively. In the treatment performance experiments, bench-scale reed beds treating synthetic wastewater were used to investigate the quantification of each removal pathway of PTBP by exogenous REs under various impact factors, i.e. RE/PTBP ratio, RE presence, RE type, and plant presence. In the rhizosphere effect experiments, soil sorption and biodegradation test, plant uptake test, microbial abundance and diversity analyses, and enzyme activity analyses were studied to clarify the microbial response and enzymatic mechanism on PTBP-PREs system. To the best of our knowledge, it is the first time that the quantified removal pathway and microbial co-metabolic mechanism of aromatic compounds such as Alkylphenols by PREs has been reported. The knowledges obtained from this study will be helpful not only for the further research of co-metabolism between aromatic compounds and PREs, but also for the development application of bioremediation on the contamination of aromatic compounds.

2. Materials and methods

2.1. Setup of reed beds and synthetic wastewater

Eight bench-scale reed beds were set up in a phytotron in Nov. 2018 at Sun Yat-sen University, in Guangzhou City, South China, as depicted in Fig. 1. Each bed consisted of plastic column (20 cm diameter, 60 cm height), and was filled from bottom to top with pumice stones (1.5–3.0 cm diameter, 5 cm depth) and pollution-free soils (0.5 cm diameter, 50 cm depth), while the height of the submerged layer of wastewater above the soils was 2 cm approximately. Six of eight beds were planted with common reed (*Phragmites australis*, 338–774 g and 47–63 cm), and the others were unplanted. These emergent plants were purchased from a local market. Seedlings of common reed were planted with density of 1 plant/bed in Dec. 2018 and grown by synthetic wastewater before the treatment effect experiment. The conditions of phytotron were kept as 25 ℃ temperature, 70% humidity, 12 h/d light (06:00–18:00), and 15,000 lux light intensity, to simulate the local tropical and subtropical climate. Synthetic wastewater was used for treatment performance experiment, and the composition is in Table A.1.

2.2. Bench-scale experiment for treatment performance

Four pairwise comparative groups were conducted using reed beds, while each group had two repetitions (Fig. 1). Among them, two exogenously applied REs, i.e. PCA and OA, were added respectively to group PCA (beds 1–2) and group OA (beds 3–4), to compare the influence of different RE types on PTBP removal; blank control group (group CK, beds 5–6) without REs addition were performed, to estimate the effect of REs presence; unplanted control group (group UP, beds 7–8) with PCA addition were also performed, to evaluate the effect of plant presence. Synthetic wastewater with 10 mg/L PTBP was fed as the raw influent during the experiment. Since the addition of 50 mg/L phenolic root exudates (PREs) was demonstrated to be effective for accelerating the PTBP degradation in our previous study (A et al., 2020), 10–100 mg/L REs were used here to further investigate the influence of different strength ratios between exogenous RE and target PTBP. Three strengths of REs were performed, i.e. 10 mg/L, 50 mg/L, and 100 mg/L, corresponding to three RE/PTBP ratios, i.e. 1:1, 5:1, and 10:1, respectively.

After a stabilization period of half a year (Jan.–Jun. 2019) for plant growth using synthetic wastewater, the treatment performance experiment was conducted during Jul.–Aug. 2019. On the first day of a batch cycle, raw influent (2.5 L) was poured into each bed. After a batch cycle (2 days), treated water was drained for 10 min from the beds, and then fresh influent was poured into the beds. This batch treatment was repeated for all the beds in every 2 days. All the beds were operated at a specific RE/PTBP ratio for two weeks, while influent (0 h) and effluent (3, 12, 24, 48 h) samples were collected during the last three cycles when the beds had stabilized under the particular RE/PTBP ratio.

Water temperature (WT), pH, and oxidation-reduction potential (ORP) were measured simultaneously by a pH meter (FE28-Standard,

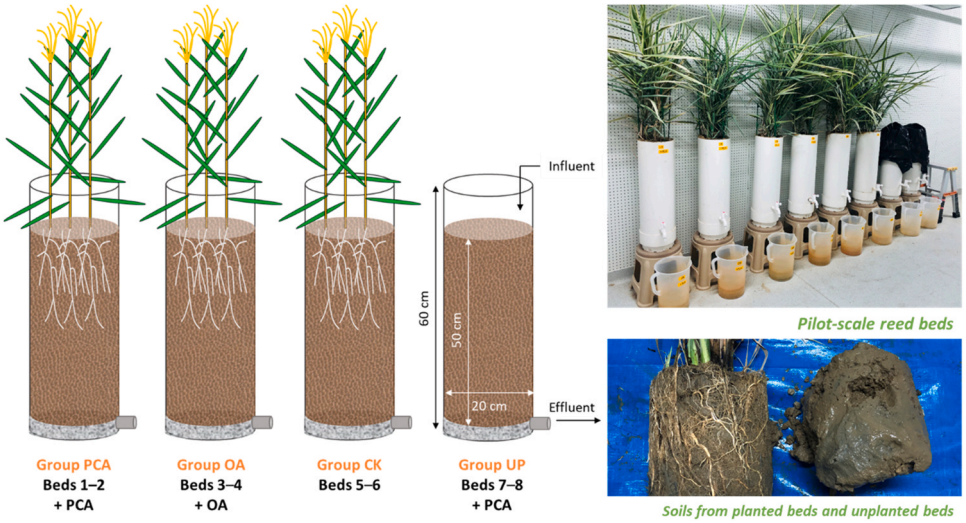

Fig. 1. Schematic diagram (left) and photo (right) of bench-scale reed beds. Common reed (*Phragmites australis*) was planted in beds 1–6.

