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Short Communication

Vertical up-flow constructed wetlands exhibited efficient antibiotic removal but induced antibiotic resistance genes in effluent

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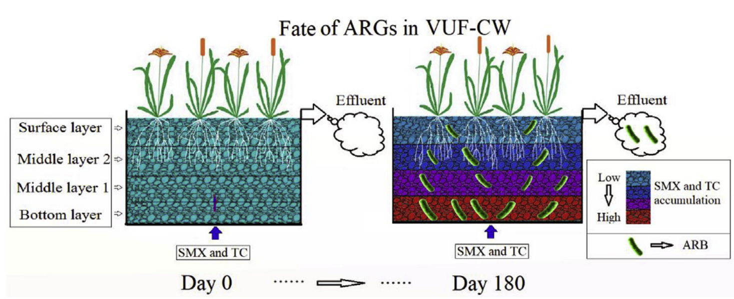
HIGHLIGHTS

- *Sul* and *tet* genes showed an increase in wetland substances.
- Effluent possessed lower abundances of ARGs than that in wetland media.
- Positive correlations between 16S rRNA and ARG copy numbers in effluent.
- ARGs may be spread via effluent due to antibiotics removal by VUF-CW.
- Elimination of total microbes in effluent might inhibit spread of ARGs.

GRAPHICAL ABSTRACT



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ABSTRACT

The intensive use of antibiotics results in their continuous release into the environment and the subsequent widespread dissemination of antibiotic resistance genes (ARGs), thus posing potential risks for public health. Although vertical up-flow constructed wetlands (VUF-CWs) have been widely used to treat wastewater in remote or rural regions, few studies have assessed the potential risks of ARG dissemination when VUF-CWs are applied to treat wastewaters containing antibiotics. In this study, the removal performance of two typical antibiotics (sulfamethoxazole (SMX) and tetracycline (TC)) and the fate of ARGs were evaluated in three lab-scale VUF-CWs. The results indicated that high removal efficiencies (>98%) could be achieved for both SMX and TC. However, the exposure of antibiotics resulted in harboring abundant ARGs (mainly *sul*- and *tet*-related genes), even with increasing abundances with operation time. The abundances of ARGs had a positive correlation with the accumulation of SMX and TC in different layers of VUF-CWs, where the *tet* and *sul* genes have the highest abundance in the bottom layer due to the highest antibiotic exposure concentration. Positive correlations were observed between the abundance of *tet* gene and antibiotic concentration in effluent. Although the effluent had lower abundances of the ARGs than that in the wetland media, the occurrence of ARGs in effluent might still

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pose risk for public health. Further studies are required to explore effective control strategies to eliminate ARGs from VUF-CWs.

1. Introduction

The intensive use of antibiotics in medical, veterinary or agriculture and aquaculture purposes results in the continuous release of antibiotics into the environment, leading to the increasingly widespread occurrence of antibiotic resistance. In particular, the release of antibiotics into water environments has caused general concern because it directly promotes the acquisition of antibiotic resistance genes (ARGs) by microorganisms, even if they are present in low concentrations (Storteboom et al., 2010; Fernandes et al., 2015; Rodriguez-Mozaz et al., 2015). Microorganisms could acquire ARGs through horizontal gene transfer (HGT), including conjugative transfer, transformation and transduction Conjugative transfer is a major pathway for the transfer of ARGs between nonpathogenic and pathogenic microbes (McKinney et al., 2010; Wu et al., 2015), which is often mediated by mobile genetic elements such as plasmids, transposons and integrons. In addition, when antibiotic resistant bacteria (ARB) are killed, DNA released to the environment can persist to be protected from DNAse, eventually transforming into other microbes (Crecchio et al., 2005; McKinney et al., 2010).

Previous studies have been conducted to investigate the occurrence, abundance and diversity of ARGs in various engineered systems, mainly focusing on activated sludge processes (Guo et al., 2017). These studies have reported wastewater treatment plants (WWTPs) could be hotspots of ARGs (Neudorf et al., 2017; Guo et al., 2018). Firstly, WWTPs receive wastewater from households and hospitals where antibiotics are used frequently, likely causing a significant ARGs presence (Rodriguez-Mozaz et al., 2015). Secondly, the persistent selective pressure from the antibiotic residues at sub-inhibitory concentrations in wastewater, as well as the dense and diverse microbial population in activated sludge will likely favor the HGT of ARGs among different microorganisms (Kim et al., 2014).

