Ecological Engineering 91 (2016) 85–92

Contents lists available at ScienceDirect

Ecological Engineering

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

Removal characteristics and mechanism of antibiotics using constructed wetlands☆

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ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form 9 December 2015
Accepted 26 January 2016
Available online 28 February 2016

Keywords:
Antibiotics
Microcosm
Phragmites australis
Adsorption
Soil
p*K*a
Photodegradation

ABSTRACT

The occurrence and removal of antibiotics (sulfamethoxazole (SMZ), sulfathiazole (SFI), sulfamethazine (SMA), trimethoprim (TMP), tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), and enrofloxacin (EFX)) using constructed wetlands (CWs) for treating livestock wastewater were examined. The levels of antibiotics in the effluents of the CWs were in the order of CTC, SFI, SMZ, SMA, TMP, OTC, EFX and TC, ranging from 47.98 to 6834.66 μg/L, respectively. There was an inverse correlation ($p < 0.0493$) in the removal of between sulfonamide group (SMZ, SFI, and SMA) and tetracycline group (TC, OTC, and CTC) antibiotics in the effluents of the CWs, indicating that sulfonamide-type antibiotics were more effectively removed in the CWs. Sulfonamide-type antibiotics also have higher p*K*a values, resulting in more effective adsorption into negatively charged soils through electrostatic interaction. Sunlight photodegradation experiment showed that EFX was effectively removed (70%) compared to other antibiotics. The microcosm adsorption experiment using wetland soils under biotic and abiotic conditions showed that antibiotics in the biotic system were more effectively removed than abiotic system, indicating that soil-mediated microbial degradation can be a major removal mechanism in the CW soils. The microcosm adsorption experiments using wetland plants (*Phragmites australis*) showed that the biotic system also removed sulfonamide-type antibiotics more effectively compared to the abiotic system. Our results suggest that the removals of antibiotics in the CWs are mainly mediated by biodegradation and adsorption onto soil and plants. Also, the physicochemical property can be the important factors in the removal of antibiotics in the CWs. Our results imply that the CW system can be used for the removal of antibiotics for treating livestock wastewaters.

1. Introduction

Numerous pharmaceutical compounds are classified as emerging contaminants compared to legacy contaminants, and have become a major threat because of their widespread use, continuous release, persistence, and the increasing evidence for their ecotoxicological (if not human health) effects (Buser et al., 1999). Among these pharmaceuticals, the global use of antibiotics was reported as 100,000–200,000 t in 2003 (Kümmerer, 2003). Antibiotic usage for animal feeds varies from 3.0 to 220.0 g/kg depending on the size and type of animal (Zhao et al., 2010). The occurrence and fate of antibiotics in livestock wastewater are major concerns, and the detection of antibiotics has been reported in a sustained manner.

Antibiotics have been widely used in livestock farms as prophylaxis (therapy) and growth promoters, and their usage in Korea was reported as 1460 t in 2003 (Jung, 2003). According to health insurance claim data in 2003, Korea had the sixth highest antibiotic usage in OECD countries (Kim et al., 2006; Kim et al., 2011). The most frequently used antibiotics in livestock farms are sulfonamide antibiotics and fluoroquinolones, which are most commonly found in swine wastewater and livestock farms, respectively (Zhao et al., 2010; Chen et al., 2012). Although trimethoprim is not actually a sulfonamide, it is often used in combination with sulfamethoxazole as cotrimoxazole (Dan et al., 2013).

The reported concentration range in swine wastewater ranged from 23.80 to 685 μg/L, and concentrations of some antibiotics varied from trace amounts to as high as ppm levels in manure slurry and wastewater (Kumar et al., 2005; Wei et al., 2011; Zhang et al., 2011; Chen et al., 2012). The National Institute of Environmental Research (NIER) in Korea reported concentrations of sulfathiazole

☆ The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: http://www.textcheck.com/certificate/JZa7Jj.

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http://dx.doi.org/10.1016/j.ecoleng.2016.01.058
0925-8574/

and chlortetracycline in livestock wastewater effluents of 659.74 and 523.60 μg/L, respectively. The high concentrations of antibiotics found in swine wastewater are caused by the poor antibiotic absorption rate of animals as 30–90% of antibiotics in the diet are excreted in unchanged form or as metabolites (Wei et al., 2011). Antibiotic contamination in sewage, agricultural wastewater, surface water, influent, and treated drinking water pose a threat to the management of water quality due to their potential adverse effects on the environment and human health (Adams et al., 2003; Yang and Carlson, 2003; Qiang and Adams, 2004; Choi et al., 2007). They may cause microbial resistance among pathogen organisms or the death of microorganisms, which are important for wastewater treatment (Aksu and Tunç, 2005). When untreated antibiotics reach rivers or lakes, the potential toxicity of antibiotics to humans through drinking water treatment plants becomes a concern (Yang and Carlson, 2003). Therefore, it is important to investigate the occurrence and removal of antibiotics from the environment, especially near livestock farms.

Wetlands are used globally to treat domestic and industrial sewage wastewater, which have been used to treat wastewater ranging from raw sewage to tertiary-treated waste streams (White et al., 2006). Among the wetland systems, constructed wetlands (CWs) have been used in swine wastewater treatment processing as post-treatment due to their ease of operation, low input requirements, and low operational cost compared to conventional technical solutions for water treatment (Kadlec and Knight, 1995; Knight et al., 2000; Poach et al., 2003). Several studies have been performed using CWs for the treatment of wastewater effluents to control organics, nutrients, and heavy metals, as well as many other components (Brix and Arias, 2005; Vymazal, 2005; Maine et al., 2006; Song et al., 2006). CWs commonly show 60–99% removal of organics, as reflected by biological oxygen demand (BOD) and chemical oxygen demand (COD), and moderate (or low) removal efficiencies for nutrients, such as ammonia, nitrate, and total phosphate (Brix and Arias, 2005; Vymazal, 2005).

