Water Research 110 (2017) 241–251

Contents lists available at ScienceDirect

Water Research

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

Functionality of microbial communities in constructed wetlands used for pesticide remediation: Influence of system design and sampling strategy

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ARTICLE INFO

Article history:
Received 3 August 2016
Received in revised form
29 November 2016
Accepted 14 December 2016
Available online 18 December 2016

Keywords:
Community level physiological profiling (CLPP)
Constructed wetland
Emerging organic contaminants
Pesticides
Phytoremediation
Wetland plant

ABSTRACT

The objective of this study was to compare the microbial community metabolic function from both unsaturated and saturated constructed wetland mesocosms (CWs) when treating the pesticide tebuconazole. The comparison was performed for both interstitial water and substrate biofilm by community level physiological profiling (CLPP) via BIOLOG™ EcoPlates. For each CW design (saturated or unsaturated), six mesocosms were established including one unplanted and five planted individually with either *Juncus effusus, Typha latifolia*, *Berula erecta, Phragmites australis* or *Iris pseudacorus*. Microbial activity and metabolic richness of interstitial water from unsaturated CWs were significantly lower than that from saturated CWs. However, in general, the opposite result was observed for biofilm samples. Wetland plants promoted significantly higher biofilm microbial activity and metabolic richness than unplanted CWs in both CW designs. Differences in the microbial community functional profiles between plant species were only found for saturated CWs. Biofilm microbial metabolic richness was generally statistically higher than that of interstitial water in both unsaturated (1.4–24 times higher) and saturated (1.2–1.7 times higher) CWs. Carbon source (guild) utilization patterns were generally different between interstitial water and biofilm samples. Functionality of the biofilm microbial community was positively correlated to the removal of all pollutants (TN, NH_4^+-N, TP, TOC and tebuconazole) for both unsaturated and saturated CWs, suggesting the biofilm plays a more important role in pollutant removal than the interstitial water microbial community. Thus, merely observing the interstitial water microbial communities may underestimate the role of the microbial community in CW performance. Interestingly, the ability for the biofilm microbial community to utilize amino acids and amines/amides was positively correlated with tebuconazole removal in all system types.

1. Introduction

Constructed wetlands (CWs), as a cost-effective, robust and sustainable technology, have become one of the most commonly employed decentralized wastewater treatments for various inorganic and organic pollutants (Brix, 1999; Vymazal and Březinová, 2015). Microorganisms have been demonstrated to play a key role on pollutant removal in CWs, such as organic matter (Zhang et al., 2015), nitrogen (Kaseva, 2004), industrial organic pollutants (Lin et al., 2012) and emerging organic contaminants (Fernandes et al., 2015). Recently, there has been increased interest in the study of microorganism in CWs for wastewater treatment to fully understand the pollutant removal mechanisms.

Microbial community structure was shown to be altered after exposure to a cocktail of the pesticides diuron, 3, 4-dichloroaniline and glyphosate in a natural saturated surface flow CW application (stormwater basin) (Bois et al., 2011). However, to our knowledge, there is no study that compares microbial communities in different CW designs with different wetland plant species treating pesticides. The presence of different plants (*Phragmites australis* and *Phalaris arundinacea*) can alter microbial community metabolic function in saturated horizontal subsurface flow CWs (Button et al.,

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http://dx.doi.org/10.1016/j.watres.2016.12.021
0043-1354/

2016). A previous study performed by the authors (Lv et al., 2016a) indicated that plant species and not pesticide (imazalil or tebuconazole) presence significantly drive the microbial community activity and richness in saturated CWs. Moreover, the microbial community metabolic function was demonstrated to be significantly different between different CW designs (unsaturated vertical flow and saturated horizontal flow CWs) when treating domestic wastewater (Button et al., 2015). However, the microbial community functionality in different CW designs when treating pesticides are still unknown. Additionally, the plant effect on microbial community metabolic function has been mainly studied in saturated CWs (Button et al., 2016; Lv et al., 2016a). There is a large knowledge gap regarding whether the plant effects still occur in unsaturated CWs, such as vertical flow CWs. To the best of our knowledge, the present study is the first to address this point.

Microbial communities can exist as free-floating microorganisms in interstitial water or attached to the substrate and plant roots in CWs (Weber and Gagnon, 2014). Previous studies have analysed independently water samples (Ibekwe et al., 2016; Lloyd et al., 2004) or substrate samples (Chen et al., 2015; Coban et al., 2015) to understand the microorganism characteristic in CWs. To the best of our knowledge, there are only two studies in the literature focused on saturated CWs (Gagnon et al., 2007; Weber and Legge, 2013), in which quantitative observations of microbial activity supported the idea that the interstitial water microbial community, although present, played a relatively small role in contaminant removal when compared to substrate or rhizospheric bound microbial communities. However, in different CW designs, comparison of microorganisms in water and substrate biofilm samples has not been studied. Different CW designs, namely traditional saturated (horizontal subsurface and free water-surface systems) and unsaturated (vertical systems) with different oxygen levels and water contact time are expected to differently shape the microbial community. Thus, study of microbial communities in both water and substrate biofilm in different CW designs would contribute to better understand pollutant removal mechanisms.

