Bioresource Technology 182 (2015) 26–33

Contents lists available at ScienceDirect

Bioresource Technology

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

Microbial community dynamics associated with veterinary antibiotics removal in constructed wetlands microcosms

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HIGHLIGHTS

- Constructed wetlands (CWs) microcosms were used to remove veterinary antibiotics.
- Response of the microbial community to enrofloxacin and tetracycline was evaluated.
- CWs microbial communities were able to adapt to the presence of antibiotics.
- No significant changes in microbial abundance or diversity were observed.
- Depuration capacity was not affected by the presence of antibiotics.

ARTICLE INFO

Article history:
Received 14 November 2014
Received in revised form 17 January 2015
Accepted 23 January 2015
Available online 31 January 2015

Keywords:
Constructed wetlands
Microbial community
Veterinary antibiotics

ABSTRACT

This study aimed to evaluate the response of the microbial community from CWs microcosms tested for the removal of two veterinary antibiotics, enrofloxacin (ENR) and tetracycline (TET), from livestock industry wastewater. Three treatments were tested (control, ENR or TET (100 μg L^{-1})) over 12 weeks in microcosms unplanted and planted with *Phragmites australis*. CWs removal efficiency was relatively stable along time, with removals higher than 98% for ENR and 94% for TET. In addition, CWs were able to reduce wastewater toxicity, independently of antibiotics presence. Despite no significant differences were observed in terms of microbial abundance, bacterial richness or diversity, analysis of similarities (two-way crossed ANOSIM) showed a significant effect of both time and treatments in bacterial community structure. This study points to CWs applicability for veterinary antibiotics removal from livestock wastewaters, showing that CWs microbial communities were able to adapt without significant changes in their diversity or depuration capacity.

1. Introduction

Pharmaceuticals are classified as emerging environmental pollutants being used in large scale in human and veterinary medicine. These compounds are produced to have a low biodegradability and high water solubility (Zhou et al., 2009). Pharmaceuticals use in livestock industries has increased over past few years to protect from or cure various diseases. In several parts of the world, like Europe, US or UK, the presence of numerous classes of antibiotics in water matrixes has been reported, being some of them known for their environmental persistence (Zhang et al., 2014).

Antibiotics or their active compounds can enter the water system, either directly through effluent discharges or indirectly through soil lixiviation when manure is used as organic fertilizer in agriculture (Carvalho et al., 2014). In the first case, conventional wastewater treatment plants are generally not capable or equipped to remove these compounds from wastewater, therefore, they are released without efficient treatment. Other effective technologies do exist such as advanced oxidation processes, adsorption by activated carbon or membrane bioreactors (MBR) but they entail cost effectiveness (Luo et al., 2014; Zhang et al., 2014).

Even though antibiotics are found at low concentrations in the environment, they can cause serious toxic effects in organisms and promote antibiotic resistance. Furthermore, high concentrations of antibiotics in wastewater can affect biological wastewater treatment in terms of stability and performance due to the resilient

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http://dx.doi.org/10.1016/j.biortech.2015.01.096
0960-8524/

bacteriostatic effects of antibiotics (Deng et al., 2011). In addition, antibiotics can cause changes in the microbial community present in the biological treatment (Deng et al., 2011).

A potential and sustainable alternative to remove antibiotics from wastewaters is constructed wetlands (CWs). This technology can be used as a secondary or tertiary treatment and is designed to mimic natural wetlands, being based on the interactions among soil/sediment, plant and microorganisms to remove contaminants from effluents. Advantages of this technology are low costs, easiness of operation and maintenance, high quality effluent with less energy dissipation and strong potential for application in developing countries, particularly in small rural communities (Carvalho et al., 2012). However, this technology viability requires ample understanding of mechanisms removal, toxicity risks, environmental factors influence, removal efficiencies and design impacts (Li et al., 2014). These planted systems rely on the simultaneous occurrence of several complexes physical, chemical and biological processes, including sorption and sedimentation, photolysis, hydrolysis, volatilization, plant uptake and accumulation, plant exudation and microbial degradation (Garcia-Rodríguez et al., 2014).

Constructed wetlands efficiency for removal of conventional parameters like biochemical oxygen demand, chemical oxygen demand (COD), total suspended solids (TSS), and nutrients from different wastewaters, including livestock industry wastewaters, was already reported (Meers et al., 2008). In addition, application of CWs for pharmaceutical compounds removal from urban wastewaters has also been widely reported (e.g. Garcia-Rodríguez et al., 2014; Li et al., 2014; Zhang et al., 2014). However, pharmaceuticals removal from livestock industry wastewaters in CWs has been only recently reported and by very few works (Xian et al., 2010; Hussain et al., 2012; Carvalho et al., 2013a). These effluents normally have much higher organic contents (including hardly degradable organic compounds) than those from domestic wastewaters, which makes them more difficult to treat.

