CONCENTRATION PROFILES OF TETRACYCLINE ANTIBIOTICS IN ANIMAL WASTES AND WATER RESOURCES PROXIMAL TO SWINE FARM IN JAPAN

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Abstract

The occurrence of tetracyclines (TCs), such as tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC) was investigated in aqueous samples collected from the manure treatment tanks in the swine farm by using solid-phase extraction and high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS). TCs were detected in most samples analyzed in this study; CTC showed the highest concentrations (82.2 μg/L), followed by TC and OTC. This result suggests that CTC have been used frequently in the swine farm for veterinary medicines. The concentrations of TCs in liquid samples decreased during the manure treatment process. This indicates that the activated sludge treatment of liquid manure is available to remove TCs in the swine farm. However, TCs concentrations in aqueous samples of a treatment tank containing solid manure were relatively high, implying an adsorption of TCs in sludge particles during the manure treatment process. The CTC concentrations in aqueous samples in this study were greater than the EC_{50} level of the aerobic sludge bacteria which reported previously. This indicates that TCs may have a potential risk to bacteria during the sludge treatment process and investigations on the environmental effects of these antibiotics are necessary.

Introduction

In recent years, the occurrence of pharmaceuticals and personal care products (PPCPs) has been reported in the environment[1]. While the PPCPs are detected at low concentrations in natural waters, high concentrations of antibiotics have been determined in the sewage sludge and the effluents of wastewater treatment plant (WWTP)[2]. This suggests that WWTP is the major source of PPCPs in the aquatic environment.

In addition to the WWTP, livestock farms are considered to be a potential source of pharmaceuticals and antibiotics in the environment. Veterinary antibiotics are widely used to livestock at therapeutic doses and to prevent illness in animal production. The antibiotics are also added to animal feed as growth promoting drugs. Among various antibiotics, TCs are widely used in the animal production (Fig. 1). In the United States, three million pounds of TCs are used in animal agriculture per year, and more than one million pounds are used in the poultry industry[3]. Recent studies have reported the concentrations of TCs in both liquid and solid samples from livestock farm. The high concentrations of TCs were detected in liquid manure[4] and in aqueous samples from swine waste lagoon[5]. Further, TC and OTC were determined in manure-amended soils at the concentrations of several ng/g levels[6]. These studies examined TCs concentrations in livestock samples, but little information is available on the concentrations and degradation fate of TCs during the manure treatment processes in livestock farm.

The aim of this study is to understand the status of contamination by TCs in aqueous samples from manure treatment of a large-scale swine farm in Japan. The degradation pattern of TCs was also examined during the manure treatment processes in this study.

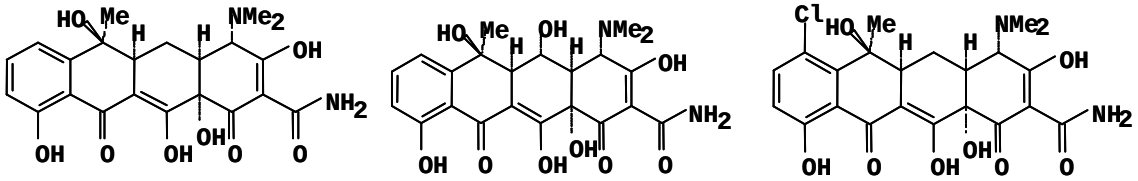

Figure 1. Chemical structures of tetracycline antibiotics (TCs) analyzed in this study

Materials and Methods

Samples: The aqueous samples collected in the manure treatment plant in the swine farm were analyzed in this study. This manure treatment plant is composed of three types of the treatment tanks. The treatment processes at the farm were shown in Figure 2. Ten aqueous samples were collected from these treatment tanks, such as 1st treatment (n=2), 2nd treatment (n=3), and 3rd treatment (n=2) tanks, effluent of the farm (n=1), and river waters proximal to the farm (n=2) in September 2006. All samples were collected in plastic bottles and stored in deep freezer until analysis.

Analytical method: Aqueous samples were analyzed using a modification of the method[5,7] with some modifications. Approximately 150-200 ml of aqueous samples was centrifuged at 7,200 *g* for 10 min to remove suspended solid in the aqueous layers. The supernatant was transferred into a glass bottle and then 2 g/L EDTA was added. The sample solvent was adjusted to pH 4 with formic acid. The sample clean up of aqueous samples was carried out with tandem solid phase extraction (SPE) cartridges, a SAX cartridge (Varian, CA, USA) and an Oasis HLB cartridge (Waters, Midford, USA). After extraction, an Oasis cartridge was dried and then eluted with 10 ml of MeOH. The eluate collected in a 10 ml plastic bottle was evaporated to near-dryness under a gentle stream of nitrogen, and it was reconstituted with 2ml of water for injection into LC-MS/MS. Mobile phases of solvents were 0.1% formic acid (solvent A) and MeOH (Solvent B). The flow rate of HPLC was 0.2 ml/min. Ionization was performed with electrospray ionization (ESI). The LC column, Waters SunFire C18 (2.1 x 150 mm, 3.5 μm) was used in this study. Quality control samples included spike recovery tests through the entire analytical procedure. As a result, recoveries of TCs ranged from 86 to 102 %.

