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Occurrence and Removal of Veterinary Antibiotics in Livestock Wastewater Treatment Plants, South Korea

Jin-Pil Kim [1], Dal Rae Jin [2], Wonseok Lee [3], Minhee Chae [1] and Junwon Park [4,*]

1 Geum River Environment Research Center, 182-18 Jiyong-ro, Okcheon-eup, Okcheon-gun, Chungcheongbuk-do 29027, Korea; jpkim@korea.kr (J.-P.K.); minhiec@korea.kr (M.C.)
2 Division of Water Environmental Engineering Research, National Institute of Environmental Research, 42, Hwangyeong-ro, Seo-gu, Incheon 22689, Korea; jin7425@korea.kr
3 Division of Waste-to-Energy Research, National Institute of Environmental Research, 42, Hwangyeong-ro, Seo-gu, Incheon 22689, Korea; boystone@korea.kr
4 Division of Monitoring and Analysis, Yeongsan River Basin Environmental Office, 31, Gyesuro, Seo-gu, Gwangju 61945, Korea
* Correspondence: newjjun@korea.kr

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Abstract: In this study, livestock wastewater treatment plants in South Korea were monitored to determine the characteristics of influent and effluent wastewater, containing four types of veterinary antibiotics (sulfamethazine, sulfathiazole, chlortetracycline, oxytetracycline), and the removal efficiencies of different treatment processes. Chlortetracycline had the highest average influent concentration (483.7 µg/L), followed by sulfamethazine (251.2 µg/L), sulfathiazole (230.8 µg/L) and oxytetracycline (25.7 µg/L), at five livestock wastewater treatment plants. Sulfathiazole had the highest average effluent concentration (28.2 µg/L), followed by sulfamethazine (20.8 µg/L) and chlortetracycline (11.5 µg/L), while no oxytetracycline was detected. For veterinary antibiotics in the wastewater, a removal efficiency of at least 90% was observed with five types of treatment processes, including a bio-ceramic sequencing batch reactor, liquid-phase flotation, membrane bioreactor, bioreactor plus ultrafiltration (BIOSUF) and bio best bacillus systems. Moreover, this study evaluated the removal efficiency via laboratory-scale experiments on the conventional contaminants, such as organic matter, nitrogen, phosphorus and veterinary antibiotics. This was done using the hydraulic retention time (HRT), under three temporal conditions (14 h, 18 h, 27 h), using the anaerobic–anoxic–oxic (A2O) process, in an attempt to assess the combined livestock wastewater treatment process where the livestock wastewater is treated until certain levels of water quality are achieved, and then the effluent is discharged to nearby sewage treatment plants for further treatment. The removal efficiencies of veterinary antibiotics, especially oxytetracycline and chlortetracycline, were 86.5–88.8% and 87.9–90.8%, respectively, exhibiting no significant differences under various HRT conditions. The removal efficiency of sulfamethazine was at least 20% higher at HRT = 27 h than at HRT = 14 h, indicating that sulfamethazine was efficiently removed in the A2O process with increased HRT. This study is expected to promote a comprehensive understanding of the behavior and removal of veterinary antibiotics in the livestock wastewater treatment plants of South Korea.

Keywords: veterinary antibiotic; livestock wastewater; removal efficiency; treatment process

1. Introduction

Frequent detection of veterinary antibiotics in aquatic environments, although these substances were originally intended for the prevention and treatment of animal diseases, has recently emerged as a major social issue [1]. Veterinary antibiotics discharged into the environment have been the cause of contamination in the aquatic environment from a variety of channels. Examples include inappropriate effluent treatment at manufacturing plants or livestock wastewater treatment plants, the disposal of unused antibiotics into sewers or trash, and drugs metabolized by animals or discharged intact. The long-term presence of such materials in aquatic environments, even in trace quantities of ng/L to μg/L, may have detrimental effects on an ecosystem [2]. Specifically, veterinary antibiotics often remain in the environment, and are likely to cause drug tolerance in living organisms. Consequently, global discussions are underway on potential countermeasures [3–5].