Microbial communities present in CWs have an important role in water quality improvement. Several biological processes occur in CWs like ammonia oxidation, denitrification and nitrogen fixation, which are mediated through different types of bacteria. Antibiotic presence, which can occur in livestock effluents, can affect depuration and purifying properties of CWs as well their functionality (Berglund et al., 2014). So, evaluating if antibiotics can affect CWs' microbial communities is necessary to fully validate this technology application.

This research purpose was to study the response of the microbial community from CWs microcosms used in a parallel study (Carvalho et al., 2013a) to evaluate removal of two veterinary drugs, enrofloxacin (ENR) and tetracycline (TET), from livestock industry wastewater. These compounds belong to two different antibiotic families: fluoroquinolones (ENR) and tetracyclines (TET). They were chosen due to their high therapeutic use in Portuguese livestock industry.

2. Methods

2.1. Sampling and microcosms assembly

Full details on sampling and microcosms' assembly can be found in Carvalho et al. (2013a). Briefly, plants (*Phragmites australis*) with sediment attached to their roots (rhizosediment) were collected in Lima River (North of Portugal) in April 2012. Sand was collected simultaneously in the river basin (within 1 m of plant stands). In the laboratory, sediment was separated from plant roots and mixed thoroughly with sand (1:1 proportion) to prepare roots' bed substrate. A small portion of the rhizosediment was maintained at −20 °C for posterior microbial community analysis (initial characterization).

Microcosms were set up in plastic containers (0.4 m × 0.3 m × 0.3 m) with 4 cm layer of gravel, 2 cm layer of lava rock and 10 cm layer of roots' bed substrate. Half of the microcosms were planted with *P. australis* (9 systems), whereas the other half was left unplanted (9 systems). Each system was wrapped in aluminum foil to avoid light penetration into the substrate, simulating a real system. Microcosms were designed to operate in batch mode having only a tap at plastic containers base for sample collection.

Wastewater (after being treated in two lagoons, first anaerobic and second aerobic) was collected every week in a pig farm, having on average 873 mg L^{-1} NH_4^+, 51 mg L^{-1} PO_4^{3-}, 1042 mg L^{-1} COD, 340 mg L^{-1} TSS, 279 mg L^{-1} VSS and pH of 8.04 (adapted from Carvalho et al., 2013a).

Three treatments were tested in parallel in planted and unplanted microcosms (in a total of 6 treatments): one only with wastewater (control), one with wastewater doped with 100 μg L^{-1} of ENR and another with wastewater doped with 100 μg L^{-1} of TET. This tested concentration, although relatively high, has already been found in wastewaters effluents (Babić et al., 2010).

The wastewater was maintained in the systems for one week (7 days, an hydraulic retention time (HRT) frequently used in full scale CWs), being replaced every week by new doped wastewater, corresponding to an influent hydraulic load of 4.95×10^{-5} $m^3/m^2/h$ (1.18×10^{-3} m^3/m^2/day). Every day the wastewater was recycled to prevent development of anoxic areas within roots' bed substrates.

Microcosms were kept under greenhouse conditions, subjected to environmental temperature variations (minimum 16 ± 2 °C and maximum 28 ± 8 °C) and environmental light exposure, along twelve weeks (April to July).

2.2. Samples collection

Water and sediment samples were collected in planted microcosms at week 1 (W1), 2 (W2), 4 (W4), 8 (W8) and 12 (W12) and only at week 1, 2 and 4 in unplanted microcosms. The unplanted systems clogged at week 6 while the planted systems continued to work until the end of the experiment (week 12).

Water samples were collected for veterinary drugs analysis as described in Carvalho et al. (2013a) as well as for toxicity screening tests. Sediment samples were collected and stored at −20 °C for DNA extraction and drugs analysis. A portion of sediment was immediately fixed for microbial abundance estimation, as described below.

2.3. Antibiotics analysis

Antibiotics, TET and ENR, in wastewater samples were analyzed by high-performance liquid chromatography (HPLC), after a pretreatment by solid – phase extraction (SPE) (Cavenati et al., 2012). The antibiotics were also analyzed in sediment (the roots' substrate bed) using ultrasonic extraction with an appropriate solvent and analysis by HPLC (Carvalho et al., 2013b). More details can be found in Carvalho et al. (2013a).

2.4. Toxicity test

To evaluate wastewater toxicity ToxScreen test was performed. This test is based on the highly sensitivity variant of the luminescent bacterium *Photobacterium leiognathi* (test control). Thus, toxicity was evaluated through bacterial luminescence of the sample relatively to the test control (Ulitzur et al., 2002).