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Can veterinary antibiotics affect constructed wetlands performance during treatment of livestock wastewater?

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ABSTRACT

Constructed wetlands (CWs) can be used to reduce various pollutants present in livestock wastewater, such as organic matter, nutrients and metals. Very recently these systems have also been used to remove the so called emergent pollutants. These pollutants can be harmful for both microorganisms and plants, two key players in CWs removal processes. Therefore, the aim of the present work was to assess the influence of emergent pollutants, namely antibiotics, on the removal of pollutants from livestock wastewaters, as antibiotics might decrease CWs performance for the treatment of this type of wastewater.

Microcosms (0.4 m × 0.3 m × 0.3 m), simulating CWs, were assembled with *Phragmites australis* to treat livestock wastewater not doped or doped with 100 μg/L of enrofloxacin and/or of ceftiofur, two antibiotics commonly used in livestock industry. Four different treatments (Control, Enr, Cef and Mix) were tested, each in triplicate. Wastewater was treated during one-week cycle, after which it was removed and replaced by new wastewater (doped or not), in a total of 8 cycles. At weeks 1, 2, 4 and 8 treated wastewater was collected and analysed to determine removal rates of nutrients (ammonia, nitrate, nitrite and phosphate), organic matter (chemical oxygen demand (COD) and biological oxygen demand (BOD)), and solids (including total suspended solids (TSS)), as well as, veterinary antibiotics (enrofloxacin and ceftiofur).

High removal rates (up to 90% depending of the parameter) were observed independently of the presence of the veterinary antibiotics, which were also significantly removed from the wastewater. Generally, measured parameters presented values lower than those expressed in the legislation for wastewater discharge into the aquatic environment.

Present results indicate that, in tested conditions, the presence of veterinary antibiotics, namely enrofloxacin and ceftiofur, did not influence significantly the biochemical removal processes that occur naturally in CWs during treatment of livestock wastewater, the systems maintaining their performance. Therefore, CWs are a valuable alternative to remove pollutants, including antibiotics, from livestock wastewaters, reducing the impact of this type of effluents into the environment. In addition, this technology can be an efficient/economically viable technology to meet the current wastewater reuse challenges.

1. Introduction

Wastewaters (urban, agricultural, industrial, livestock production, ...) are known for having various pollutants, such as the conventional pollutants organic matter, suspended solids, nutrients and metals (Meers et al., 2008). These pollutants need to be removed before these effluents are discharged in the environment and, as such, wastewaters need proper treatment. Livestock industry effluents have, in general, much higher organic contents (including hardly degradable organic compounds) than those found in urban wastewaters, making them more difficult to treat (Meers et al., 2008). In addition, these effluents can have higher loads of pharmaceutical compounds than urban wastewaters, because a higher concentration of these drugs can be administer since a considerable high number of animals are confined into small spaces. Antibiotics are among the most widely administered drugs for animal health and management, and the presence of these compounds has been reported not only in wastewaters but also in surface waters (Hussain et al., 2012).

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Constructed wetlands (CWs) can be used to reduce/remove various pollutants present in wastewaters, including in livestock wastewaters, namely the conventional pollutants (Meers et al., 2008). CWs systems have also been widely applied for the removal of the so called emergent pollutants (e.g., pharmaceuticals) from urban wastewaters (Anning et al., 2012; Garcia-Rodríguez et al., 2014). The removal of pharmaceuticals compounds from livestock wastewaters in CWs has been only recently reported and only in very few works (Carvalho et al., 2013; Hussain et al., 2012; Xian et al., 2010). But results are also promising.

CWs are engineered systems that have been designed and built to take advantage of the natural processes involving wetland vegetation, soils/substrate and their associated microbial assemblages to assist in treating wastewater. In fact, plants and microorganisms are key players in CWs, but substrate can also have a significant role. CWs are designed to take advantage of many of the processes that occur in natural wetlands, but do so within a more controlled environment (Vymazal, 2007). Pollutants removal is achieved by a combination of physical, chemical and biological processes: sedimentation, filtration, precipitation, sorption, plant uptake, microbial decomposition, and microbial nitrogen transformations (Meers et al., 2005), in which all three CWs components (plants, microorganisms and substrates) can have a significant influence.

Emergent pollutants, particularly antibiotics, can be harmful for both microorganisms and plants, which, as mentioned, are key players in CWs removal processes. For example, there is an increasing body of evidence documenting a reduction of microbial diversity in soils contaminated with antibiotics (Jechalke et al., 2014). In addition, plant exposure to pharmaceuticals, including antibiotics, may influence, for example, plant development, due to phytotoxicity (Carvalho et al., 2014). Moreover, antibiotics in the soil may influence the plant development indirectly by disrupting soil communities (the decay in the number of soil bacteria leads to a lack of feed for soil fauna) which will influence soil functions: plant residues are decomposed slower, denitrification is slower, and therefore nutrients are recycled more slowly (Fatta-Kassinos et al., 2011). Thus, the presence of antibiotics in wastewater may affect CWs performance for the removal of conventional pollutants.