Mettler Toledo, Switzerland). Ammonia nitrogen (NH_4-N) concentrations were determined by the indophenol method. Total organic carbon (TOC) concentrations were determined by a TOC analyzer (TOC-VCPH, Shimadzu, Japan). PTBP concentrations were determined by a high-performance liquid chromatography (LC-10A series, Shimadzu, Japan) (Ogata et al., 2013).

Removal rate constant of pollutants (k, /d) was calculated as:

$$k = \frac{lnC_1 - lnC_2}{t_2 - t_1}$$

where C_t (mg/L) stands for the concentration of pollutants in the water sample at time t.

2.3. Lab-scale experiment for rhizosphere effect

2.3.1. Sampling of soils, plants, and microbes

After the treatment performance experiment, all the beds were resolved as soon as possible (Fig. 1). The soil samples of the planted and unplanted beds were collected from the rhizosphere and from the upper layer (15 cm from the top), respectively. The emergent plants (853–1252 g and 100–140 cm) were also entirely harvested and gently cleaned by tap water. Then, root tissue samples were collected and washed thoroughly by ultrapure water. The soil samples were used for soil sorption and biodegradation test, microbial abundance and diversity analyses, and enzyme activity analyses; and the plant tissue samples were used for plant uptake test, as explained below.

2.3.2. Soil sorption and biodegradation test

The soil sample (10 g) collected from the planted bed or the unplanted bed was added to a 500 mL capped flask containing 250 mL microbial mineral basal medium (A et al., 2017) (the composition is in Table A.2) with 10 mg/L PTBP as the sole carbon source. Flasks were incubated on a rotary shaker (25 ℃ and 120 rpm) for two days. Sterile control experiments (after autoclaving) were also performed simultaneously. The biodegradation test was obtained by non-sterile treatment, and the soil sorption effect was acquired from the sterile control experiments. Water samples (2 mL) were collected periodically (0, 3, 9, 24, 48 h) from all the flasks for chemical analyses (PTBP and TOC).

2.3.3. Plant uptake test

The sterile root tissue sample (10 g) collected from the emergent plant was added to a 500 mL capped flask containing 250 mL autoclaved plant inorganic nutrient solution (the composition is in Table A.3) with 10 mg/L PTBP as the sole carbon source. Flasks were incubated on a rotary shaker (25 ℃ and 120 rpm) for two days. Water samples (2 mL) were collected periodically (0, 3, 9, 24, 48 h) from all the flasks for chemical analyses (PTBP and TOC). Preparation methods of plant inorganic nutrient solutions and sterile plants were described elsewhere (A et al., 2020).

Then, the root tissue sample was transferred to a 100 mL centrifuge tube containing 50 mL ultrapure water. Centrifuge tubes were treated with an ultrasonic cleaning machine (KQ-700TDE, Kunshan Shumei, China) for 1 min and then with a vortex oscillator (VORTEX-5, Kylin -Bell, China) for 1 min, which was repeated for six times. Eventually, water samples (1 mL) were collected from all the tubes for chemical analyses (PTBP).

2.3.4. Microbial abundance and diversity analyses

Total microbial DNA was extracted from the soil samples using a Soil Genomic DNA Extraction Kit (Tiangen biotech (Beijing) Co., Ltd., China) according to the manufacturer's instructions. We used the primer set 341F (5′–CCTAYGGGRBGCASCAG–3′) and 806R (5′–GGACTACHVGGGTWTCTAAT–3′) that was designed to amplify V3–V4 hypervariable regions of bacterial 16S rRNA gene, while ITS1 region primers (ITS5-1737F and ITS2-2043R) were used for fungal ITS rRNA gene. Then, the library was constructed using the Ion Plus Fragment Library Kit (48 rxns) (Thermo Fisher, USA). The constructed library was subjected to Qubit quantification and library testing, and then sequenced using Ion S5™XL (Thermo Fisher, USA). Raw data were demultiplexed, quality-filtered, and analyzed using the QIIME (version 1.9.1) (Quantitative Insights In to Microbial Ecology) bioinformatics pipeline (Caporaso et al., 2010), in addition to custom Perl scripts to analyze alpha diversity. All of the reads were quality-filtered by QIIME quality filters (Min et al., 2017). Sequences with ≥ 97% similarity were assigned to the same operational taxonomic units. The low abundance operational taxonomic units were eliminated from the operational taxonomic unit table if they did not have a total of at least 2 counts across all the samples in the experiment (Jiao et al., 2016).

2.3.5. Enzyme activity analyses

PTBP-related degrading enzymes, i.e. monophenol oxidase, polyphenol oxidase, monooxygenase, and dioxygenase, were collected with soil samples from the planted or unplanted beds. Soil samples were treated using a specific ELISA kit (Shanghai Huding Biotechnology, China) according to the manufacturer's instructions, and then their corresponding enzyme activities were determined using a microplate reader (SpectraMax M2, Molecular Devices, USA) at 450 nm.

2.4. Statistical analysis

The software package SPSS (Statistic Package for Social Science; version 13.0) was used for statistical data analysis. Differences and correlations between two variables were analyzed using the one-way ANOVA and Pearson's coefficient, respectively.

