Chemosphere 212 (2018) 245–253

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Effects of pharmaceuticals on microbial communities and activity of soil enzymes in mesocosm-scale constructed wetlands

Qing Yan [a, c, 1], Yufeng Xu [b, 1], Yonghong Yu [a, c], Zhi Wei Zhu [a, c, **], Guozhong Feng [a, *]

[a] China National Rice Research Institute, Hangzhou, China
[b] College of Energy and Environmental Engineering, Hebei University of Engineering, Handan 056038, China
[c] Laboratory of Quality & Safety Risk Assessment for Rice (Hangzhou), Ministry of Agriculture, Hangzhou 310006, China

HIGHLIGHTS

- The microbial community and enzyme activity were evaluated during long-term stress.
- Enzyme activity was not inhibited in the experimental range (0–500 μg/L).
- The main microbial florae were not affected in laboratory experiments.
- The total PLFAs did not differ obviously in response to low pharmaceuticals in CWs.

ARTICLE INFO

Article history:
Received 14 May 2018
Received in revised form
10 August 2018
Accepted 13 August 2018
Available online 18 August 2018

Handling Editor: T. Cutright

Keywords:
Constructed wetland
Enzyme activity
Pharmaceutical
Cyperus alternifolius
Removal

ABSTRACT

Cyperus alternifolius based mesocosm-scale constructed wetland was employed to remove pharmaceuticals. We investigated the microbial community composition using phosphor lipid fatty acids (PFLAs) analysis and substrate enzyme activity during long-term exposure to pharmaceuticals in mesocosm-scale constructed wetlands. The results showed that there was no visible inhibition effect of pharmaceuticals on CW substrate enzymes activities in the experimental range (0–500 μg/L). Microbial communities, as revealed by PFLAs, were enhanced by the presence of plants, while the PFLAs content was highest when the pharmaceutical concentration was 10 μg/L or 30 μg/L at CWs. Except for anaerobic bacteria and Saturated fatty acids, the maximum PLFAs levels were reached when the pharmaceuticals were 10 μg/L or 30 μg/L, while Bacteria, G (−), fungal bacteria, Aerobic bacteria and Monounsaturated fatty acids were remarkably affected by high pharmaceuticals (100–500 μg/L). However, the main microbial florae were not changed among the treatments. In this study, the removal efficiencies of the studied pharmaceuticals in Planted (30) was greatest, which could be attributed to the higher microbial biomass. These results indicate that *C. alternifolius* can phytoremediate pharmaceutical-contaminated waters in CWs. Individual fatty acid cannot be used to represent specific species; therefore, more approaches to species identification such as rRNA-based methods must be included in future studies to better understand the metabolic mechanisms of microorganisms involved in the removal of studied pharmaceuticals and improve the performance of CWs.

1. Introduction

Pharmaceuticals as emerging environmental contaminants have increasingly gained attention over the last two decades because of their omnipresence in the environment and their potential detrimental effects on human health and aquatic life (Petrie et al., 2017; Yang et al., 2017b; Binh et al., 2018; Zhang et al., 2016). Particularly, the release of antibiotics into aquatic environments can promote the acquisition of antibiotic resistance genes (ARGs) by microorganisms after a long exposure time, even at trace levels (D'Alessio et al., 2018; Liang et al., 2018; Song et al., 2018). It is known that pharmaceuticals are not completely removed by conventional wastewater treatment plants (WWTPs, not designed to remove these emerging pollutants), and that WWTP effluents are an

* Corresponding author.
** Corresponding author. China National Rice Research Institute, Hangzhou, China.
E-mail addresses: qyan2005@hotmail.com (Q. Yan), zhuzhiwei@caas.cn (Z.W. Zhu), fengguozhong@caas.cn (G. Feng).
[1] The first two authors contributed equally to this paper.

https://doi.org/10.1016/j.chemosphere.2018.08.059
0045-6535/

important source of pharmaceuticals in the environment (Rivera-Utrilla et al., 2013; Mirzaei et al., 2017; Kanakaraju et al., 2018).

During the last few decades, constructed wetlands (CWs) have become popular, and they are now seen as a promising tertiary option for WWTPs. In comparison with other advanced treatment technologies (e.g., advanced oxidation, carbon adsorption, ozonation, and membrane filtration), CWs are eco-friendly and cost-effective methods for removing pollutants from effluents (Verlicchi and Zambello, 2014; Tokumura et al., 2016; Weedon, 2017; He et al., 2018a; Kanakaraju et al., 2018; Schierano et al., 2018). CWs have received increased attention because of their potential for application to treatment of pharmaceutical-containing wastewaters, and the removal efficiency of pharmaceuticals in CWs has been demonstrated to be at least as good as that observed in conventional wastewater treatment processes (Verlicchi and Zambello, 2014; Yan et al., 2016a).

Several processes are involved in the removal of pharmaceuticals in CWs, including plant uptake, sorption, precipitation, hydrolysis and microbial degradation (Li et al., 2014b; Verlicchi and Zambello, 2014). Moreover, microbial metabolism and matrix enzymes play vital roles in the mineralization of pollutants in CWs. Soil biological parameters such as enzyme activity and the microbial community are sensitive indicators of soil quality in CWs that can be utilized to evaluate the nutrient requirement of microbes and predict their functional feedbacks to environmental changes (Zhang et al., 2011b; Yang et al., 2013, 2017a). Therefore, a better understanding of the microbial community and soil enzyme activity in CWs would be beneficial for optimizing or improving the removal of pharmaceuticals by CWs. However, most studies of pharmaceutical removal by CWs conducted to date have investigated the effects of different types of wetland configurations, plants and treatment strategies on the removal efficiencies and behavior of different pharmaceuticals (Li et al., 2014b; Verlicchi and Zambello, 2014). Moreover, study of soil enzymes have focused on their responses to heavy metals, pesticides, PAHs and other persistent organic pollutants (Lebrun et al., 2012; Sivakumar et al., 2012; Lessard et al., 2013; Yang et al., 2013; Oleszczuk et al., 2014). To the best of our knowledge, no attention has been paid to the effects of pharmaceuticals on microbial enzymatic activity. Till now, there have been no studies published relevant to microbial enzymatic activity in CWs under the pharmaceutical stress. Furthermore, the relationship between microbial communities and removal of pharmaceuticals from wastewaters in CWs is poorly understood. Thus, it is necessary to evaluate the microbial consortium and its functionality to help us better understand and control CWs.

