Science of the Total Environment 651 (2019) 1926–1934

Contents lists available at ScienceDirect

Science of the Total Environment

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

Microbial community metabolic profiles in saturated constructed wetlands treating iohexol and ibuprofen

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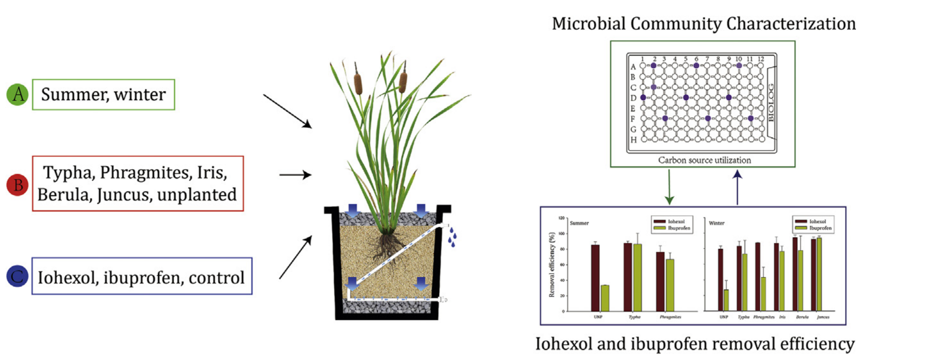
HIGHLIGHTS

- Iohexol and ibuprofen at 100 μg L^{-1} does not influence the microbial community.
- Microbial community metabolic profiles are shaped by plant presence and species.
- Microbial activity and richness were higher in planted mesocosms.
- Utilization of specific carbon sources was linked with iohexol and ibuprofen removal.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 April 2018
Received in revised form 8 October 2018
Accepted 8 October 2018
Available online 10 October 2018

Keywords:
Carbon source
Community-level physiological profiling
Pharmaceuticals
Plant species
Season
Water treatment

ABSTRACT

The aim of the present study was to elucidate the microbial community metabolic profiles in saturated constructed wetland (CW) mesocosms planted with five different wetland plant species fed with water individually spiked with 100 μg L^{-1} ibuprofen or iohexol. Community-level physiological profiling (CLPP) using Biolog Ecoplates was performed and coupled with the assessment of water quality parameters (water temperature, pH, DO and TOC, TN, NH_4-N, PO_4-P removal efficiency). The microbial community metabolic profiles (microbial activity, richness, and carbon source utilization), as well as the water quality parameters revealed similar trends among the control mesocosms and the mesocosms fed with water spiked with iohexol and ibuprofen. Significant differences were observed between the planted and unplanted mesocosms and between seasons (summer and winter) within each of the feeding lines (control, iohexol or ibuprofen). The microbial community metabolic profiles in the saturated CW were shaped by plant presence and plant species, while no negative impact of iohexol and ibuprofen presence was noticed at the 100 μg L^{-1}. In addition, the microbial activity and richness were generally higher in planted mesocosms than in the unplanted systems in the summer. For the first time, a positive correlation between iohexol removal and the microbial community metabolic profiles (activity, richness and

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https://doi.org/10.1016/j.scitotenv.2018.10.103

amines and amides utilization in summer, and carbohydrates utilization in winter) in the saturated mesocosms was observed. Putrescine utilization in the summer and D-cellobiose, D,L-alpha-glycerol phosphate in winter were linked with the metabolic processing of iohexol, while glycogen in summer and L-phenylalanine, Glycyl-L-glutamic acid in winter were linked with ibuprofen removal efficiency in the saturated CW.

1. Introduction

Emerging organic pollutants, such as pharmaceuticals have been detected in the aquatic environment (Xu et al., 2007). Toxic effects of these compounds have been observed in aquatic organisms (Mottaleb, 2015), which could eventually pose a risk to human health and the environment (Hernando et al., 2006; Safe, 2000). Ibuprofen is an anti-inflammatory agent frequently used and demonstrated to be toxic to aquatic organisms at the μg L^{-1} concentration level (Ericson et al., 2010; Santos et al., 2010). Ibuprofen has been quantified in WWTP effluent on the order of ng to μg L^{-1} (Pal et al., 2010). Iohexol is an iodinated contrast media agent commonly used in hospitals. Even though no direct evidence has shown adverse effects on aquatic organisms or humans, high concentrations are found in European WWTPs, which has led to general concern and further research requirements (Loos et al., 2013). Overall, pharmaceutical removal has been a principal issue of wastewater treatment over the last decade.

