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Removal of antibiotics and antibiotic resistance genes from domestic sewage by constructed wetlands: Optimization of wetland substrates and hydraulic loading

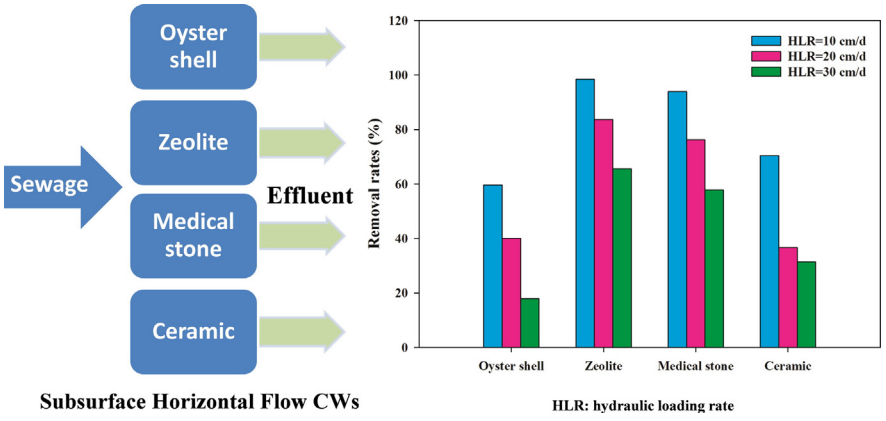
Jun Chen, Xiao-Dong Wei, You-Sheng Liu *, Guang-Guo Ying *, Shuang-Shuang Liu, Liang-Ying He, Hao-Chang Su, Li-Xin Hu, Fan-Rong Chen, Yong-Qiang Yang

State Key Laboratory of Organic Geochemistry, CAS Research Centre for Pearl River Delta Environmental Pollution and Control, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

HIGHLIGHTS

- Horizontal subsurface flow CWs with 4 substrates and 3 hydraulic loadings
- 7 antibiotics and 18 ARGs in domestic sewage significantly reduced by the CWs
- The CWs with zeolite and HLR 20 cm/day was the best choice for chemical removal.
- Sorption and biodegradation contributed to the removal of antibiotics.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aimed to assess removal potential of antibiotics and antibiotic resistance genes (ARGs) in raw domestic wastewater by various mesocosm-scale horizontal subsurface-flow constructed wetlands (CWs) planted *Cyperus alternifolius* L. with different design parameters. Twelve CWs with three hydraulic loading rates (HLR 10, 20 and 30 cm/day) and four substrates (oyster shell, zeolite, medical stone and ceramic) were set up in order to select the best optimized wetland. The result showed that 7 target antibiotics compounds including erythromycin-H_2O, lincomycin, monensin, ofloxacin, sulfamerazine, sulfamethazine and novobiocin were detected, and all selected 18 genes (three sulfonamide resistance genes (*sul*1, *sul*2 and *sul*3), four tetracycline resistance genes (*tet*G, *tet*M, *tet*O and *tet*X), two macrolide resistance genes (*erm*B and *erm*C), three quinolone resistance genes (*qnr*B, *qnr*D and *qnr*S) and four chloramphenicol resistance genes (*cml*A, *fex*A, *fex*B and *flo*R)) and two integrase genes (*int*1 and *int*2) were positively detected in the domestic wastewaters. The aqueous removal rates of the total antibiotics ranged from17.9 to 98.5%, while those for the total ARGs varied between 50.0 and 85.8% by the mesocosm-scale CWs. After considering their aqueous removal rates in combination with their mass removals, the CW with zeolite as the substrate and HLR of 20 cm/day was selected as the best choice. Combined chemical and biological analyses indicate that both microbial degradation and physical

* Corresponding authors.
E-mail addresses: guangguo.ying@gmail.com, guang-guo.ying@gig.ac.cn (G.-G. Ying).

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sorption processes were responsible for the fate of antibiotics and ARGs in the wetlands. The findings from this study suggest constructed wetlands could be a promising technology for the removal of emerging contaminants such as antibiotics and ARGs in domestic wastewater.

1. Introduction

Constructed wetlands (CWs) are artificial wetlands which are designed and constructed to manipulate the natural processes to treat wastewater, and can be classified into surface flow and subsurface flow wetlands (vertical or horizontal) according to their hydrology and flow path (Ji et al., 2002; Truu et al., 2009; Vymazal, 2010; Zhi and Ji, 2012). Compared to conventional wastewater treatment technologies, CWs have economical and eco-friendly advantages due to their low-cost, easy operation and low maintenance (Puigagut et al., 2008; Faulwetter et al., 2009). Constructed wetland systems have already been used in the treatment of a wide range of wastewaters originated from domestic (Bahgat et al., 1999; Decamp and Warren, 2001; Keffala and Ghrabi, 2005; Nurk et al., 2005; Reyes-Contreras et al., 2012; Adrados et al., 2014; Younger and Henderson, 2014), industrial (Calheiros et al., 2007; Tao et al., 2007; Younger and Henderson, 2014), and agricultural sources (Nguyen, 2000; Tanner et al., 2002; Yeh et al., 2009; Liu et al., 2013a), as well as landfill leachate (Kozub and Liehr, 1999; Sundberg et al., 2007).