3. Results

3.1. Treatment performance experiment

Common reeds grew well with roots flourishing throughout the experiment (Fig. 1), and their biomass growth (weight and length) are presented in Fig. A.1. The total increases of plant weight and length were 489–778 g and 55–87 cm, respectively, which was ranked in the following order: group OA > group CK > group PCA.

3.1.1. Physicochemical parameters

Time courses of physicochemical parameters, i.e. WT, pH, and ORP, in the reed beds at different RE/PTBP ratios are presented in Fig. A.2.

The WT, pH, and ORP values in all the reed beds were, respectively, warm (22.8–24.9 ℃), neutral (6.59–7.54), and anaerobic (148–212 mV) conditions. No significant difference ($p > 0.05$) was found in WT between influent (23.5–24.7 ℃) and effluent (22.8–24.9 ℃) among all the groups for simulating a subtropical climate, i.e. 25 ± 1 ℃ air temperature during the whole experiment. With the increasing ratios of RE/PTBP, the pH values of influent decreased notably in group OA from 6.58 to 3.77, and reduced slightly in group PCA from 6.67 to 6.29. However, all the reed beds presented pH-neutralizing capacities (6.59–7.54) against both weak acidic PCA and strong acidic OA, possibly owing to the buffering capacity of the soil (Nelson et al., 2010). Since the saturated flow of reed beds, anaerobic conditions were maintained in all the groups, with relatively low ORP (148–212 mV) that is beneficial to the activity of denitrifying bacteria (Dušek et al., 2008).

3.1.2. Chemical pollutants

Time courses of concentrations for chemical pollutants, i.e. NH_4-N, TOC, and PTBP, in the reed beds at different RE/PTBP ratios are also presented in Fig. A.3, and their removal rates among four groups and three RE/PTBP ratios are depicted in Fig. 2.

At RE/PTBP ratio of 1:1, no significant difference ($p > 0.05$) was observed in the removal rates of NH_4-N, TOC, and PTBP among all the groups, with the values of 0.28–0.69 /d, 0.09–0.21 /d, and 0.81–1.08 /d, respectively.

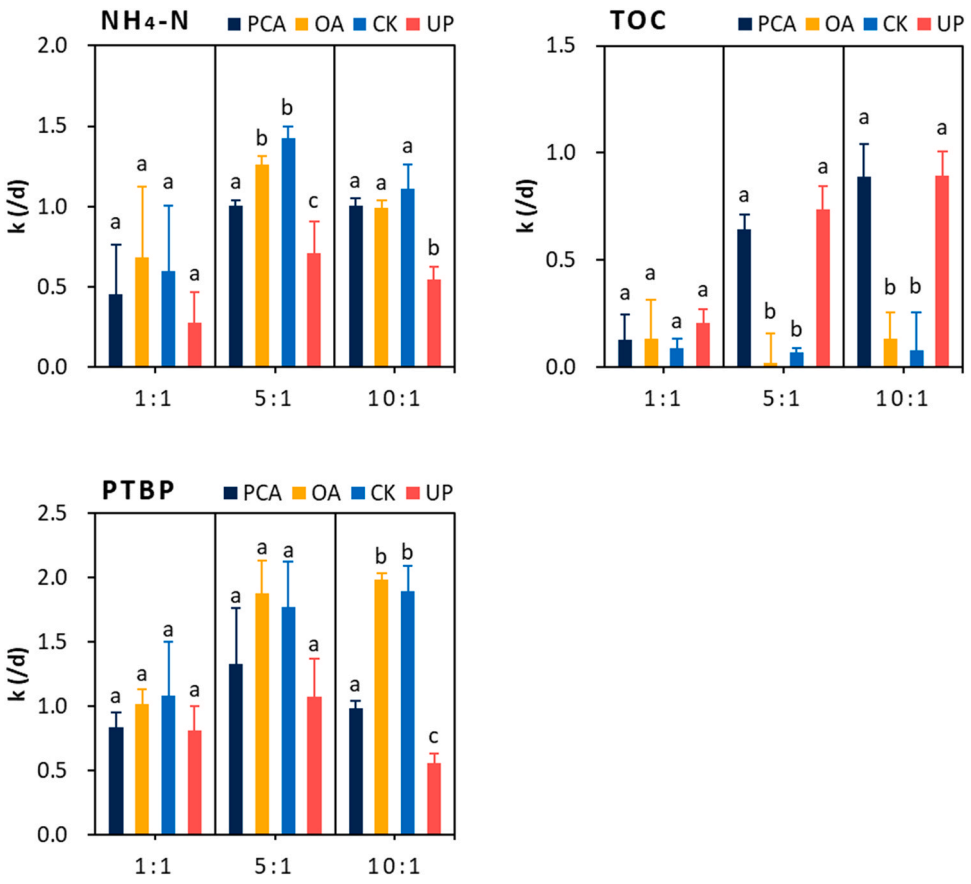

Fig. 2. Removal rate constant (k) of NH_4-N, TOC, and PTBP in the planted and unplanted beds at different RE/PTBP ratios (1:1, 5:1, and 10:1) ($n = 6$). Different letters on the top of the column indicate significant differences of pollutant removal rate constant among different groups according to a one-way ANOVA at $p < 0.05$.

