Environmental Science and Pollution Research (2018) 25:23009–23017
https://doi.org/10.1007/s11356-018-2384-5

RESEARCH ARTICLE

Removal efficiency and enzymatic mechanism of dibutyl phthalate (DBP) by constructed wetlands

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Received: 15 March 2018 / Accepted: 22 May 2018 / Published online: 1 June 2018

Abstract

Four vertical-flow constructed wetland systems were set up in the field in order to study the removal efficiency and possible enzymatic mechanism of the constructed wetlands in treating sewage containing different concentrations of dibutyl phthalate (DBP). Under DBP spiked concentrations of 0.5, 1.0, and 2.0 mg/L, good DBP removal rates of 62.08, 82.17, and 84.17% were achieved, respectively. Meanwhile, certain removal effects of general water quality parameters were observed in all four constructed wetlands: with high average removal rates of nitrate nitrogen (NO_3^--N) and chemical oxygen demand (COD) of 91.10~93.89 and 82.83~89.17%, respectively, with moderate removal efficiencies of total nitrogen (TN), total phosphorus (TP), ammonia nitrogen (NH_4^+-N) of 44.59~49.67, 30.58~37.18, and 28.52~37.45%, respectively. Compared to the control, an increase of enzyme activities of urease, phosphatase, dehydrogenase, and nitrate reductase was observed in the treatments with DBP addition. In the presence of 0.5 mg/L of DBP concentration, the urease, phosphatase, and dehydrogenase activities reached the highest levels, with an increase of 350.02, 36.57, and 417.88% compared with the control, respectively. It appeared that the low concentration of DBP might better stimulate the release of enzymes.

Keywords Constructed wetlands · Dibutyl phthalate (DBP) · Removal efficiency · Enzyme activity

Introduction

Phthalic acid esters (PAEs) are one of the most important environmental endocrine disruptors. As a common plasticizer, it has been widely applied in the production of plastic products, rubber, coatings, and toys, and it also can be used for the material of liquor, insect repellent, cosmetics, and so on (Net et al. 2015; Heudorf et al. 2007; Staples et al. 1997). PAEs are easily released from plastic and transferred into the environment, which can be detected in the atmosphere, water, soil, and other media (Watanabe 2001). The concentrations of 16 kinds of PAEs in the central urban area of Guangzhou ranged from 1.67 to 322 μg/g, with an average concentration of 17.7 μg/g (Zeng et al. 2009). A survey showed that total phthalate concentrations in indoor dusts ranged from 269 to 4831 mg/kg in Palermo city and were higher in older homes compared to those constructed later (Santino et al. 2013). In China, the extent of pollution of PAEs varied among watersheds, and the total concentrations in Changjiang River Estuary samples ranged from 180.3 to 3421 ng/L (Zhang et al. 2017). Due to the carcinogenicity, teratogenicity, and mutagenicity (Tyler et al. 1998; Beliles et al. 1989), both the Environmental Protection Agency (EPA) of the USA and the China State Environmental Protection Administration (SEPA) have listed a variety of phthalates as environmental priority pollutants (Xu et al. 2008). Phthalic acid esters have become one of the most important environmental organic pollutants in the world and intensive attention has been paid (Tang et al. 2015).

As a new sewage treatment method, constructed wetland (CW) has been widely applied since the 1970s. Numerous studies showed that CWs had good purification effects on PAHs, pesticides, heavy metals, antibiotics, and other pollutants (Fountoulakis et al. 2009; Agudelo 2010; Mungur et al. 1997; Matamoros et al. 2009; Reyes-Contreras et al. 2011), but little information on the long-term removal

Responsible editor: Philippe Garrigues

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efficiencies and substrate enzyme activities of CWs treating PAEs is available. As one of the most commonly used PAEs, dibutyl phthalate (DBP) was classified as priority pollutants by the EPA and listed as environment priority-controlling pollutants in China. In previous studies, DBP has become widely distributed in water, soil, and atmosphere. The highest concentration of DBP in some parts of the Yellow River and Songhua River reached 330×10^{-6} g/L (Liu et al. 2014). In summary, due to the high toxicity and extensive distribution, DBP was selected as the target pollutant in this study, and four vertical-flow constructed wetland systems were set up in the field. The aims were (1) to evaluate the long-term purification effect of DBP by the CWs and (2) to demonstrate the change pattern of substrate enzyme activities of CWs treating sewage spiked with DBP.