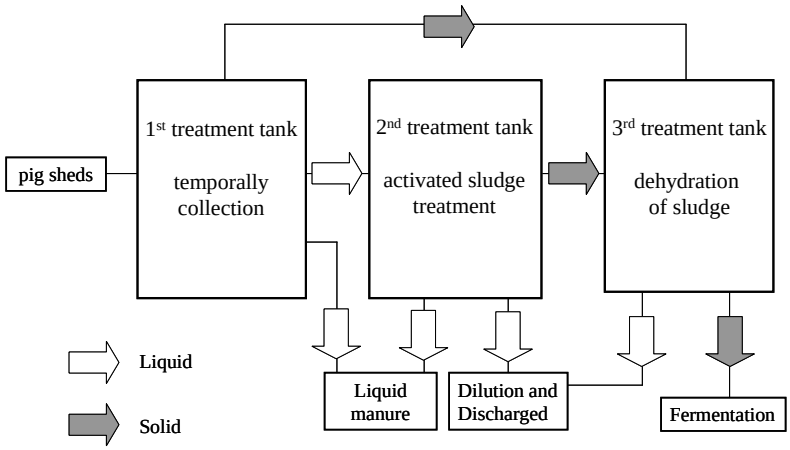

Figure 2. Schematic diagram of manure treatment tanks

Results and Discussion

The concentrations of TCs and the chromatograms were shown in Table 1 and Figure 3, respectively. Among the TCs analyzed in this study, TC, OTC and CTC were detected in all samples of the swine farm. CTC was detected

in the highest concentration (82.2 μg/L), followed by TC and OTC in 1st treatment tank. This suggests that TCs, especially CTC, have been used frequently in the swine farm for veterinarian applications. The CTC concentrations in this study are comparable with those in liquid samples of a swine lagoon in the US[8]. The concentrations of TCs in samples decreased during the manure treatment process (Table 1). The CTC concentration of aqueous samples in 1st treatment tanks was 69 μg/L, but the level decreased to 2.85 μg/L in 2nd treatment tank. The significant declines of OTC and TC concentrations were also found in samples between 1st and 2nd treatment tanks. These results indicate that the activated sludge treatment in 2nd treatment tank is available to remove TCs in the liquid layers of manure in the swine farm. In contrast, the concentrations of TCs in aqueous samples of 3rd treatment tank were almost equal to those in 1st treatment tank. Because the 3rd treatment tank contains a large amount of solid manure precipitated from 1st and 2nd treatment tanks, high TCs concentrations in 3rd tank samples implies the adsorption of TCs in sludge particles during the treatment process. The low concentrations of TC, OTC and CTC were identified in the effluent of the swine farm, and no TCs were detected in river waters at 30m and 60 m distance from the drain of the swine farm.

The concentrations of CTC in aqueous samples ranged from 48.8 to 82.2 μg/L in this study (Table 1.). These values are greater than the EC_{50} obtained in the aerobic sludge bacteria (30 μg/L[9]).These results may imply that CTC gives adverse effects to microorganisms in the manure treatment tanks. In addition, animal waste applied to agricultural field as fertilizer may act as a non-point source of antimicrobial residues in water resources.

This study reported high concentrations of TCs in liquid samples of manure treatment tanks of a swine farm. While TCs were removed from liquid samples due to the activated sludge treatment, they were potentially adsorbed by sludge particle and accumulated in solid manure. To understand the degradation and accumulation fate of TCs in manure treatment process, it is necessary to analyze solid samples in the swine farm.

Table 1. Concentrations of TCs (μg/L) in aqueous samples of manure treatment tanks and river waters proximal to a swine farm in Japan

	TC	OTC	CTC	sum
1st treatment tank				
1	19.6	10.9	55.8	86.3
2	18.8	10.2	82.2	111.2
2nd treatment tank				
1	1.13	1.34	3.08	5.55
2	0.98	1.50	2.70	5.18
3	1.03	1.54	2.77	5.34
3rd treatment tank				
1	15.8	22.3	57.1	95.2
2	14.3	18.3	48.8	81.4
Effluent of swine farm	0.61	0.78	0.88	2.27
river water				
30 m downstream	N.D.	N.D.	N.D.	
60 m downsream	N.D.	N.D.	N.D.	

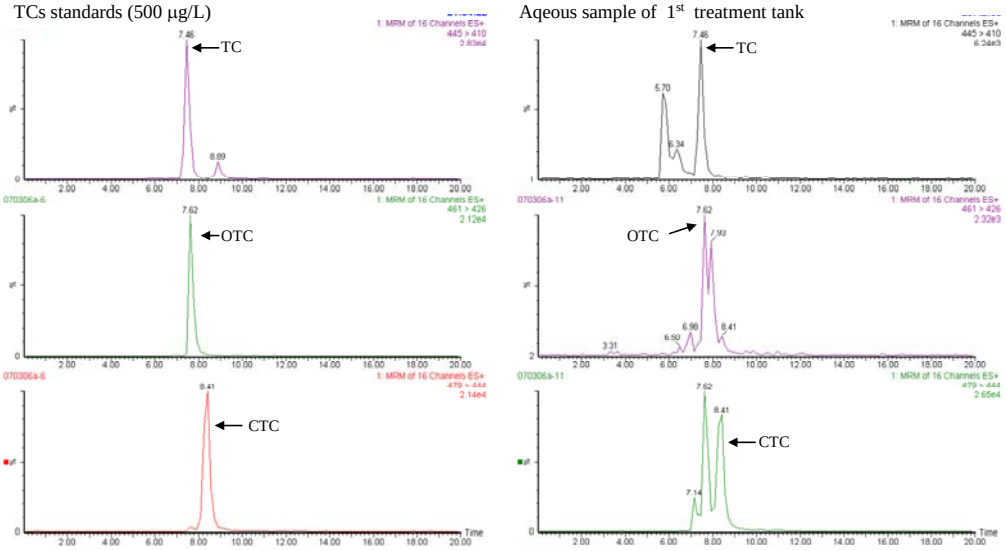

Figure 3. Chromatograms of TCs standards and aqueous sample of 1st treatment tank analyzed in this study

Acknowledgements

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