



Behavior of tetracycline and sulfamethazine with corresponding resistance genes from swine wastewater in pilot-scale constructed wetlands



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HIGHLIGHTS

- Removal rate of VAs from swine wastewater in CWs of different flow configurations.
- Comparison of VAs removal efficiencies of CWs at different seasonal condition.
- Response of *tet* genes and *sul* genes in pilot-scale CWs with different types.

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ABSTRACT

Four pilot-scale constructed wetlands (free water surface, SF; horizontal subsurface flow, HSF; vertical subsurface flows with different water level, VSF-L and VSF-H) were operated to assess their ability to remove sulfamethazine (SMZ) and tetracycline (TC) from wastewaters, and to investigate the abundance level of corresponding resistance genes (*sulI*, *sulII*, *tetM*, *tetW* and *tetO*) in the CWs. The results indicated that CWs could significantly reduce the concentration of antibiotics in wastewater, and the mass removal rate range of SMZ and TC were respectively 11%–95% and 85%–95% in the four systems on the basis of hydraulic equilibrium; further relatively high removal rate was observed in VSF with low water level. Seasonal condition had a significant effect on SMZ removal in the CWs (especially SMZ in SF), but TC removal in VSFs were not considered to have statistically significant differences in winter and summer. At the end period, the relative abundances of target genes in the CWs showed obvious increases compared to initial levels, ranging from 2.98×10^{-5} to 1.27×10^{-1} for *sul* genes and 4.68×10^{-6} to 1.54×10^{-1} for *tet* genes after treatment, and those abundances showed close relation to both characteristic of wastewater and configuration of CWs.

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1. Introduction

Constructed wetlands (CWs) are land-based wastewater treatment systems that have been designed and constructed to utilize natural processes to assist in treating wastewater; further three main types of CWs are typically used worldwide including: free water surface constructed wetlands (SF), horizontal subsurface flow constructed wetlands (HSF) and vertical subsurface flow constructed wetlands (VSF) [1]. With the advantages of relatively low operating costs and effectiveness in reducing nutrient mass load, CWs have proven to be alternatives or useful complements to traditional technology for treating various types of wastewater [1,2].

In recent years, concern regarding potential pollution by veterinary antibiotics (VAs) and antibiotic resistance genes (ARGs) in swine wastewater have significantly grown after confirmation of their high concentrations in wastewater, manure, soil around livestock farms and producer lagoons [3–5]. In order to decrease the load of VAs and ARGs from livestock industries discharge into the environment, a number of treatment technologies have been attempted, and the potential use of CWs also has been partially explored [6–8]. However, presently, much of the existing research on emerging contaminants removal capacities of CWs is limited to that of pharmaceuticals and personal care products (PPCPs) or low concentrations of antibiotics in urban wastewater. Currently, there is little available information about CWs treatment of swine wastewater containing higher residues of VAs, and the subsequent behavior of VAs and corresponding resistance genes from wastewater with higher concentrations of organics and nutrients in CWs

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also require further evaluation. Meanwhile, different types of CWs showed notably different specific advantages that would not only have an effect on microbial characteristics, reduction of organics and nutrients, but possibly also on VAs and ARGs behavior [1,9,10].

Thus, the objectives of this study were (1) to assess the removal efficiencies of VAs from wastewater in different pilot-scale CWs in outdoor operation for a period of more than one year; (2) to investigate the accumulation and correlation of VAs and corresponding resistance genes in CWs without effect of ARGs and metal input. The target VAs including tetracycline (TC) and sulfamethazine (SMZ), which belong to two families (tetracyclines and sulfonamides) with notably different subsurface mobility, were selected due to their extensive use in the livestock industry and their higher detection frequencies in swine wastewater [4,5,11]. Quantitative real-time PCR (Q-PCR) analysis was used to detect corresponding resistance genes: three tetracycline resistance (*tet*) genes and two sulfonamides resistance (*sul*) genes, in wetland medias; these genes were chosen because they have a greater relative abundance in both swine wastewater and soils adjacent to representative swine farms in China [5,12].

2. Materials and methods

2.1. Experimental design and setup

Four pilot-scale CWs were set up in the open air within the testing grounds of Institute of Urban Environment (Xiamen, China), and each CW differed from the other in their design parameters and flow path, which are summarized in Fig. 1 and the supporting information. The theoretical hydraulic retention time (HRT) values of SF, HSF, VSF-L and VSF-H were 15.5, 16.4, 7.3 and 14.2 days, respectively. The wastewater was infused into the four CWs by peristaltic pump, and the hydraulic loading rate (HLR) of each system was 2 cm/d. A synthetic swine wastewater prepared with tapwater was used, and the average values of composition were as follows: chemical oxygen demand (1980 mg/L), total nitrogen (722 mg/L), ammonia nitrogen (551 mg/L) and total phosphorus (83 mg/L). The average value of influent pH was 7.23. The physicochemical properties of TC and SMZ, which are shown in Table A.1 and Fig. A.1, were spiked into the wastewater to produce individual concentrations of about 30 µg/L in accordance with the relative contents level of commonly detected VAs in swine wastewater [6,11].