Wastewater treatment systems operating within livestock wastewater treatment plants must be investigated to ensure efficient livestock effluent management. In South Korea, livestock wastewater treatment systems are largely divided into two types: an individual treatment method, that reduces effluent contaminant concentrations below those required by water quality standards in the livestock wastewater treatment plants, and a combined treatment method, in which the livestock wastewater is treated until certain levels of water quality are achieved in the livestock wastewater treatment plants, and then the effluent is injected into nearby sewage treatment plants for additional treatment. The combined treatment method for livestock wastewater can reduce construction costs, as well as maintenance costs, thereby facilitating wastewater treatment operations. On the other hand, the combined treatment method may degrade the overall performance of the treatment processes of sewage treatment plants, because the livestock wastewater, which is pretreated and from the livestock wastewater plant, is likely to contain high contaminant loads and highly concentrated antibiotics. Therefore, it is important to remove these substances efficiently in the combined treatment process.

Over the past decade, South Korea has seen both a decrease in the use of antibiotics (during 2011), and a rebound (following 2014), for different livestock species (Table 1), and these are due to the transitory impact of the foot-and-mouth disease outbreak in 2011 [6]. These figures indicate a higher level of consumption of antibiotics per capita for livestock in South Korea compared to other countries [7], thereby drawing attention to the issue of antibiotic overuse. The consumption of livestock antibiotics in South Korea is significantly higher than that of Sweden (80 t, 2003), Denmark (one of the world's major dairy producing countries) (170 t, 1997), and even higher than England (735 t, 2003) [8].

Table 1. Sales performance of antibiotics for different livestock species (Unit: kg) [6].

Year	Cattle	Swine	Poultry	Fishery	Total
2009	63,066	551,109	205,622	178,370	998,167
2010	57,443	581,507	204,472	203,490	1,046,912
2011	57,726	459,320	199,929	239,316	956,291
2012	65,456	448,676	194,309	227,928	936,369
2013	63,538	384,296	159,290	213,235	820,359
2014	72,414	428,283	150,580	241,855	893,132
2015	71,133	480,718	156,903	200,933	909,687
2016	69,419	502,068	156,554	235,776	963,817
2017	88,741	536,431	153,563	247,841	1,026,576
2018	93,398	490,528	157,401	241,889	983,216

With the danger of antibiotics becoming a global issue, antibiotic consumption has had to be managed at the national level. In the United States, the U.S. Geological Survey (USGS) conducts full-scale research on the streams near large cities and agro-livestock industries [9], and the EU updates 33 kinds of preferred compounds every four years, and continually adds new items [10]. Other countries have actively engaged in research on the treatment of pharmaceutical wastewater, as well as the monitoring of these in public waters, together with on-going research on the pharmaceutical characteristics of different treatment processes [11,12]. Similarly, in South Korea, efforts have been made to investigate the development of pharmaceutical analytical methods, together with monitoring efforts for pharmaceuticals in public waters and in influents and effluents from sewage/wastewater treatment plants [13–17]. Despite these efforts, there is still insufficient research addressing the occurrence and removal of veterinary antibiotics in livestock wastewater treatment. In previous studies targeting antibiotics in sewage treatment plants, the detection limit precluded an accurate representation of the concentration of veterinary antibiotics. The influent concentrations in sewage treatment plants were limit of quantitation (LOQ)–0.645 μg/L for sulfathiazole, LOQ–0.343 μg/L for sulfamethazine, LOQ–0.06 μg/L for oxytetracycline, and LOQ–0.302 μg/L for chlortetracycline [16–20]. Low or even negative removal efficiencies were also obtained for sulfathiazole (ranging from −163.4% to 53.0%), sulfamethazine (12.2% to 67.4%) and chlortetracycline (−88.1% to 0%) [16–20]. The reported concentration of antibiotics detected in sewage treatment plants and the negative removal efficiency make it difficult to clearly understand the removal of antibiotics from these treatment plants, in order to then develop an efficient way of removing the livestock wastewater in combined treatment plants.

Therefore, this study monitored the livestock wastewater treatment plants operated by five major treatment processes in South Korea, in order to determine the characteristics of influent and effluent for four antibiotics, and the removal efficiencies of different treatment processes. This study conducted laboratory-scale experiments to evaluate the combined treatment method for livestock wastewater in the anaerobic–noxic–oxic (A2O) process, which is commonly used by the sewage treatment plants in South Korea. It also investigated the removal efficiency of conventional contaminants, such as organic matter, nitrogen and phosphorus, as well as veterinary antibiotics, based on the hydraulic retention time (HRT) of the treatment plants.

