Water Research 102 (2016) 294–304

Contents lists available at ScienceDirect

Water Research

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

# Phytoextraction, phytotransformation and rhizodegradation of ibuprofen associated with *Typha angustifolia* in a horizontal subsurface flow constructed wetland

Yifei Li [a, b, *], Jiefeng Zhang [a, c], Guibing Zhu [d, **], Yu Liu [a, b], Bing Wu [e], Wun Jern Ng [a, c], Adhityan Appan [c], Soon Keat Tan [a, c, f]

[a] School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore
[b] Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One, 637141, Singapore
[c] Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One, 637141, Singapore
[d] Key Laboratory of Drinking Water Science and Technology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, PR China
[e] Singapore Membrane Technology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One, 637141, Singapore
[f] Maritime Research Centre, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore

ARTICLE INFO

*Article history:*
Received 4 May 2016
Received in revised form
23 June 2016
Accepted 24 June 2016
Available online 25 June 2016

*Keywords:*
Organic micropollutant
Non-steroidal anti-inflammatory drug
Phytoremediation
Macrophyte
Microorganism

ABSTRACT

Widespread occurrence of trace pharmaceutical residues in aquatic environments is of great concerns due to the potential chronic toxicity of certain pharmaceuticals including ibuprofen on aquatic organisms even at environmental levels. In this study, the phytoextraction, phytotransformation and rhizodegradation of ibuprofen associated with *Typha angustifolia* were investigated in a horizontal subsurface flow constructed wetland system. The experimental wetland system consisted of a planted bed with *Typha angustifolia* and an unplanted bed (control) to treat ibuprofen-loaded wastewater (~107.2 μg $L^{-1}$). Over a period of 342 days, ibuprofen was accumulated in leaf sheath and lamina tissues at a mean concentration of 160.7 ng $g^{-1}$, indicating the occurrence of the phytoextraction of ibuprofen. Root-uptake ibuprofen was partially transformed to ibuprofen carboxylic acid, 2-hydroxy ibuprofen and 1-hydroxy ibuprofen which were found to be 1374.9, 235.6 and 301.5 ng $g^{-1}$ in the sheath, respectively, while they were 1051.1, 693.6 and 178.7 ng $g^{-1}$ in the lamina. The findings from pyrosequencing analysis of the rhizosphere bacteria suggest that the *Dechloromonas* sp., the *Clostridium* sp. (e.g. *Clostridium saccharobutylicum*), the order Sphingobacteriales, and the *Cytophaga* sp. in the order Cytophagales were most probably responsible for the rhizodegradation of ibuprofen.



## 1. Introduction

Pharmaceutical residues have been frequently observed in various aquatic environments such as wastewater, surface water, ground water and even drinking water (Cizmas et al., 2015; Luo et al., 2014). These compounds, regarded as emerging pollutants, are at trace levels (ng $L^{-1}$ to μg $L^{-1}$) in the aquatic environment, normally at least one order of magnitude lower than the levels to cause acute ecotoxicity (Corcoran et al., 2010). However, the non-target aquatic organisms including fish have a high probability of suffering unintended direct or side effects induced by such pharmaceuticals that are generally regarded to have a low toxic effect on humans or livestock (Corcoran et al., 2010; Liu et al., 2015). This is perhaps attributed to (1) the biological active nature of some pharmaceuticals in non-target organisms unlike the principal function to the target organisms, especially under long-term exposure resulted by the continuous release of pharmaceuticals into the aquatic environment; and (2) the additive toxic effects of the combinations of various pharmaceuticals and other pollutants (Cizmas et al., 2015; Corcoran et al., 2010; Fent et al., 2006).

* Corresponding author. School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore.
** Corresponding author.
*E-mail addresses:* yli19@e.ntu.edu.sg (Y. Li), gbzhu@rcees.ac.cn (G. Zhu).

http://dx.doi.org/10.1016/j.watres.2016.06.049
0043-1354/

Therefore, developing efficient methods to control pharmaceutical pollution in aquatic environments is of great urgency and significance.

As a secondary wastewater treatment or a wastewater polishing process, constructed wetlands have received increasing attention on their application in treating pharmaceutical-containing wastewaters (Li et al., 2014; Verlicchi and Zambello, 2014). The ecological perspective and economical operation of constructed wetlands make for attractive application alternatives in regions where there are lacking of appropriate infrastructure to set up centralized wastewater treatment plants (WWTPs) (Babatunde et al., 2008; Vymazal, 2011). To date, however, most of the reported work is related to the performance of constructed wetlands for the removal of pharmaceutical contaminants. There has not been a comprehensive understanding of the relevant mechanisms involving the substrate, plants and microorganisms of constructed wetlands. In particular, there are large gaps on the plant-associated mechanisms in constructed wetlands such as phytoextraction (uptake, translocation and accumulation), phytotransformation (degradation in plant tissues) and rhizodegradation (microbial degradation in rhizosphere) for the removal of pharmaceuticals from wastewater.

This study selected ibuprofen as the target pharmaceutical, a non-steroidal anti-inflammatory drug (Corcoran et al., 2010; Flippin et al., 2007). Ibuprofen is one of the most widely used over-the-counter drugs and is one of the most frequently detected pharmaceuticals in aquatic environments such as raw wastewaters, effluents of WWTPs and surface waters (Corcoran et al., 2010; Luo et al., 2014; Saravanan et al., 2012). It has been reported that ibuprofen is toxic to reproduction of some aquatic organisms, for example, changing the spawning pattern of Medaka at environmental concentrations of 1–100 μg $L^{-1}$ (Flippin et al., 2007), eliciting embryo abnormalities of zebrafish if at a concentration higher than 10 μg $L^{-1}$ (David and Pancharatna, 2009), and altering egg hatching of *Daphnia magna* at a concentration range of 0.5–50 μg $L^{-1}$ (Wang et al., 2016).