2.2. Veterinary antibiotics analysis and quantification of ARGs in samples

Liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS) was used to analyze the concentrations of SMZ and TC in water and wetland media (soil and oyster shell). Veterinary antibiotics analysis in the samples was based on the published method [7], and the brief direction is summarized in the supporting information. At the initial and end period (1st day and 420th), Q-PCR analysis was used to detect the corresponding resistance genes: *sull*, *sullII*, *tetM*, *tetW* and *tetO*. The determination of ARGs in samples was based on the published method [13,14], and the brief direction is summarized in the supporting information. The sampling sites were defined by the wastewater flow path in the four CWs (Fig. 1), and three samples from the wetland media (soil and oyster shell) were collected and mixed to form a composite sample during the sampling period.

2.3. Removal efficiency and statistical analysis

Due to the contaminant concentrations variation in influent and different evapotranspiration water loss in the CWs, the comparisons of more reliable VAs removal efficiencies in the CWs were

reflected by mass removal rate (MR) [15], which were calculated using the following formula: $MR (\%) = (C_i Q_i - C_e Q_e) / C_i Q_i \times 100\%$, where C_i and C_e (µg/L) were influent and effluent concentrations respectively, and Q_i and Q_e (L/day) were inlet water amount and outlet water amount respectively.

All experimental values reported in this study are the means ± S.D. of three measurements for each composite sample. Statistical analyses were conducted using SPSS Version 16.0. Comparisons of CWs removal efficiencies and the linear correlations between variables were performed with non-parametric Mann-Whitney *U* tests and Spearman coefficient (non-parametric statistics), respectively. Comparisons were considered to have statistically significant differences for $P < 0.01$.

3. Results

3.1. Removal efficiencies of SMZ and TC in CWs

The target VAs concentrations in the influent and effluent of four CWs are shown in Fig. 2. During an operation period of more than one year, the average effluent concentrations in SF, HSF, VSF-L and VSF-H were 25.58, 13.11, 4.47 and 10.94 µg/L for SMZ, and 3.91, 1.26, 0.54 and 0.77 µg/L for TC, respectively. The effluent concentration of SMZ was notably higher than that of TC in all CWs, and the effluent concentration trend for both VAs was SF > HSF > VSF-H > VSF-L. In terms of mass removal efficiency (Fig. 3), the average mass removal rates of SMZ and TC were 40% and 92% for SF, 59% and 92% for HSF, 87% and 99% for VSF-L, and 70% and 98% for VSF-H, respectively. Similar to the VAs content in effluent, TC showed a better removal efficiency in all CWs compared to SMZ, while VSF-L offered the best year-round removal rate for both SMZ and TC.

During the experiment operation, average temperatures were 13 °C in winter and 30 °C in summer. Thus, the comparisons of VAs effluent content and removal efficiencies of the four CWs at different seasonal conditions were conducted (Table 1). The results indicated that the effluent concentrations of SMZ in all CWs and TC in HSF were strongly influenced by seasonality, yet there is no correlation between TC effluent concentrations in the CWs (SF and VSFs) and seasonal condition. Meanwhile, compared to winter, summer conditions had a significantly positive effect on the removal rate of TC in SF and HSF, and SMZ in all CWs (especially SMZ in SF, $P < 0.01$). However, comparisons of removal rate of TC in the two VSFs were not considered of statistically significant difference ($P > 0.4$) in winter and summer.

3.2. Accumulation of SMZ and TC in CWs

The accumulated concentrations of SMZ and TC in the soil and oyster shell of CWs with different sampling sites were measured at the end period of our experiment (Table 2). The data illustrated that SMZ and TC in all samples, except TC in surface soil of HSF, were detected. However, the VAs concentrations in CWs were positively correlated with the type of antibiotics, and soils showed a higher sorption capacity of antibiotics from the aqueous phase than did oyster shell. SMZ concentration ranges in soil and oyster shell were 0.84–26.89 µg/kg and 1.15–10.24 µg/kg respectively, which were noticeably lower than TC (52.39–118.56 µg/kg for soil, 4.19–28.19 µg/kg for oyster shell). In term of VAs accumulated concentrations in different CWs sites, VAs content level was affected by the flow path in HSF and two VSFs, yet retained a similar content level in the two sites of SF.

3.3. Abundances of *tet* genes and *sul* genes in CWs

In the present study, the comparison and subsequent discussion were with respect to the relative abundances of ARGs, which could

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