Therefore, this study was conducted to investigate the changes in CW biological properties, such as soil enzyme activities and microbial community structure, under pharmaceutical pressure during wastewater treatment. The enzymes involved in the degradation of exogenous pollutants are catalase (CAT) (H_2O_2-oxidoreductase), dehydrogenase (DHA) and urease; therefore, these three enzymes were analyzed in the present study. DHA is an important oxidoreductase in soils that is the catalyst for important metabolic processes, including decomposition of organic inputs and detoxification of xenobiotics (Maliszewska-Kordybach and Smreczak, 2003; Li et al., 2012). Currently, DHA activity is commonly applied to assess microbial activity. Catalase is possessed by almost all aerobic and facultative anaerobic microorganisms (Margesin et al., 2000). Urease catalyzes the hydrolysis of carbon-nitrogen (C–N) bonds of some amides and urea (Madejon et al., 2001). Phospholipid fatty acids (PLFAs) are biomarkers of viable microbial biomass and structure of in situ microbial communities that can reflect the actual condition of the microbial community (Kato et al., 2005). Therefore, microbial community composition under the long-term exposure of pharmaceuticals was investigated using PLFA analysis in this study. To the best of our knowledge, this is the first study to investigate the PLFAs composition and substrate enzyme activity under pharmaceutical stress during treatment in CWs.

2. Materials and methods

2.1. Selected pharmaceuticals

Pharmaceuticals (Table 2) were selected based on their persistence, production volumes and occurrence in the environment. Thus, the neuroleptic pharmaceutical carbamazepine (CBZ), which is known to be persistent and nearly ubiquitous in aquatic systems, was included, as well as the antibiotics sulfamethoxazole (SMZ), ofloxacin (OFX), and roxithromycin (ROX).

2.2. Mesocosm-scale constructed wetlands (CWs)

The experimental setup was established in a controlled greenhouse environment (photoperiod: 12 h, temperature: $28 \pm 2\,^{\circ}C$, illumination: 2800 ± 250 lux). Similarly sized *Cyperus alternifolius* (height of 0.7–0.8 m, 6 tillers per plant) were selected and planted at a density of 30 plants/m^2 in the CWs after being rinsed thoroughly. *C. alternifolius*, with the common names of umbrella papyrus, umbrella sedge or umbrella palm, is a perennial herb and grows in humid areas or swamp land. There were no significant differences in the growth parameters of *C. alternifolius* during treatment (see Supplementary Table S1). It grows fast with strong root system and it can form a good landscape. Each CW consisted of a stainless steel container (length: 0.6 m, width: 0.5 m, height: 0.6 m) filled with 30 cm top layer of ceramisite with a D_{60} of 5 mm, followed by a 5 cm high gravel layer (particle size Ø 1–1.5 cm), and then a 5 cm bottom layer of coarse gravel (particle size Ø 5–8 cm) as a supporting layer. The water level was maintained immediately below the medium surface, corresponding to a flooding rate of approximately 100%. Perforated PVC pipes with a diameter of 5 cm were installed in the middle of the reactor to collect water samples. The synthetic water simulating municipal WWTP secondary effluent was prepared with the following composition (in mg/L): 5 $MgSO_4$, 3.8 $CaCl_2$, 1.1 KH_2PO_4, 1.85 K_2PHO_4, glucose, 10.1 NH_4Cl, 36.11 KNO_3, 54.16 $NaHCO_3$, 5 $FeSO_4$, 0.75 H_3BO_3, 5 EDTA-Na_2, 0.09 KI, 0.03 $Na_2MoO_4 \cdot 2H_2O$, 0.075 $CoCl_2 \cdot 6H_2O$, 0.05 $MnSO_4 \cdot H_2O$, 0.06 $ZnSO_4 \cdot 7H_2O$, 0.015 $CuSO_4 \cdot 5H_2O$. Then, the composition of the influent was: 60 mg/L COD; 20 mg/L total nitrogen (TN); 8 mg/L NH_4^+–N; and 1 mg/L total phosphorus. The tap water that needs to be used the next day was all prepared in a 350 L influent water storage tank the day before. Six CWs (five planted CWs [Planted (0), Planted (10), Planted (30), Planted (100), Planted (500)] and one unplanted control) together with a 350 L influent water storage tank constituted a working line in the experiment, and the working lines were used in triplicate. The entire system was acclimatized for four months before the start of the experimental period. The wastewater was maintained in the CWs for 12 h and refilled every 12 h. Synthetic wastewater was fortified with a mixture of the studied pharmaceuticals at different test concentrations (0, 10, 30, 100, and 500 μg/L). Thus, the experimental CWs were designated as Planted (0), Planted (10), Planted (30), Planted (100), Planted (500), and Unplanted (0) according to the Pharmaceuticals concentration in influent and whether *C. alternifolius* was planted in the CWs or not.

2.3. Enzyme activity analysis

Urease activity was measured by the colorimetric method as