Materials and methods

Experimental setup

Four parallel experimental vertical-flow constructed wetland systems were set up at the experimental base of Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China (30° 30.39′ N, 114° 28.09′ E). The wetland chamber (1.25 m × 1 m × 1 m; length × width × depth) was filled with a 40-cm-thick layer of gravel (Ø = 10–20 mm) at the bottom, followed by a 35-cm-thick layer of granolithic (Ø = 5–10 mm) at the upper layer. The wetland was planted with *Phragmites australis*, at a density of 20 plants/m^2. Figure 1 shows the layout of the experimental CWs.

Wastewater of 420 L/day with a hydraulic loading rate (HLR) of 330 mm/day was intermittently introduced into each CW unit twice a day. The effective content of each CW is approximately 0.425 m^3, which yielded a nominal hydraulic retention time (HRT) of 1 day.

Influent water quality

Synthetic wastewater was used to minimize fluctuation of the influent quality. $C_6H_{12}O_6$, NH_4Cl, $NaNO_3$, and KH_2PO_4 were dosed as carbon, nitrogen, and phosphorus source respectively. The influent characteristics were summarized in Table 1. In consideration of the concentrations of DBP found in related industrial wastewater and typical polluted surface water bodies (Zeng et al. 2008; Fromme et al. 2002), three concentration levels (0.5, 1.0, and 2.0 mg/L) of DBP were used. The experimental CWs were designated as DBP (0), DBP (0.5), DBP (1), and DBP (2), according to the DBP concentration in influent. DBP (0) was established as control.

Sample collection and analysis

Water samples were collected weekly from the inlet and outlet of each CW system after a stabilization period of 5 months. Water temperature (T), dissolved oxygen (DO) concentration, pH, and electric conductivity (EC) were determined immediately using YSI 556 Multiparameter System (Yellow Springs Instrument Company, USA). Chemical oxygen demand (COD), total phosphorus (TP), total nitrogen (TN),

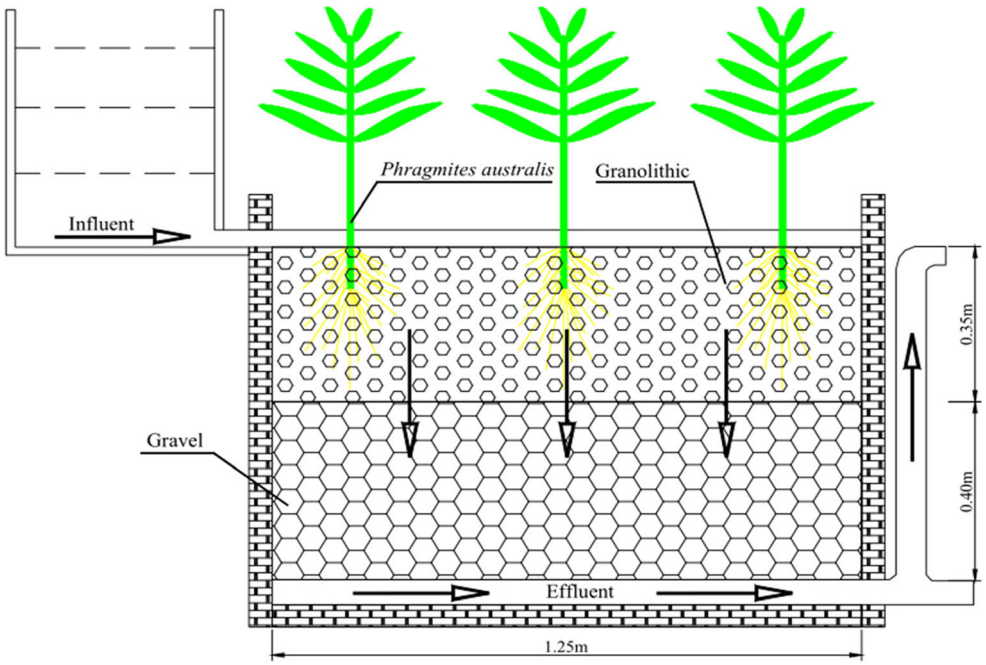

Fig. 1 Schematic presentation of the experimental CW used in this study

Table 1 Characteristics of the influent ($n = 105$)

Parameter	TN (mg/L)	TP (mg/L)	NH_4^+-N (mg/L)	NO_3^--N (mg/L)	COD (mg/L)
Mean value (± sd)	16.78 (± 3.04)	1.97 (± 0.44)	9.95 (± 1.10)	6.56 (± 1.87)	278.48 (± 167.66)

ammonium nitrogen (NH_4^+-N), and nitrate nitrogen (NO_3-N) were measured according to the methods of APHA (1998).