Constructed wetlands (CWs) have been designed and constructed to treat wastewater by a series of natural processes involving plants, soil/sediment, and microorganisms (Vymazal, 2011). Compared to activated sludge processes, the advantages of CWs include better wastewater purification efficiency, less energy consumption, low construction and maintenance costs, and less labor-intensive. These advantages make the CWs be environmental-friendly and economic-feasible technology for wastewater treatment in developing countries, in particular in remote or rural areas (Fernandes et al., 2015; Huang et al., 2015). While substantial studies have focused on the application of CWs for removing chemical oxygen demand (COD) and nutrients from sewage, recent research also suggested CWs potentially offer a technical option for cost-effective removal of antibiotics from various wastewaters (Huang et al., 2015). For instance, it has been reported that higher antibiotic removal efficiency was achieved in CW, compared to conventional wastewater treatment processes (Zhang et al., 2013; Liu et al., 2014; Huang et al., 2015). However, few studies assess potential removals of both antibiotics and ARGs (Anderson et al., 2013; Chen et al., 2015, 2016). In particular, the temporal and spatial changes of ARGs have not been investigated in CWs when treating wastewaters containing antibiotics.

The aims of this study are to assess the removal potential of antibiotics and ARGs, and to reveal the temporal and spatial pattern of ARGs in CWs. Considering CWs have been widely used to treat wastewater from the livestock industries that have very high concentrations of sulfamethoxazole (SMX) and tetracycline (TC)) in China (Huang et al., 2013a; Wu et al., 2015), three lab-scale vertical up-flow constructed wetlands (VUF-CWs) were established to evaluate the removal of antibiotics and the dynamic fate of ARGs during long-term operation (6 months). In addition, the occurrence and abundance of ARGs in effluent were also assessed through quantitative polymerase chain reaction (qPCR). It is expected that our results would offer insights of potential roles of CWs on the elimination or spread of ARGs.

2. Materials and methods

2.1. Configuration and operation of VUF-CWs

Experiments were performed in three lab-scale VUF-CWs with 20 cm in diameter and 55 cm in height (Fig. 1) at room temperature (28 ± 2 °C). The VUF-CW columns were filled with sand and soil (soil was obtained from the Southeast University, Nanjing, China. sand: soil volume ratio = 40:1; sand particles were 2–3 mm in diameter), which consisted of four layers, i.e. bottom layer, middle layer 1, middle layer 2, and surface layer. All layers were of the same thickness (13 cm). 10 *Oenanthe javanica* was planted in the top layer and grew vigorously. The synthetic domestic wastewater containing glucose (0.225 g L^{-1}), KH_2PO_4 (0.004 g L^{-1}), NH_4Cl (0.020 g L^{-1}), NaCl (0.15 g L^{-1}), and 0.20 mL concentrated trace element solution (Cao et al., 2015; Zhang et al., 2016). The synthetic domestic was fed continuously into the VUF-CWs at the bottom inlet using peristaltic pumps. Three systems were fed with different concentrations of SMX and TC. CW1 was used to treat wastewater with SMX of 200 μg L^{-1} and TC of 200 μg L^{-1}; CW2 was set to treat wastewater with SMX of 500 μg $L^{-1}$and TC of 500 μg L^{-1}; while CW3 was adopted to treat wastewater with SMX of 800 μg $L^{-1}$and TC of 800 μg L^{-1}. The reactors were operated indoor at 30 ± 2 °C with a relative humidity of 55–65%. The data was obtained after 1 week of stable operation. Although different antibiotic concentrations were

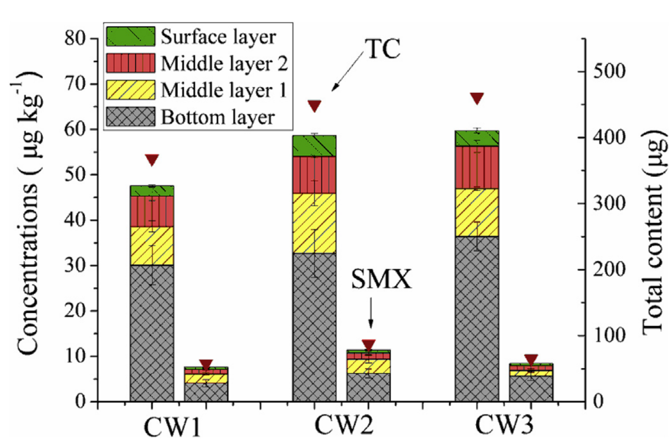

Fig. 1. Accumulation of SMX and TC in the different layers and VUF-CW.