At RE/PTBP ratio of 5:1, the removal rates of NH_4-N were higher in the planted beds (groups PCA, OA, CK) (1.01–1.43 /d) than in the unplanted beds (group UP) (0.71 /d), in the -PRE beds (groups OA, CK) (1.26–1.43 /d) than in the +PRE beds (groups PCA, UP) (0.71–1.01 /d); and the removal rates of TOC were higher in the +PRE beds (0.65–0.74 /d) than that in the -PRE beds (0.02–0.07 /d); PTBP was markedly removed by all the reed beds with a relatively high removal rate of 1.07–1.88 /d.

At RE/PTBP ratio of 10:1, the planted beds (0.99–1.11 /d) removed NH_4-N more efficiently than the unplanted beds (0.54 /d); and the +PRE beds (0.89 /d) removed TOC more efficiently than the -PRE beds (0.08–0.13 /d); PTBP was removed more efficiently in the planted beds (0.99–1.98 /d) than in the unplanted beds (0.55 /d), in the -PRE beds (1.89–1.98 /d) than in the +PRE beds (0.55–0.99 /d).

In summary, NH_4-N was efficiently removed in all the groups at different RE/PTBP ratios (42–94%), while the NH_4-N removals were higher in the planted beds (56–94%) than in the unplanted beds (42–68%), as well as at the high RE/PTBP ratios (66–94%) than at the low RE/PTBP ratio (42–72%). TOC could be efficiently removed in the +PRE beds at high RE/PTBP ratios (72–83%), but inefficiently removed in the -PRE beds (4–24%) or at low RE/PTBP ratio (16–34%). PTBP was greatly removed in all the reed beds at different RE/PTBP ratios (67–100%), with the highest removal of 88–98% at RE/PTBP ratio of 5:1. This is because a certain content of growth substrates can stimulate the release of key enzymes by functional bacteria for co-metabolizing hardly-degrading organic pollutants, but the excess growth substrates will cause competitive inhibition that may reduce the removal rate of contaminants (Wang et al., 2019). Time courses of decreasing concentrations for chemical pollutants in the reed beds at different RE/PTBP ratios was very similar, and significant correlations ($p < 0.05$) were found among the NH_4-N, TOC, and PTBP removals, indicating that the inherent interaction of their attenuation.

3.2. Rhizosphere effect experiment

3.2.1. The effect of soil sorption and biodegradation

Time courses of concentrations for PTBP and TOC in the liquid phase in the soil sorption and biodegradation test are portrayed in Figs. 3a and A.4, respectively. The organic pollutants were removed from the liquid phase by both adsorption onto soil with biofilm collected from the reed beds and degradation by biofilm microbes in the test, but only by adsorption onto soil in the sterilized control test.

The PTBP removals were comparable by soils collected from each treatment group, whereas PTBP was removed more efficiently in the unsterilized test (62–71%) than in the sterilized test (28–35%). No intermediate was detected by high-performance liquid chromatography analysis in the PTBP-mineral basal medium inoculated with soil samples of each group (data not shown), suggesting the occurrence of aromatic ring cleavage. However, several intermediates of PTBP were commonly detected by high-performance liquid chromatography analysis in the sterilized test (data not shown), indicating the incompletely decomposition without microbial influence.

3.2.2. The effect of plant uptake

Time courses of concentrations for PTBP and TOC in the liquid phase in the plant uptake test are given in Figs. 3b and A.5, respectively. Among them, the mass removal amount of PTBP after the plant uptake and the subsequent ultrasound-oscillation treatment were used as the total PTBP removal content and the root-absorbed PTBP component, respectively; and the difference between two of them was used as the water-soluble PTBP component.

About 38–41% of PTBP was removed by plants collected from the -PRE beds (groups OA, CK), which was mainly reduced by plant uptake (77–83%) and partially adsorbed onto the root surface (17–23%).