The concentrations of DBP were determined by gas chromatography-mass spectrometry (Agilent GC-MS, 7980A+5975C). The pretreatment methods were as follows: a suitable amount of water in the glass centrifuge tube is taken, 4 ml n-hexane for extraction is added, and then the tube is put into a sonicator for 10 min and at a 250 rpm frequency in oscillator for 0.5 h. After that, the upper organic phase is sucked into sample bottles for DBP analysis.

The activities of substrate enzyme were measured once a month. The substrate samples were taken from five random spots of the wetland surface (0~5 cm) and well mixed before stored in a freezer at 4 °C. Urease, phosphatase, dehydrogenase, and nitrate reductase activities were determined according to the references (Tabatabai and Bremner 1969; Zantua and Bremner 1975; Xu and Zheng 1986).

Statistical analysis

The experiment lasted for 12 months from October 2016 to September 2017. All statistical analysis was calculated using SPSS version 19.0 for Windows and Origin 8.0 was conducted to draw pictures. One-way analysis of variance (ANOVA) and correlation analysis were performed to test the differences and relationships between related factors. Value of $p < 0.05$ was defined as significant and $p < 0.01$ as highly significant.

Results and discussion

Removal efficiencies for DBP

The removal efficiencies of DBP are plotted in Fig. 2. Due to the low solubility of DBP in water and the small amount of spiked DBP compared with the influent wastewater, the measured DBP concentration in the inflow fluctuated, but the change tendency among the treatments was similar. The actual concentrations of DBP in the CWs were 0.08~0.41, 0.28~0.86, and 0.48~1.45 mg/L in DBP (0.5), DBP (1), and DBP (2) treatment, respectively.

As shown in Fig. 2, the average removal rates for DBP under the treatment of DBP (0.5), DBP (1), and DBP (2) were 62.08, 82.17, and 84.17%, respectively. High influent DBP concentrations were associated with high removal rates, and there were significant differences between them (DBP (1), DBP (2) versus DBP (0.5), $p < 0.05$). In the later experimental period, the removal rates of DBP were more than 80% and relatively stable in the three treatments, indicating that the CWs were capable of purifying DBP with high performance.

Some studies reported that the degradation process of organic pollutants in CWs consists of two stages (Yan et al. 2016; Dordio et al. 2010): the adsorption into medium in the first stage and the combined effects of decomposition/ uptake by plants and microorganisms in the second stage. However, biodegradation is considered to be the main removal mechanism for DBP, which relies on the microorganisms attached to