There is a general consensus that constructed wetlands (CWs), as a sustainable and cost-efficient alternative for wastewater treatment, can also efficiently degrade a number of pharmaceuticals (Verlicchi and Zambello, 2013). The relevant removal mechanisms of pharmaceuticals in CWs have been attributed to phytodegradation, plant uptake, biodegradation, sorption to the substrate, and photodegradation, depending on the CW design used (García et al., 2010; Li et al., 2014). Previous studies have shown that ibuprofen is a biodegradable compound within CWs (Dordio et al., 2010; Zhang et al., 2016), and more specifically in saturated CW mesocosms (Zhang et al., 2016). Seitz et al. (2008) showed that iohexol can be recalcitrant and only partially removed by ozonation. However, we demonstrated that iohexol can be efficiently removed from hydroponic solution by *Phragmites australis* (Zhang et al., 2015), as well as in saturated constructed wetlands (Zhang et al., 2016). Additionally, the mass balance and regression analysis pointed to biodegradation as the main mechanism for its removal, while for both compounds ibuprofen and iohexol, the removal rates differed according to plant species (Zhang et al., 2016). However, previously, it was not possible to conclude whether the biodegradation resulted from microbial degradation and/or metabolization within plant tissue is still unclear. Up to 2016, from the 32 publications studying pharmaceutical and microbial communities in CWs, only 7 studies have looked at microbial community function (Weber, 2016). The majority of these studies were looking to understand the impacts of compounds such as antibiotics on the inherent microbial community and any potential long-term impacts to CW health and water treatment capabilities. However, the role of microbial community function and metabolic pathways for pharmaceutical biodegradation in CWs are still unknown.

Previous research has shown that plant species selection has an influence on the microbial community functional profiles in CWs treating pharmaceuticals (Zhang et al., 2017). Lyu et al. (2016) demonstrated that plant species determined the microbial community profiles in CWs with no impact from the presence of pesticides. As for concentration, Weber et al. (2011) showed the presence of ciprofloxacin had an adverse effect on the microbial communities in CWs at a concentration of 2 mg L^{-1}. However, relatively little is known as to whether pharmaceutical presence at realistic environmental concentrations (ng L^{-1}–μg L^{-1}) can result in a discernable microbial community response. For example, by impacting the behavior of plants that shape the biofilm microbial community and subsequent microbial biodegradation. Additionally, season is widely accepted as a main factor for influencing microbial community in CWs (Stein and Hook, 2005), including when pesticides are present in the treated water (Lyu et al., 2016). However, research on seasonal effects on microbial communities in CWs treating pharmaceuticals has not yet been conducted. There is a need for better understanding the microbial community metabolic profiles in CWs, both in relation to the role of plants and seasonality. In addition, little is known regarding the relationship between microbial community function and the biodegradation of pharmaceutical compounds, including iohexol and ibuprofen.

The main aim of the present study was to elucidate the microbial community metabolic profile in saturated CW mesocosms planted with five different wetland plant species fed with water individually spiked with ibuprofen and iohexol in both summer and winter. In addition, the relationship between the microbial community metabolic profiles and pharmaceutical removal was assessed.

2. Materials and methods

2.1. Experimental setup

The experiment was conducted under a glass-roof for protection against rain and snow but ensuring outdoor environmental conditions. Five emergent plant species were used: *Typha latifolia*, *Phragmites australis*, *Iris pseudacorus*, *Berula erecta* and *Juncus effusus*. Full details of the experimental setup are described elsewhere (Zhang et al., 2016). Briefly, eighteen mesocosms (5 planted, 1 unplanted, triplicated) filled with quartz sand (particle size 0.5–1 mm with average porosity of 37%), were connected to a 350 L influent tank via a PE pipe (Fig. 1). The influent water was prepared with tap water, 100 mg L^{-1} "Pioner Grøn" (Brøste Group, Denmark) N: P: K full strength nutrients and acetic acid (20 mg L^{-1} total organic carbon (TOC)). Dedicated influent tanks were used for each pharmaceutical spiking and for the uncontaminated control. The compounds were spiked in the influent tanks periodically, as needed to replenish the tank capacity. The systems have been previously tested with 10 and 100 μg L^{-1} pharmaceutical concentration level, and subject to different hydraulic loading rates (HLRs) (0.7–13.8 cm d^{-1}) in both summer and winter as detailed elsewhere (Zhang et al., 2016). For the present study, mesocosms were sampled in September 2014 (Summer) and in March 2015 (Winter) after two weeks' stabilization at a HLR of 3.4 cm d^{-1} and a pharmaceutical exposure concertation of 100 μg L^{-1}. Sampling was performed at the end of each season to avoid disturbances to system performance. The daytime in summer and winter was 14 h and 6 h, respectively. The average measured air temperature and relative humidity were 26.7 ± 4.3 °C and 51.8 ± 12.7% respectively in summer and 6.1 ± 2.2 °C and 82.3 ± 5.4% respectively in winter.

2.2. Sampling strategy

The three working lines (ibuprofen, iohexol and unspiked control) were sampled in both seasons for water quality parameters (as described elsewhere (Zhang et al., 2016)) and for community-level physiological profiling (CLPP). For the CLPP, before collection, each mesocosm was shaken laterally. Afterwards, the initial interstitial water was discharged from the bottom outlet of the mesocosm. The