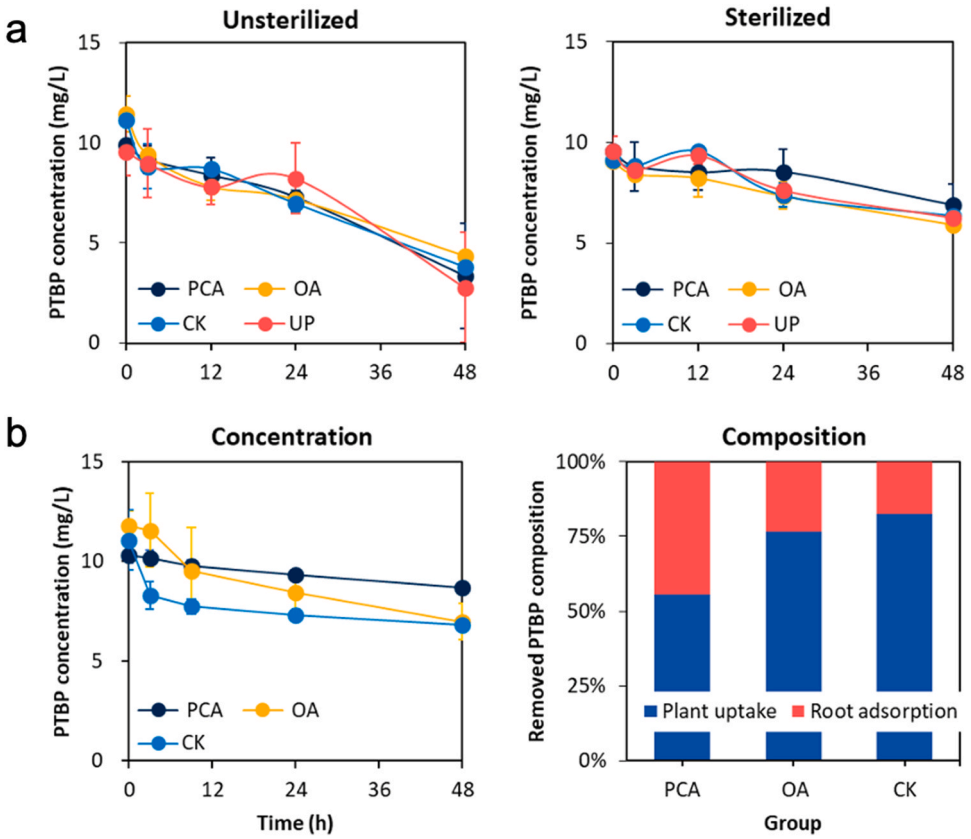

Fig. 3. The results of soil sorption and biodegradation test and plant uptake test. (a) Adsorption and biodegradation of PTBP by soils collected from the planted and unplanted beds at unsterilized and sterilized conditions ($n = 2$). (b) Uptake of PTBP by emergent plants (*Phragmites australis*) collected from the planted beds at sterilized condition ($n = 2$).

However, only 16% of PTBP was removed by plants collected from the +PRE beds (group PCA), which was evenly eliminated by both the plant uptake (56%) and root sorption (44%). This suggests plants may play a certain role in PTBP removal for -PREs beds but not for +PRE beds.

3.2.3. Soil microbial abundance and diversity

Chao1 index and Shannon index were used to represent microbial abundance and diversity, respectively, of soil collected from the reed beds, as depicted in Fig. 4a.

The bacterial abundance and diversity were larger in the +RE beds (groups PCA, OA, UP) (1055–1191 of abundance and 8.46–9.06 of diversity) than in the -RE beds (group CK) (524 of abundance and 6.86 of diversity), indicating the addition of REs may promote bacterial growth and enrich their composition. Generally speaking, bacterial populations in the planted systems is significantly larger than those in the unplanted system (A et al., 2017). However, no significant difference was found in bacterial community between planted and unplanted beds in this study, suggesting the presence of REs is the key factor affecting the growth and activity of rhizosphere bacteria, whereas the exogenous REs may make up the lack of plants.

The fungi abundance and diversity of all reed beds were comparable, indicating that the changes of fungi in each reed bed were relatively stable under the influence of REs.

3.2.4. Soil enzyme activity

The activities of PTBP degrading-related enzymes, i.e. monophenol oxidase and polyphenol oxidase according to the objects, and monooxygenase and dioxygenase according to the functions, as presented in Fig. 4b.

Monophenol oxidase activities were markedly higher in the +PRE beds (groups PCA, UP) than that in -PRE beds (groups OA, CK); while polyphenol oxidase activities were notably higher in the +RE beds (groups PCA, OA, UP) than in the -RE beds (group CK). No significant difference was found in monooxygenase activity among different treatment groups; the best performance of dioxygenase activity was observed in the unplanted beds (group UP). Furthermore, significant positive correlations ($p < 0.05$) were found between polyphenol oxidase activity and bacterial abundance, and between monooxygenase activity and fungal abundance.

4. Discussion

4.1. Removal behavior quantification

Generally, substrate sorption and biological degradation are considered to be the major processes affecting the removal of organic contaminants in wetland systems (Stefanakis et al., 2014). Moreover, processes such as plant uptake, photochemical oxidation, and volatilization may be relevant for the chemical structure and chemical-physical properties of organic compounds (Imfeld et al., 2009).

PTBP could be removed by both the soil adsorption and microbial degradation, with the removal percentage of 31–37% and 27–37%, respectively (Fig. 3a). The PTBP eliminations were comparable by soils collected from different reed beds (Fig. 3a), due to the steady physicochemical properties of soils (i.e. pH, Eh and TOC) (Fig. A.6) determining the adsorption capacity of organic pollutants (Schwarzenbach and Westall, 1981). Like our previous findings (A et al., 2020, 2017), PTBP was efficiently degraded from liquid phase by rhizosphere microbes of *Phragmites australis* (Fig. 3a); unlike that, PTBP was removed by the unplanted beds (group UP) as well as the planted beds (groups PCA, OA, CK) (Fig. 2). This indicates not only phenolic compounds secreted from plant roots can accelerate the biodegradation of PTBP in the rhizosphere (Toyama et al., 2011; Hoang et al., 2010), but also the exogenous PREs in the unplanted