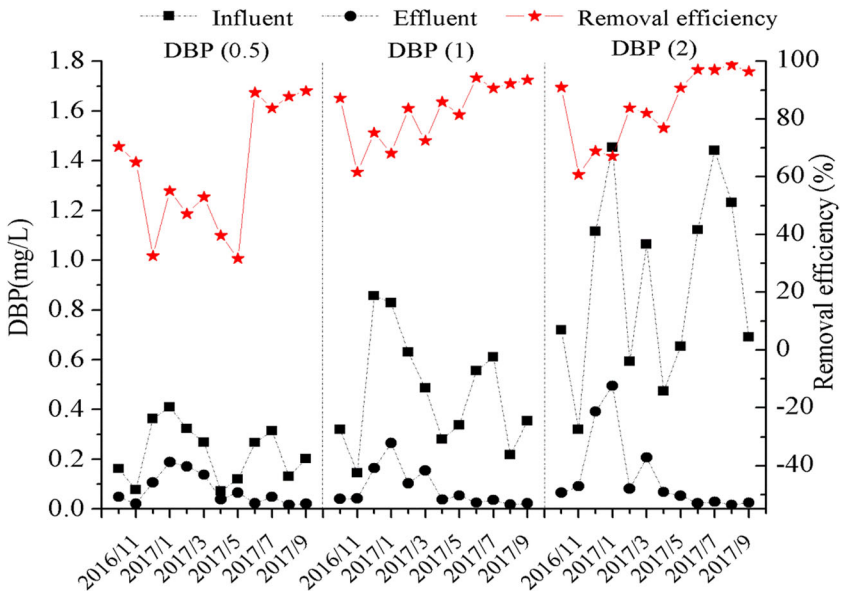

Fig. 2 Removal efficiencies of CWs for DBP

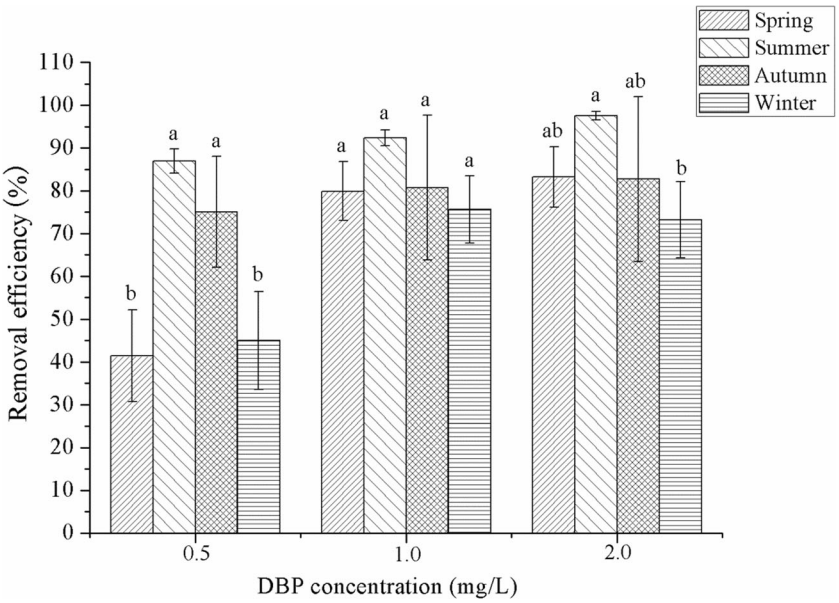

Fig. 3 Removal efficiencies (%) of DBP in the four seasons

the substrates and plant roots (Liang et al. 2003; Hammer 1989). Xu et al. (2008) indicated that DBP biodegradation might begin by ester hydrolysis to produce monobutyl phthalate (MBP) and corresponding alcohol. The conversion of MBP to phthalic acid or butyl benzoate might be due to microbial decarboxylation. The two derivatives of MBP degrade to form protocatechuate through ring cleavage.

Generally speaking, high summer temperature increases microbial activities and also increases the vigorous growth of wetland plants which provides more oxygen to accelerate the oxidative decomposition of organic matters in CWs. Therefore, as expected, the summer removal rate of DBP found in this study was relatively high. While the winter low temperature may have an inhibitory effect on DBP degrading bacteria in wetlands, resulting in a lower removal rate to a certain extent. Figure 3 shows the removal rates of DBP in different seasons. It can be seen that the change trend of DBP removal rates was consistent with the above conclusions. The highest DBP removal efficiencies were achieved in summer, with mean values of 86.88, 92.38,

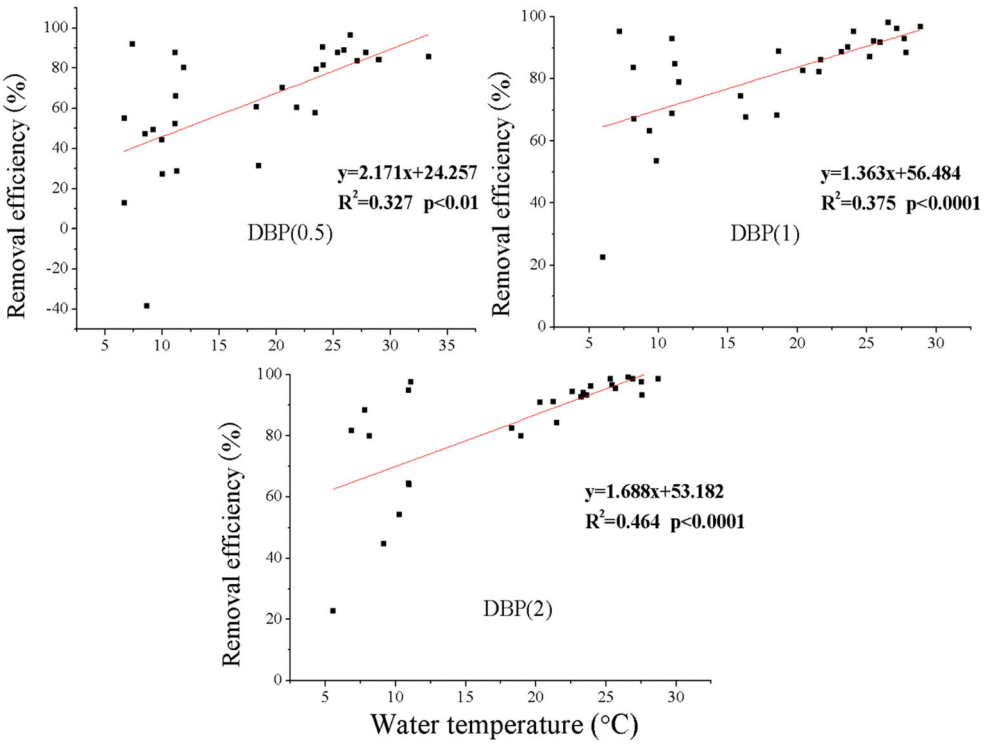

Fig. 4 Correlation between outflow water temperature (°C) and removal efficiencies (%) of DBP with different concentrations