We used community level physiological profiling (CLPP), using BIOLOG™ EcoPlate with multiple sole-carbon sources to evaluate microbial community metabolic function. CLPP, as an easy, accurate and rapid determination technology, has already been used to determine microbial community stabilization time of CWs (Weber and Legge, 2011) to understand the plant and seasonal effect on microbial community in CWs (Bissegger et al., 2014; Chazarenc et al., 2010), to investigate microbial community metabolic function spatial dynamics in CWs (Button et al., 2015; Weber and Legge, 2013) and to evaluate the influent wastewater quality effects on microbial community in CWs (Zhao et al., 2010).

The objective of this study was to compare the microbial community metabolic function in unsaturated and saturated constructed wetland mesocosms treating the pesticide tebuconazole. The physico-chemical properties of tebuconazole are shown in Table S1. The comparison was assessed on both interstitial water and substrate biofilm samples. Moreover, a correlational study was performed to determine the relationship between microbial community metabolic function and water quality/pesticide remediation.

2. Materials and methods

2.1. Mesocosm setup

Unsaturated and saturated constructed wetland mesocosms (CWs) were set up at the greenhouse facility of Aarhus University, Denmark, to study the removal of tebuconazole in these setups. Each CW design (saturated and unsaturated) consisted of a dedicated influent tank and 6 triplicated mesocosm types: unplanted and planted with *Juncus effusus (Juncus)*, *Typha latifolia (Typha)*, *Berula erecta (Berula)*, *Phragmites australis (Phragmites)* and *Iris pseudacorus (Iris)*. A detailed description of the experimental setup can be found in Lv et al. (2016c). Briefly, each mesocosm was set up in a 6 L plastic container (surface area of 300 cm^2) filled with a 4 cm bottom layer of gravel (8–12 mm particle size), main 10 cm layer of sand (0.5–1 mm particle size) and finally a 4 cm top layer of gravel, reaching a total depth of 18 cm. Synthetic wastewater was fed onto the mesocosm surface. For the present work, mesocosms outlet height was set at 15 and 3 cm for saturated and unsaturated CWs, respectively. The systems were initially setup in July 2014 and used for previous experiments throughout summer 2014 and winter 2015. The present work was conducted in late summer of 2015 (September), after the plants natural growing season (April to June).

The set of CWs was rain protected, but exposed to natural daily air temperature (minimum 11 °C and maximum 37 °C) and environmental light exposure variations. The systems were fed with "Pioner Grøn" (Brøste Group, Denmark) N:P:K full strength nutrient solution prepared with tap water having the following composition (mg L^{-1}): Total-N 19.3; NH_4^+-N 7.4; NO_3^--N 11.9; P 2.3; Mg 3.0; K 15.4; S 3.9. A carbon feed using acetic acid was used to simulate a 12.6 mg L^{-1} TOC influent concentration. Additionally, two different influent concentrations of tebuconazole (10 and 100 μg L^{-1}) and four different hydraulic loading rates (HLR: 1.8, 3.4, 6.9 and 13.8 cm d^{-1}) were tested along a two-month period from July to August 2015 for pollutant dynamics studies. For the present microbial study, samples were collected on September 2015 after two weeks stabilization at a HLR of 3.4 cm d^{-1} and 100 μg L^{-1} tebuconazole influent concentration level.

2.2. Sample collection

Influent and effluent samples were collected prior to the sampling for microbial studies. The pH, dissolved oxygen (DO), water temperature and electrical conductivity (EC) were measured *in*-situ using a Multi-Parameter HQ40d and a sensION + EC5 meters (HACH, Düsseldorf, Germany). Fifty milliliters for each water sample was transferred into PE bottles and preserved at −18 °C until analysis. Total nitrogen (TN) and total organic carbon (TOC) were measured by a TOC-V analyzer equipped also with a TNM-1 unit (Shimadzu, Japan). NH_4^+-N, NO_3^--N and PO_4^{3+}-P were analysed by QuikChem Methods® (10-107-06-3-D, 10-107-04-1-C, 10-115-01-1-A, respectively) on an automated flow injection analyzer (QuikChem FIA+ 8000 Series, Lachat instruments, Milwaukee, USA). For tebuconazole analysis, water samples (0.5 L) were stored at 4 °C until being processed within 48 h, while substrate samples (200 g) were kept at −20 °C until being lyophilized and further processed. Tebuconazole was analysed by an HPLC system (Thermo Scientific Ultimate 3000) equipped with a diode array detector (DAD) according to Lv et al. (2016c). Plant height and leaf chlorophyll (measured using a calibrated hand-held chlorophyll content meter, CCM-200, Opti-Science, USA) were monitored at the beginning and end of the experiment.

Before the interstitial water sample collection, each mesocosm was shaken for 1 min. For unsaturated CWs, mesocosms were shaken after plugging the bottom output and filling up with tap water. Then a mesocosm bottom output was unplugged, the first pulse (20–30 mL) discarded and the remaining effluent collected in a 1 L sterilized amber bottle. Tap water use was seen as a compromise to ensure a representative sample at a single time point, as opposed to what would be a composite collection over a period of sampling. For biofilm sampling, 300 g of substrate was taken at the middle depth (9 cm) of each mesocosm and stored in