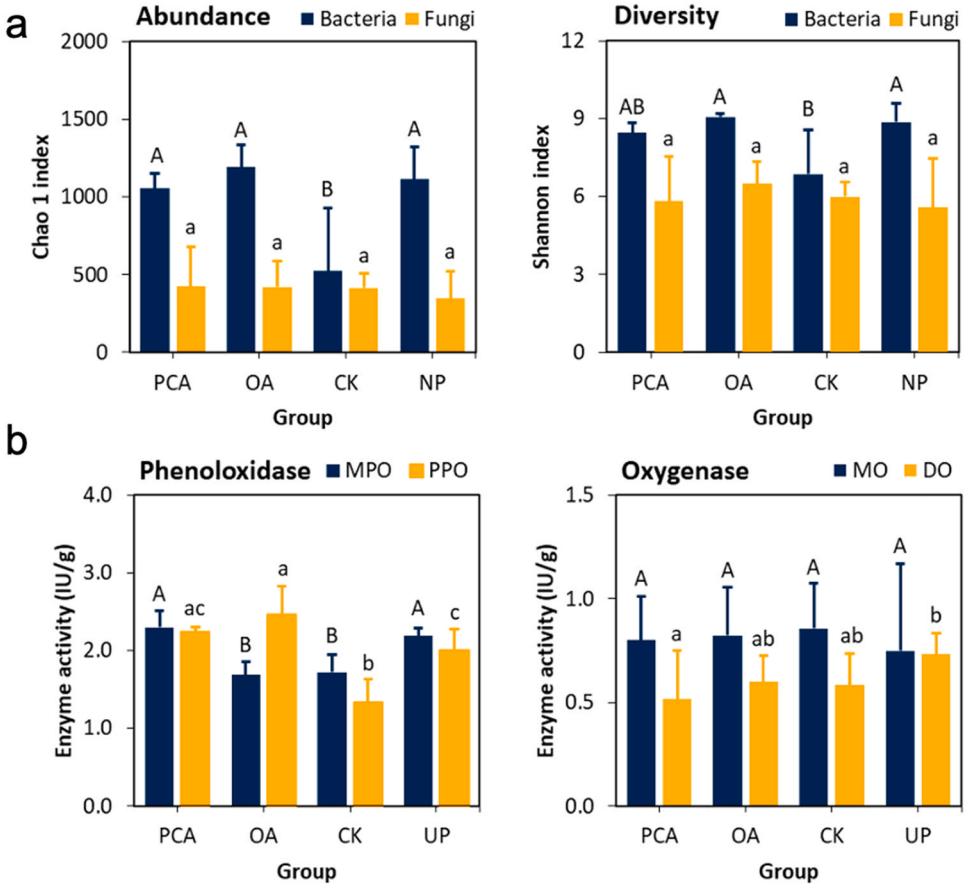

Fig. 4. The results of microbial abundance and diversity and soil enzyme activity. (a) Abundance and diversity of bacteria and fungi of soils collected from the planted and unplanted beds ($n = 3$). (b) Activity of monophenol oxidase (MPO), polyphenol oxidase (PPO), monooxygenase (MO), and dioxygenase (DO) of soils collected from the planted and unplanted beds ($n = 3$). Different letters on the top of the column indicate significant differences of microbial index or enzyme activity among different groups according to a one-way ANOVA at $p < 0.05$.

soil may also enrich the PTBP degrading-related bacteria (Fig. 4a), and therefore enhance the co-metabolism effect of PRE-PTBP system.

Plant uptake is expected to play an important role on the PTBP removal, due to that the octanol-water partition ($\mathrm{Log}K_{ow}$) implies PTBP ($\mathrm{Log}K_{ow} = 3.31$) is able to be significantly taken up through the plant cell membrane (Imfeld et al., 2009). The efficient removal of PTBP by plant uptake was observed in the -PRE beds (groups OA, CK) (38–41%) but not in the +PRE beds (group PCA) (16%) (Fig. 3b), which is accordance with their growth trend of plant biomass (Fig. A.1). Among them, 9–32% and 7–9% of total PTBP were removed via root uptake and root sorption, respectively (Fig. 6). Furthermore, the capacity of plant uptake for organic chemicals will be impacted by the plant species as well (Serra et al., 2019); hence, the removal of PTBP by PREs will be further enhanced by using wetland plants with strong tolerance to phenols.

Normally, REs secreted from plants by contaminant stimulation, resulting in a release of key enzymes by microbes for promoting biodegradation of pollutants (Xie et al., 2012). However, even in the lack of plant effect (group PCA) or plant presence (group UP), the +PRE beds still presented comparable removal efficiencies of PTBP as the -PRE beds, and significantly higher removal efficiencies of TOC (Fig. A.3). This is because PREs may promote the microbial diversity (Fig. 4a) and community composition (Fig. 5) in the planted and unplanted soils, and stimulate the enrichment of PTBP-degrading bacteria (Fig. 4a) and the activity of phenoloxidase (Fig. 4b). This suggests the addition of PREs may potentially instead of the plant presence on the removal of aromatic compounds such as PTBP.

Additionally, because the nature of PTBP is hardly to be photodegraded or volatilized (A et al., 2020), the performance of photochemical catalysis, volatilization and plant volatilization were ignored in this study. Therefore, soil adsorption, microbial degradation, and plant uptake are regarded as the major removal pathways of PTBP by PREs in the *Phragmites australis* rhizosphere, with the removal percentages of 31–37%, 27–37%, and 16–41%, respectively (Fig. 6).

4.2. Co-metabolic mechanism evaluation

The metabolism behavior of alkylphenols such as PTBP is mainly divided into two steps (Arora et al., 2010; Dey et al., 2016): the decomposition starts by the phenolic ring hydroxylation via monooxygenase that generated by fungi, which followed by a cleavage pathway through dioxygenase that created by bacteria (Fig. 6).