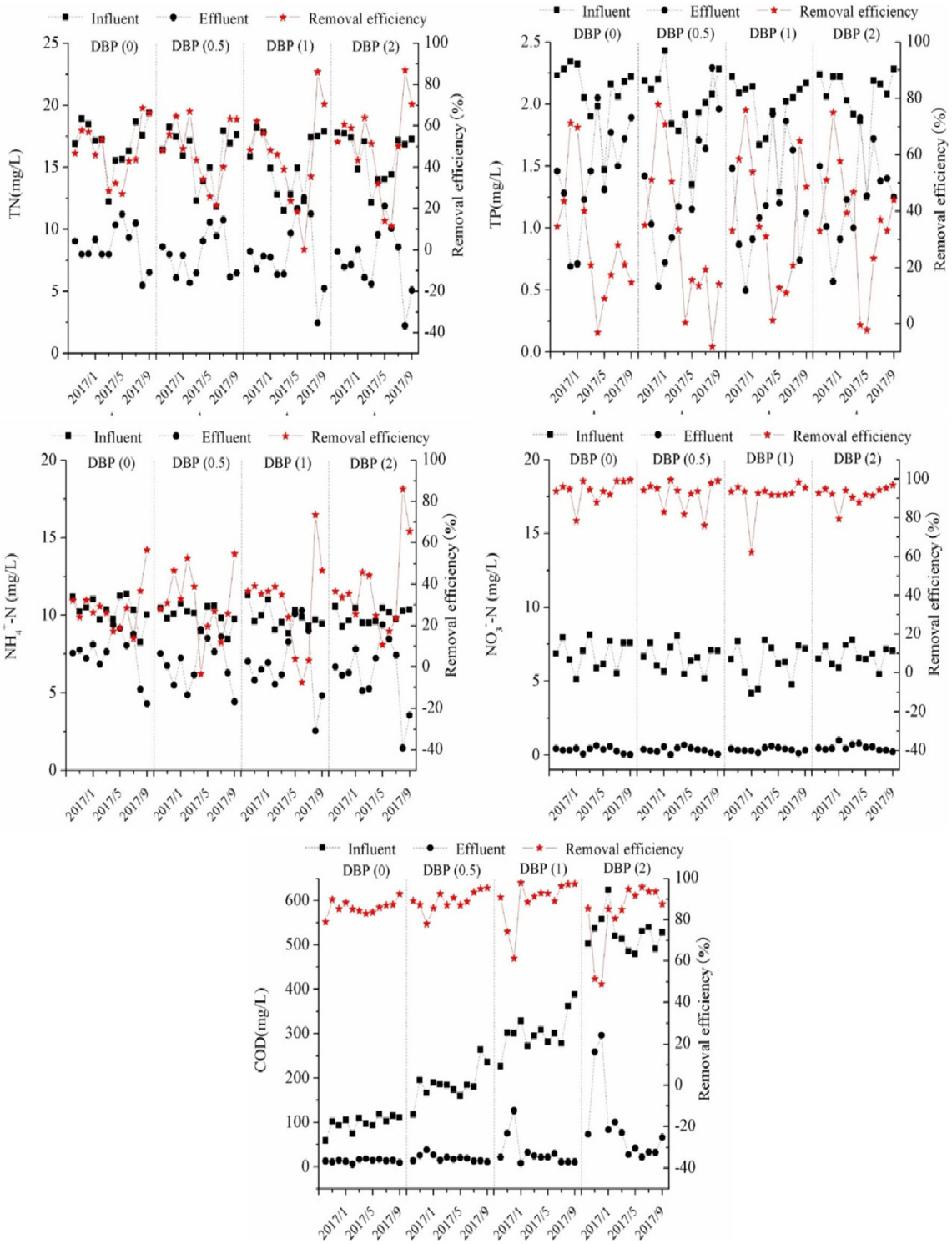

Fig. 5 Removal efficiencies (%) of CWs for conventional pollutants under different DBP concentration

and 97.57% under the treatment of DBP (0.5), DBP (1), and DBP (2), respectively. Meanwhile, the poorest removal performance was observed during winter. The removal efficiencies in summer were significantly higher than that in winter ($p < 0.05$) except DBP (1) treatment. In addition, when the concentration of DBP was 0.5 mg/L, the removal rates in autumn were significantly higher than that in spring ($p < 0.05$). The relationships between DBP removal rates and temperature are shown in Fig. 4. Significant linear relationships ($p < 0.01$) between outflow water temperature and DBP removal efficiencies were obtained. The r^2 values were 0.327, 0.375, and 0.464 in the three treatments,