The objective of this study was to assess the phytoextraction, phytotransformation and rhizodegradation of ibuprofen associated with the macrophyte *Typha angustifolia* in constructed wetlands. For this purpose, a mesocosm-scale horizontal subsurface flow constructed wetland system was constructed for the treatment of ibuprofen-loaded wastewater. The system consisted of a planted bed with *Typha angustifolia* and an unplanted bed as the control. The concentrations of ibuprofen and three of its metabolites including ibuprofen carboxylic acid, 2-hydroxy ibuprofen and 1-hydroxy ibuprofen in the water samples and the leaf tissues (sheath and lamina) were tested to evaluate the mechanisms of phytoextraction and phytotransformation. The bacterial DNA templates were periodically isolated from the rhizosphere samples for 454 high-throughput pyrosequencing analysis to identify the bacteria responsible for the rhizodegradation of ibuprofen.

## 2. Materials and methods

### 2.1. Chemicals

The chemicals used in this study included the followings. Glucose (anhydrous), ammonium sulfate (99+%), potassium dihydrogen phosphate (98+%), magnesium sulfate heptahydrate (99+%), calcium chloride dihydrate (99–105%), sodium hydrogen carbonate (99%) and sodium carbonate (98%) were acquired from Alfa Aesar (USA) and were used for the synthesis of municipal wastewater. High purity (≥98%) ibuprofen (IBP), 2-hydroxy ibuprofen (2-OH IBP), 1-hydroxy ibuprofen (1-OH IBP) and ibuprofen-$d_3$ (IBP-$d_3$) were supplied by Sigma-Aldrich (USA). High purity (≥98%) ibuprofen carboxylic acid (CBX IBP) was provided by Toronto Research Chemicals (Canada). HPLC-grade methanol, *n*-hexane, hydrochloric acid (37%) and ammonium acetate 5 M solution were acquired from Merck (USA).

### 2.2. Experimental wetland system

A mesocosm-scale horizontal subsurface flow constructed wetland (HSSF-CW) system was set up at Nanyang Technological University (NTU) campus in Singapore under tropical climate. The system was installed in a semi-sheltered environment with a transparent roof and insect nettings on all sides. The system consisted of a 200-L wastewater holding tank and two identical fiberglass HSSF-CW beds (Fig. 1). One bed was planted with *Typha angustifolia* (initial density: 15 plants $m^{-2}$), and the other was unplanted (control). Both beds were packed with a 300 mm deep gravel layer containing rough gravels (size of 20–24 mm) at the inlet and outlet sections and fine gravels (size of 4–10 mm) at the treatment section. The influent was pumped from the wastewater holding tank to the surface of the inlet section to ensure 250 mm deep water below the gravel surface. Two perforated pipes (20 mm i.d.) were installed vertically into the gravel to collect interstitial water at the one-third length (1/3L) and two-third length (2/3L) of the treatment section along the flow path. The treatment section was divided accordingly into three zones: upstream 1/3 zone (inlet to 1/3L), middle 1/3 zone (1/3L to 2/3L) and downstream 1/3 zone (2/3L to outlet). The two sampling pipes also worked as ventilation pipes to enhance the aeration process in the beds to some degree.

### 2.3. System operation and sampling

After the *Typha angustifolia* was acclimated in the planted bed, the HSSF-CW system was fed continuously with IBP-free synthetic wastewater at the rate (7.64 mL $min^{-1}$) equivalent to a 4-day hydraulic retention time. The synthetic wastewater was designed to contain 300 mg $L^{-1}$ of chemical oxygen demand (COD), 27 mg $L^{-1}$ of ammonium nitrogen ($NH_4^+$–N) and 18 mg $L^{-1}$ of total phosphate (TP). From the 30$^{th}$ day onwards, the influent water was spiked with IBP (~100 μg $L^{-1}$). It has been reported that the IBP concentration ranged from <0.004 to 603 μg $L^{-1}$ in the influent wastewater of conventional WWTPs (Luo et al., 2014). To clearly elucidate IBP transportation pathway associated with the plants in wetland, a relatively high concentration of IBP (~100 μg $L^{-1}$) was used in this study. The sampling campaign commenced on the 30$^{th}$ day at four sampling locations in each bed: inlet, 1/3L, 2/3L and outlet. A 40 mL of water sample was collected from each location every 4 days to test certain water-quality parameters: temperature, pH, dissolved oxygen (DO), oxidation reduction potential (ORP), COD, $NH_4^+$–N and TP. After the system had adapted to the IBP injection (about 30 days), from the 62nd day onwards, the sampling interval was changed to once every 8 days. An additional 50 mL of water sample was collected from each location to monitor the concentrations of IBP and its metabolites (CBX IBP, 2-OH IBP and 1-OH IBP). Besides, every 8 days, one or two entire leaves of *Typha angustifolia* were randomly collected at 1/3L and 2/3L, respectively. The leaf samples were separated into lamina (the blade part of leaf) and sheath (the basal part of leaf) tissues and the concentrations of IBP and its metabolites inside these tissues were measured. The system was continuously operated over a long-term period of 342 days. From the 62nd day onwards, the rhizosphere samples (sediment mixture) near plant roots at 1/3L and 2/3L were collected using peristaltic pump, respectively, in five sampling periods with an interval of 40 days. In each period that lasted 24 days, rhizosphere samples (50 mL each) were collected every eight days (including the initial day) and the total four samples at each location were mixed as a composite sample for bacterial DNA extraction. In the