In a world in which natural resources are becoming scarce, wastewater reclamation and water reuse are of great importance. This question is of particular relevance in and rural and agriculture areas where livestock industries can be found. CWs can be a green and cheap option to treat wastewater, reducing pollutants concentrations to their minimum, with potentialities for water reuse. These systems can be used not only for removal of conventional pollutants, but also for the removal of emergent pollutants, as long as the CWs performance is not affected by the presence of these new pollutants in wastewaters.

Therefore, the main objective of the present work was to assess the influence of emergent pollutants, namely antibiotics, on the removal of conventional pollutants from livestock wastewater in CWs, as one must investigate if antibiotics decrease CWs performance for the treatment of this type of wastewater. Taking into consideration that more than one antibiotic can be present in the wastewaters and that either antagonistic or synergetic effects can occur, the influence of a mixture of two antibiotics on CWs performance was also assessed.

To attain the mentioned aim a series of experiments was carried out in controlled conditions using microcosms to simulate a sub-surface vertical flow CWs, in which livestock wastewater was doped or not with veterinary antibiotics (alone or in mixture). Wastewater was treated during one-week cycles simulating the cumulative effect of adding repetitively a new pollutant load. Two antibiotics commonly used for therapeutic and prophylactic purposes in Portuguese livestock industry were selected: enrofloxacin (Enr) and ceftiofur (Cef). These antibiotics belong to different families (fluoroquinolone (Enr) and cephalosporin (Cef)) presenting different physical-chemical properties. Small-scale experiments, like the microcosms systems used in present study, allow to fully control the experimental conditions when intending to study the influence of specific variables (in the present case the presence of antibiotics in wastewater treatment). Microcosms were planted with *Phragmites australis*, one of the plants most frequently used in CWs (Stottmeister et al., 2003). Systems were evaluated not only for the removal of conventional pollutants (organic matter, suspended solids and nutrients), but also for the removal of antibiotics.

2. Materials and methods

2.1. Microcosms experiments

Experiments were carried out in controlled conditions in microcosms simulating CWs.

Livestock wastewater (after treatment in two lagoons, one anaerobic and another aerobic), was collected every week in a pig farm. The wastewater was used as collected or spiked with one or both antibiotics.

P. australis plants were collected, in Lima River in NW Portugal. To preserve plants' rhizosphere, plants were collected with the sediment attached to their roots. In the laboratory sediment was hand removed and mixed with river sand (in a 1:2 proportion) to obtain plants roots bed substrate into which plants were transplanted (each microcosms had ca. 80 plants). The mixing of sediment with sand aimed to increase the substrate porosity.

Microcosms (0.4 m × 0.3 m × 0.3 m), simulating CWs, were assembled to treat livestock wastewater not doped (Control) or doped with 100 μg/L of enrofloxacin (Enr) or with 100 μg/L of ceftiofur (Cef) or doped with a mixture of Enr and Cef, each antibiotic with a concentration of 100 μg/L (Mix). This concentration, although relatively high, has already been found in wastewaters effluents (Babić et al., 2010). Three microcosms replicates per treatment (4 treatments: control, ENr, Cef and Mix) were assembled.

All CWs microcosms had three layers: 1st with gravel (4 cm), 2nd with lava rock (2 cm) and 3rd with the plants roots bed substrate (11 cm height). These CWs systems were based on previously used ones (Carvalho et al., 2013), that have shown to properly simulate CWs systems. All microcosms had a tap at the bottom and worked in batch mode. The wastewater was poured on top, to percolate through the different layers of the solid matrix (ca. 1 L of wastewater to have 100% water saturation, with water just below the surface) and drained out through the tap when necessary, simulating a sub-surface vertical flow CW.

The livestock wastewater was treated during 8 one-week cycles. At the beginning of each week, new wastewater (doped or not with veterinary antibiotics) was added. The wastewater was recirculated every day to prevent the formation of anaerobic areas. After each one-week cycle, treated wastewater was removed and replaced by new wastewater (doped or not). Each one-week cycle simulated a 7 days hydraulic retention time as well as the cumulative effect of adding a new pollutant load to the system each week, similar to a real CW system.

At the end of weeks 1, 2, 4 and 8 the CWs treated wastewater was collected for analysis to determine pH and removal rates of nutrients (ammonia, nitrate, nitrite and phosphate), organic matter (chemical oxygen demand (COD) and biological oxygen demand (BOD)), solids (including total suspended solids (TSS), only measured in week 8), and veterinary antibiotics (Enr and Cef). The treated wastewaters of the other weeks were discarded.