The monooxygenase activity was comparable among all the treatment groups (Fig. 5) and was positively correlated with fungal abundance ($p < 0.05$). Among them, *Penicillium* and *Fusarium* are reported as the degrading-related fungi of alkylphenols such as octylphenol and nonylphenol (Rajendran et al., 2020; Zhang et al., 2016), which may release monooxygenase to stimulate PTBP to convert into catechol structure by hydroxylation (Fig. 6). On the other hand, the dioxygenase activity was observed the highest in group UP (Fig. 4b), possibly due to the enrichment of *Sphingomonas* and *Gemmatimonas* (Fig. 4a). Reportedly, extensive study on the biodegradation of alkylphenol revealed that octylphenols and nonylphenols can be degraded by some *Sphingomonas* strain (Corvini et al., 2004; Gabriel et al., 2005); *Gemmatimonas* was responsible for pyrene degradation in the mixed microbiome that can produce dioxygenase, metabolize pyrene, and enhance the soil microbial function (Wang et al., 2018). Hence, PRE may accelerate PTBP biodegradation through enriching these degrading-related bacteria catalyzing the open-loop cleavage of PTBP by generating dioxygenase (Fig. 6).

Polyphenol oxidase is a representative phenol oxidase and widely distributed in nature (Mayer, 2006), in which monophenol oxidase can be used both as a monophenase to convert monophenol and its derivatives into catechol structure by ortho hydroxylation, and as a

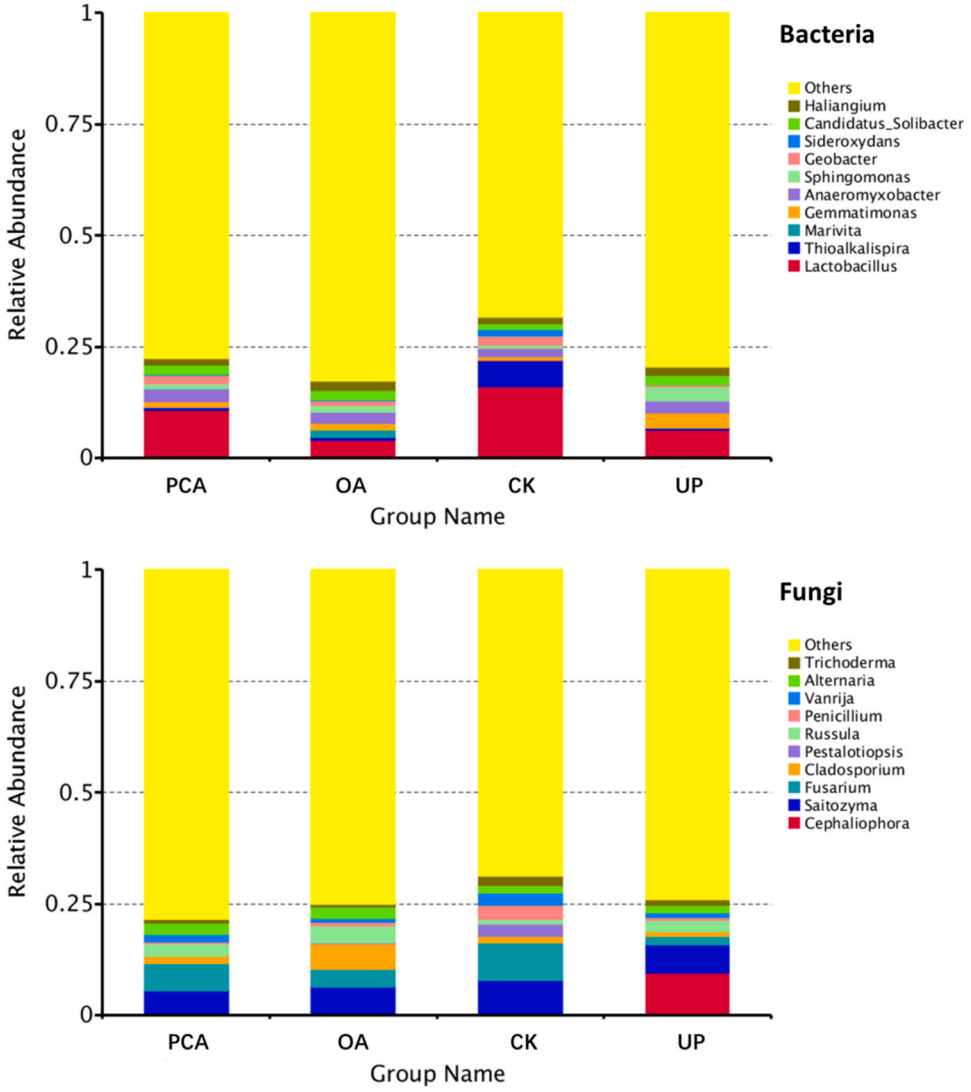

Fig. 5. Distribution of the top 10 genera of bacteria (above) and fungi (below) of soils collected from the planted and unplanted beds ($n = 3$).

diphenolase to oxidize these catechol chemicals for generating quinones (Li and Nappi, 1991). Polyphenol oxidase activity in the +RE beds (groups PCA, OA, UP) was apparently higher than in the -RE beds (group CK) (Fig. 4b), owning a significant positive correlation ($p < 0.05$) with bacterial abundance (Fig. 4a). Meanwhile, monophenol oxidase activity in the +PRE beds (groups PCA, UP) were significantly higher than in the -PRE beds (groups OA, CK) (Fig. 4b). That is to say, these REs also enhanced the activity of non-specific key enzyme such as polyphenol oxidase, but only PRE stimulated the activity of specific key enzyme such as monophenol oxidase catalyzing the phenolic ring hydroxylation of PTBP followed by a dehydrogenation route (Fig. 6).