Table 2 Pollutant removal efficiencies under different treatments (mean values ± sd)

Pollutant	Removal efficiency (%)			
	DBP (0)	DBP (0.5)	DBP (1)	DBP (2)
TN	47.60 ± 13.74	48.07 ± 15.52	44.59 ± 23.52	49.67 ± 21.98
TP	30.58 ± 22.73	30.98 ± 24.97	37.18 ± 23.40	36.45 ± 22.08
NH_4^+-N	28.52 ± 11.04	30.45 ± 16.73	30.46 ± 22.03	37.45 ± 21.09
NO_3^--N	93.89 ± 5.96	91.87 ± 7.52	91.10 ± 9.26	91.79 ± 4.56
COD	85.96 ± 3.46	89.17 ± 4.80	89.14 ± 10.85	82.83 ± 15.96

respectively. Furthermore, the higher the temperature, the better the correlations between the two factors.

Removal efficiencies for general water quality parameters

The profiles of TN, TP, NH_4^+-N, NO_3^--N, and COD removal under different concentrations of DBP were shown in Fig. 5, and the data were summarized in Table 2.

As shown in Fig. 5, the change trends of TN removal in all the wetland systems were similar. Initially, some slight fluctuations of TN removal efficiencies were obtained, then followed by sharp decreases in March, April, and May. At the end of the experiment, the abilities of removing TN increased and reached 66.32, 63.31, 70.69, and 70.56% under DBP (0), DBP (0.5), DBP (1), and DBP (2) treatments, respectively. When it came to TP, the highest removal efficiencies were achieved in winter for all treatments (60.36, 66.40, 54.72, and 57.34%, respectively), which were consistent with the findings of Liang et al. (2017). Similar variations of removal efficiencies to TN were observed for NH_4^+-N, with the average removal rate of 28.52, 30.45, 30.46, and 37.45% (shown in Table 2), respectively. As for NO_3^--N removal, good performances were observed in the beginning, and over 90% of NO_3^--N reduction was achieved in all treatments in the first 3 months. Then a decline occurred in January. After that, the NO_3^--N removal increased and maintained stably with the mean removal rates of 93.89, 91.87, 91.10, and 91.79%, and