Recently, the suitability of CWs for the removal of some pharmaceuticals and personal care products (PPCPs) has been assessed (Matamoros et al., 2005). Park et al. (2009) analyzed the removal of sulfapyridine and sulfamethoxazole in CWs and observed removal (30–50%) of sulfamethoxazole using full-scale surface CWs. The elimination of antibiotics from CWs can be achieved through physicochemical decomposition, photodegradation, adsorption by wetland soil and plants, and biodegradation (microbial activity). Andreozzi et al. (2003) and Matamoros et al. (2009) suggested that some PPCPs can be removed by photodegradation. Conkle et al. (2010) reported that sorption is an important removal pathway for fluoroquinolone antibiotics in wetland soil. In addition, plants growing in CWs typically exhibit several functional characteristics (e.g., transform or contain contaminants, oxygenate the systems, and provide a surface for periphyton attachment). Plants can accumulate antibiotics via water transport and passive absorption, and excessive levels of antibiotics in water or soil can exhibit significant toxicity toward plant growth and biochemical activities (Liu et al., 2009; Boonsaner and Hawker, 2010; Hillis et al., 2011; Li et al., 2011; Luo et al., 2011). The most commonly used plant in CWs is *Phragmites australis* (common reed) (Vymazal, 2011), which that can adsorb pollutants directly into its own tissues and functions as a catalyst for purification (Hadad et al., 2006).

In this study, first eight antibiotics (sulfamethoxazole (SMZ), sulfathiazole (SFI), sulfamethazine (SMA), trimethoprim (TMP), tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), and enrofloxacin (EFX)) were selected. According to Ministry of Environment report in Korea, these antibiotics were usually detected in four-river especially sulfonamide and tetracycline. Meyer et al. (1999, 2000) also reported that almost all wastewater were frequently detected chlortetracycline, sulfamethazine, lincomycin, tetracycline, and oxytetracycline in order from six pig's farm. Then, the removal characteristics and its internal mechanisms of in CWs to treat livestock wastewater flowing into the Geum River Basin in Korea were examined. The effects of physicochemical properties (e.g., molecular weight, p*K*a, and functional groups) on the removal of antibiotics in a wetland system were also examined. In addition, lab-scale microcosm experiments were performed to examine the removal mechanisms of CWs, such as sunlight photodegradation (photolysis), microbial degradation, and accumulation or adsorption by wetland soil and plants.

2. Materials and methods

2.1. Chemicals and materials

Eight antibiotics were purchased from Sigma-Aldrich (St. Louis, MO): sulfamethoxazole (SMZ; Irritant), sulfathiazole (SFI; Irritant), sulfamethazine (SMA; VETRANAL™, analytical standard), trimethoprim (TMP; ≥99% (HPLC)), tetracycline (TC; ≥98% (NT)), oxytetracycline-HCl (OTC; ≥95% (HPLC)), chlortetracycline-HCl (CTC; VETRANAL™, analytical standard), and enrofloxacin (EFX; ≥98% (HPLC)) (see Supplementary materials, Fig. S1 and Table S1). Simeton (PESTANAL®, analytical standard) from Sigma and $^{13}C_6$-sulfamethoxazole from Cambridge Isotope Labs (Andover, MA) were used as internal standards at 10 and 100 ng/L to compensate for matrix effects, respectively. Each standard solution of the antibiotics at a concentration of 0.1 g/100 mL was dissolved in methanol. All standard solutions were stored at 4 °C in the dark.

Formic acid (98%; Kanto Ltd., Tokyo, Japan), ethylenediamine tetraacetic acid (EDTA) disodium salt dehydrate, and hydrochloric acid (HCl, 37%) were purchased from Fisher Scientific Corporation (Pittsburgh, PA). All solvents and samples were filtered through 1.2 μm glass microfiber filters GF/C (47 mm circles; Whatman, Maidstone, Kent, UK) and 0.45 μm mixed cellulose ester (MCE) filters. Oasis hydrophilic–lipophilic balance (HLB) (3 cm^3, 60 mg) was purchased from Waters Oasis (Columbus, OH). Deionized water was purified using a Milli-*Q* system at 18.3 MΩ/cm in all experiments.

2.2. Constructed wetlands (CWs)

Constructed wetlands (CWs) were built in Nonsan City, Chungnamdo Province, Korea, in 2007, and its operation started in 2008. The main purpose of this wetland system was to reduce the loading of secondary piggery wastewater and stormwater runoff into nearby Geum River. The CWs consisted of six cells which were sedimentation basin (Cell 1), aeration pond (Cell 2) to enhance biological treatment, deep marsh (C3), shallow marsh (C4), another deep marsh (C5), followed by settling basin (C6) from the influent wastewater entering Cell 1 to into outlet for final discharge (Lee, 2012) (Fig. 1). The CWs has 4492 m^2 of a total surface area and 4006 m^3 of a total storage volume and 48.4 h of hydraulic retention time (HRT) from the inlet to outlet during dry days (Table S2). Two typical types of wetlands plants such as *P. australis* (PA) and *Miscanthus sacchariflorus* (MS) were planted surrounding the water zone of the CWs (Lee, 2012).

Five samples ($n = 5$) with three replicates was obtained at the CWs in May, July, September, November, and December in 2012, respectively. The samples were taken at inlet and outlet point after Cell 6 of CWs to examine removal efficiencies of antibiotics by treating the CWs.

2.3. Sunlight photodegradation experiments

Sunlight photodegradation experiments were performed in a circulating sunlight rector system consisting of a stirred reservoir