Since the anoxic condition of wetland system and the addition of exogenous REs, a considerable amount of anaerobic bacteria were enriched in the +RE beds (groups PCA, OA, UP) (Fig. 5) for anaerobic microbial pathway of PTBP. For one thing, higher distribution of anaerobic ammonium oxidation bacteria (Anammox) such as *Candidatus_Solibacter* was found in the +RE beds (Fig. 5). And a significant positive correlation ($p < 0.05$) was observed between the concentrations of PTBP and NH_4-N (Fig. A.3), suggesting the interaction of their removals. For another thing, the activity of denitrifying bacteria was stimulated by the relatively low ORP values (100 mV < ORP < 400 mV) (Dušek et al., 2008) in this study. And the ORP increased slightly after 12 h in all the treatment groups (Fig. A.2), implying the occurrence of electron transport in the denitrification. These indicate the addition of REs promoted the activity of functional anaerobic bacteria (i.e. Anammox and denitrifying bacteria) and therefore accelerate the usage of PTBP as carbon source by anaerobic ammonium oxidation and electron donor by denitrification, respectively (Fig. 6).

5. Conclusions

In this study, the best performance of PTBP removal (88–98%) by all the reed beds was observed at RE/PTBP ratio of 5:1. PTBP was removed mainly via soil adsorption (31–37%), microbial degradation (27–37%), and plant uptake (16–41%). Although the addition of REs (PCA and OA) did not contribute to the distribution of fungi, they promoted the abundance and diversity of anaerobic functional bacteria and therefore enhanced the anaerobic processes of PTBP. Meanwhile, the addition of PREs (PCA) inhibited the effect of plant uptake, but improved the enrichment of degrading-related bacteria (*Sphingomonas* and *Gemmatimonas*), particularly in unplanted soils with high activity of dioxygenase catalyzing the cleavage pathway of PTBP. Moreover, REs increased the activity of non-specific key enzyme such as polyphenol oxidase, but only PREs stimulated the activity of specific key enzyme such as monophenol oxidase catalyzing the phenolic ring hydroxylation of PTBP followed by a dehydrogenation route. The knowledge obtained from this study will

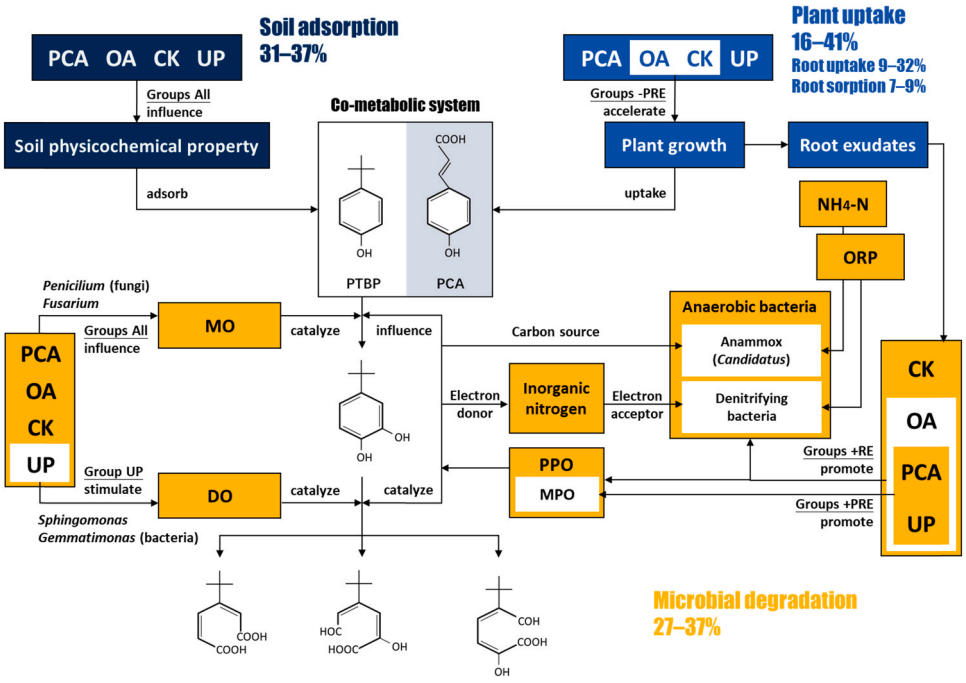

Fig. 6. Quantified removal pathways and microbial co-metabolic mechanisms of PTBP by PREs in the *Phragmites australis* rhizosphere.

be helpful for the developed remediation of aromatic compounds through microbial co-metabolism by plant-RE-microbe systems.

CRediT authorship contribution statement

Ni-Chen Zhang: Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Dan A:** Conceptualization, Methodology, Writing – original draft, Project administration. **Zhi-Feng Hong:** Investigation, Formal analysis. **Yuan-Qing Chao:** Resources. **Ya-Fei Yu:** Investigation. **Rong-Liang Qiu:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.127269.

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