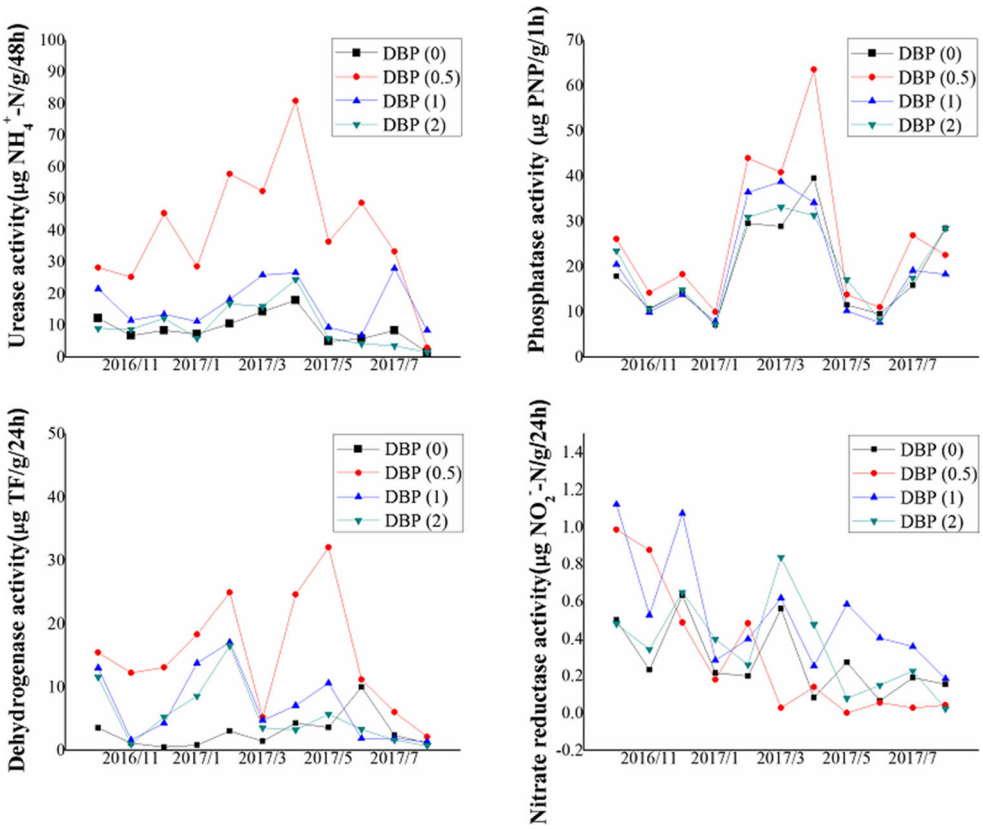

Fig. 6 Changes of four enzyme activities in the CWs

all the effluent NO_3^--N concentrations were below 1.0 mg/L. Meanwhile, the average purification efficiencies of COD were 85.96, 89.17, 89.14, and 82.83%, respectively.

The removal of nitrogen in CWs mainly depends on nitrification and denitrification processes (Gale et al. 1993; Romero et al. 1999). Thus, the removal rates of NH_4^+-N was relatively higher from May to September, since the temperature at that time was high and the microbes grew flourishingly. Compared with the high removal efficiencies of NO_3^--N, relatively poor performance of NH_4^+-N removal was observed. It indicated that the denitrification intensity was greater than nitrification in the CWs. Although the effluent DO concentration of 3.46 (± 0.98) mg/L in this study was relatively high for denitrification, the water-saturated conditions contributed to a anoxic state in the interior of CWs. This was also confirmed by previous research (Ong et al. 2013). Moreover, the degradation of organic compounds stimulated the use of oxygen by wetlands microorganisms and DBP as a kind of allelochemicals may simultaneously accelerate its consumption. Thus, the inhibition of the growth of nitrifying bacteria was appeared, so it was not conducive to the removal of NH_4^+-N but in favor of the NO_3^--N reduction. In addition, as for TP removal, while the concentrations of DBP ranged from 0 to 1 mg/L, the removal rates of TP were gradually increased, which improved 0.4 and 6.6% (DBP (0.5) and DBP (1) versus DBP (0)). But when the concentration of DBP rose to 2 mg/L, there was a decline of TP removal. In general, the path ways of phosphorus removal in constructed wetlands included plant uptake, microbial degradation, and substrate adsorption (Verhoeven and Meuleman 1999). The influent with low concentrations of DBP spiked (DBP (0.5) and DBP (1)) might facilitate the activity of phosphate accumulating bacteria, boosting the removal performance of TP. But the high DBP concentration might cause a certain toxicity to the corresponding microbes. Therefore, to a certain degree, TP removal was weakened with the increased DBP pressure.

In summary, our findings showed no significant differences in the removal efficiencies for conventional contaminants between treatments containing DBP or not ($p > 0.05$). Consequently, it is indicated that under our test conditions, the removal performance of conventional pollutants was unaffected by the different influent DBP concentrations (0.5–2.0 mg/L). The results were in agreement with many previous studies (Yan et al. 2016; Zhang et al. 2018).

The change of substrate enzyme activities

The substrate enzymes of constructed wetlands play a pivotal role in organic matter decomposition by converting macromolecules into low molecular moieties, therefore promoted the substance transformation and energy flow process in the system (Freeman et al. 1995; Sinsabaugh et al. 1993). To a certain extent, the enzyme activity can reflect the pollutants removal efficiency and biological activity of wetlands. Several researches have shown that the substrate enzymes can indicate the pollution stress of wetland systems and be used to evaluate the running state of the systems (Allison and Vitousen 2005; Reddy et al. 2010). The activities of substrate microorganisms, animals, and plant rhizosphere all contributed to the production of substrate enzymes (Kang et al. 1998). In this study, we measured the activities of four kinds of enzymes including urease, phosphatase, dehydrogenase, and nitrate reductase. Urease catalyzed the hydrolysis of urea into carbon dioxide and ammonia, which can affect the nitrogen cycle in CWs. Dehydrogenase oxidized soil organic matter by transferring electrons and protons from substrates to acceptors and was considered as an indicator of microbial activity in soil. Phosphatase catalyzed the organic-P into inorganic-P (Baddam et al. 2016). Besides, nitrate reductase contributed to the transformation from NO_3^--N to NO_2^--N.