Antibiotics have been widely used in human medicine and livestock animals for prophylactic, therapeutic and growth promoting purposes (Le-Minh et al., 2010; Hijosa-Valsero et al., 2011). After administration, antibiotic residues can be released into the receiving environments through discharge of the feces or urine, thus posing potential risks to human health and ecosystem (Costanzo et al., 2005; Kotzerke et al., 2008; Liu et al., 2009; Underwood et al., 2011). Use of antibiotics in humans and animals could also lead to development and spread of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment (Pruden et al., 2006; Tao et al., 2010; Su et al., 2012). Therefore, antibiotics and ARGs have been regarded as emerging environmental contaminants and detected in diverse environmental compartments (Tamminen et al., 2010; Su et al., 2012; Cheng et al., 2013; Coleman et al., 2013). It is found that ARGs can be disseminated among bacteria via vertical and horizontal gene transfer, and distributed from human and animal sources to receiving environment (He et al., 2014). However, previous studies have showed incomplete removal of various antibiotics and ARGs in conventional municipal WWTPs (Li and Zhang, 2010; Gao et al., 2012; Jia et al., 2012; Zhou et al., 2013). It is thus essential to understand the removal mechanisms of antibiotics and ARGs in wastewater by other treatment technologies such as CWs.

Currently, it has been proven that CWs can serve as a cost-effective and promising alternative to conventional wastewater treatment methods for removing or reducing a wide variety of contaminant such as nitrogen, phosphorous, and chemical oxygen demand (COD) (Lin et al., 2002; Hu et al., 2012b; Li et al., 2013; Wang et al., 2013), and even antibiotics (Hijosa-Valsero et al., 2011; Liu et al., 2013b; Berglund et al., 2014; Chen et al., 2014) and ARGs (Liu et al., 2013b; Chen et al., 2014). However, most of the previous studies focus on the single removal of nitrogen, or phosphorous, or antibiotics by CWs. Further studies are necessary to ensure the maximum removals of nutrients, antibiotics and ARGs together by CWs with the optimum wetland design and operating parameters.

The objectives of this study were: (1) to investigate the removals of COD, TN, NH3-N, antibiotics and ARGs in domestic wastewater by 12 mesocosm-scale CWs with three hydraulic loading rates (HLR 10, 20 and 30 cm/day) and four substrates (oyster shell, zeolite, medical stone and ceramic), and find out the optimum CW design and operating parameters for removing these contaminants, and (2) to understand the removal mechanisms of antibiotics and ARGs by the CWs. The results facilitate designing the best wetland systems for the treatment of wastewaters with these emerging contaminants.

2. Materials and methods

2.1. Construction of the mesocosm-scale CWs

Twelve mesocosm-scale CWs were set up using stainless steel containers (each CW: 60 cm wide, 80 cm long and 80 cm high) in the campus of Guangzhou Institute of Geochemistry in south China. The containers were filled with four substrates (oyster shell, zeolite, medical stone and ceramic) with three replicates. Each mesocosm-scale CW had a layer of 65 cm substrate and a layer of 60 cm water within the substrate (Fig. 1). In each mesocosm-scale CW, approximately 7.5×10^4 g oyster shell, or 5.5×10^5 g zeolite, or 4.0×10^5 g medical stone, or 3.0×10^5 g ceramic were used. The CWs were designed to be horizontal subsurface-flow systems planted with *Cyperus alternifolius* L. (6 plants in two rows in each system), but operated with three hydraulic loading rates (HLR: 10 cm, 20 cm and 30 cm/day). We achieved the specific HLR by flow meters.

The mesocosm-scale CWs were built to treat raw domestic sewage from the campus residential area which had around 300 people. The raw domestic sewage flow into a big sedimentation tank and then pumped to a stainless steel regulating tank of 4.3 m^3 before flowing into the mesocosm-scale CWs. The mesocosm-scale CWs had been complete constructed, operation parameters arranged, then started working without interruption from September 2013. The experiment for this study started from November 2014.

2.2. Sample collection

12 mesocosm-scale CWs named as CW-initial of substrate name-HLR, and the corresponding effluent samples and substrate samples were listed as following: influent (W0), CW-O-10 (W1 and S1), CW-O-20 (W2 and S2), CW-O-30 (W3 and S3), CW-Z-10 (W4 and S4), CW-Z-20 (W5 and S5), CW-Z-30 (W6 and S6), CW-M-10 (W7 and S7), CW-M-20 (W8 and S8), CW-M-30 (W9 and S9), CW-C-10 (W10 and S10), CW-C-20 (W11 and S11), and CW-C-30 (W12 and S12). Thirteen wastewater samples were collected as the 72-h composite samples (sampling once every 8 h) during a 3 day period. Twelve solid samples were collected after water sampling finished from the three substrate sampling points and then mixed into composite samples according to their depths (there were 3 sampling tubes in each CW, and each had 3 sampling depths) (please see Fig. 1-A and C).

For analysis of antibiotics, the water samples were collected from the influent and effluent of the water outlet 3 in each CW (Fig. 1-B). These samples were collected in 1 L precleaned brown glass bottles, about 50 mL of methanol was added to each bottle (1 L) of the water samples and the pH values of the samples were adjusted to 3 by using 4 M H_2SO_4. For analysis of ARGs, the water samples were collected as the composite samples of three water outlets (Fig. 1-A and B) in 0.5 L sterile polypropylene bottles.

The substrate samples were collected from each CW after wastewater sampling. For analysis of antibiotics, the substrate samples were collected in 1 L glass jars, while for analysis of ARGs, the substrate samples were collected in 50 mL sterile centrifuge tubes. One gram of sodium azide was added to each substrate sample to suppress microbial activity. After collection, all the samples were stored at 4 °C before analysis and processed within 48 h. The substrate samples were freeze-dried,