The changes of four enzyme activities were summarized in Fig. 6.

During the experimental period, in the range of the concentrations of DBP, all of the four enzyme activities were activated, even at a high level of DBP (2.0 mg/L). The urease activity increased gradually over time, and the activities reached a peak in April, which was similar to the results of Cui et al. (2011). The phosphatase activities of four treatments were lower in winter than those in other seasons and declined to the lowest in January. As the temperature went up, the phosphatase activities increased first and then decreased again. The phosphatase activities basically recovered to its original level in the end. As for the dehydrogenase, after a steady increase for several months, the activity of dehydrogenase showed an obvious downward trend in March, which may be due to the rainfall and plants replanting. Then it recovered in April and May, while the dehydrogenase activity of DBP (0) treatment was low and relatively stable during the experiment. Meanwhile, there was a relatively large volatility of nitrate reductase activity in the four systems, which showed a downward trend in general. Except the treatment of DBP (0.5), the nitrate reductase activities under the other three DBP treatments reached peaks in December and March, respectively.

We also found that the variations of the activities of urease, phosphatase, and dehydrogenase were similar except nitrate reductase. In the basis of the enzyme activities in the CWs with DBP spiked higher than that unspiked, the higher DBP concentrations, the lower the activities of enzyme with influent DBP concentrations ranging from 0.5 to 2.0 mg/L. Specifically, these three enzyme activities remarkably increased upon exposure to 0.5 mg/L of DBP, and among them, urease and dehydrogenase activities have achieved significant level ($p < 0.05$), with mean values increased by 350.02, 36.57, and 417.88% compared with the DBP (0) treatment, respectively. This indicated that low concentration of DBP may stimulate the growth of the corresponding microorganisms.

Microbes can use the lower concentration of DBP as a carbon source and a nitrogen source to maintain their own growth and reproduction, thus enhanced their abilities to secrete enzymes. Additionally, plants would also make corresponding responses upon under the stress of pollutants. Secretions released from rhizosphere of plants may increase enzyme activities in the substrate and therefore enhanced the removal efficiencies of pollutants in the CWs. However, the high concentrations of DBP may inhibit microbial activities. A significant inhibitory effect on urease and phosphatase in the soil with DBP of 50 and 100 mg/kg was detected by Pang et al. (2009). Actually, the water quality, substrate, and pollutant types could affect the activities of microbial enzymes (Peralta et al. 2013). Some studies have proved that pesticides such as fenamiphos could also stimulate dehydrogenase (Megharaj et al. 1999), and naphthalene and phenanthrene had positive effects on protease and urease (Margesin et al. 2000). The influences of PAHs on enzyme activities were related to the type of pollution source (Stanislaw et al. 2004). Yan et al. (2016) demonstrated that the enzyme activities of plants were stimulated especially at high initial PhACs concentrations ranging from 10 to 500 μg/L. As for nitrate reductase, it can be used as an important indicator to determine the nitrogen removal efficiency of a CW. In our study, the concentrations of NO_3^--N in inflow were similar, and with the operation of CWs, no significant differences in the removal rates of NO_3^--N in different treatments were found, which was consistent with the unconspicuous changes of nitrate reductase activity in each treatment. It may indicated that the nitrate reductase was not very sensitive to the addition of DBP, while the exact reason should be studied further.

Conclusions

1. With the DBP dosage of 0.5, 1.0, and 2.0 mg/L, the average removal rates were 62.08, 82.17, and 84.17%, respectively, indicating that the vertical-flow CWs can removal DBP effectively.
2. A positive correlation between the DBP removal and the temperature was detected.
3. The vertical-flow constructed wetland systems showed a good purification effect for NO_3^--N and COD, with the removal efficiencies of 91.10~93.89 and 82.83~89.17%; meanwhile, the moderate removal rates were observed for TN, TP, and NH_4^+-N of 44.59~49.67, 30.58~37.18, and 28.52~37.45%. Under our test conditions, the removal performances of general water quality parameters were not significantly affected by the presence and concentration of DBP (0.5–2.0 mg/L).
4. Low concentrations of DBP could better stimulate the release of enzymes. The activities of urease, phosphatase, and dehydrogenase increased significantly in the presence of 0.5 mg/L of DBP concentration.

Funding information This work was supported by grants from the National Natural Science Foundation of China (51578538) and Science and Science and Technology Promotion Project of Ministry of Water Resources (TG1520).

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