2. Materials and Methods

2.1. Survey Items

In this study, the selection of target indicators for monitoring was based on high levels of antibiotic resistance, high risk and high consumption. The scope of the target indicator among pharmaceuticals was limited to antibiotics because antibiotics have the greatest impact on the environment [21]. To evaluate risks, this study consulted the results of research projects on environmental risk assessment of pharmaceutical residues, conducted by the Ministry of Environment in 2011 [22]. Considering the detected concentration, ecotoxicity and unique characteristics of pharmaceuticals, this annual study selected 19 items that were expected to have high risk. Among them were 11 types of antibiotics, including amoxicillin, chlortetracycline, ciprofloxacin, enrofloxacin, erythromycin, lincomycin, neomycin, oxytetracycline, sulfamethazine, sulfathiazole and tylosin. Of these, four types of antibiotics—sulfamethazine, sulfathiazole, chlortetracycline and oxytetracycline (which are widely used in livestock farming)—were finally selected as target indicators for monitoring.

2.2. Selection of Target Treatment Plants and Sampling Method

Table 2 shows the livestock wastewater treatment plants currently operating in South Korea [23]. Survey results indicated 97 public livestock wastewater treatment plants across the country. The plants that used the five major treatment processes [bio-ceramic sequencing batch reactor (BCS), liquid-phase flotation, membrane bioreactor (MBR), bioreactor plus ultrafiltration (BIOSUF), and Biobest *Bacillus* (B3)] were selected as target monitoring plants. These five processes are popular, biological, and advanced treatment methods, and account for operations in approximately 70% of all treatment plants.

Table 2. Public livestock wastewater treatment plants in operation nationwide [23].

Treatment Process	No. of Combined Treatment Plants *	No. of Individual Treatment Plants	Total	Percentage (%)
BCS process *	14	10	24	24.7
Liquid-phase flotation process	11	11	22	22.7
HBR process *	5	1	6	6.2
MBR process *	2	1	3	3.1
BIOSUF *	7	2	9	9.3
B3 process *	4	0	4	4.1
Others	21	8	29	29.9
Total	**64**	**33**	**97**	**100.0**

* BCS: bio-ceramic sequencing batch reactor, HBR: hanmee bioreactor, MBR: membrane bioreactor, BIOSUF: bioreactor and ultrafiltration, B3: bio best bacillus. * In combined treatment plants, the livestock wastewater is treated until certain levels of water quality are achieved, and then the effluent is discharged to nearby sewage treatment plants for further treatment.

The processes and operational parameters of the target livestock wastewater treatment plants, along with the sampling sites, are shown in Table 3. The main sources of wastewater for these treatment plants are from different livestock farms (swine, poultry and fisheries). Monitoring was conducted on a seasonal basis (four times/year) from June 2013 to April 2014, and Plant E was monitored only in summer and fall. Plant A uses the BCS treatment process, in which a bioceramic carrier is placed in the sequencing batch reactor to concurrently operate inflow, aeration, sedimentation, discharge and sludge discharge. Following the biological treatment process, the influent is treated through dissolved ozone flotation (DOF), ozone, biological aerated filter (BAF), and activated carbon, before discharge. Plant B is operated by the liquid-phase flotation process, in which denitrification and nitrification take place in a liquid flotation tank. Sand filtration and activated carbon treatment processes are employed in the final clarifier after up-flow filtration using plastic filter media. Plant C treats influent wastewater using the MBR process, which immerses the pressurized ultrafiltration (UF) membrane in the aerobic tank, and the effluent discharged from the UF membrane is sent to a sewage treatment plant. Plant D operates the BIOSUF process that separates the liquids and solids in activated sludge, using a UF membrane to keep the microorganism concentration high in the aerobic tank, and to facilitate the growth of nitric oxide bacteria. This plant, without a separate post treatment facility, is linked to a sewage treatment plant. Plant E uses the B3 process, which treats wastewater with the dominant *Bacillus* bacteria cultured in column 1 of the aerobic tank. After biological treatment (the addition of coagulant and dissolved air), flotation techniques are employed for the treated effluent. In this study, the influent was collected by grab from Plants A–E after foreign substances and impurities in the livestock manure were removed by screens, detention tanks and centrifuges. Effluent was collected by grab at the site where it was released into a stream, following all the unit processes of each treatment plant or the site linked to a sewage treatment plant.

Table 3. Characteristics, treatment processes and sampling sites for different livestock wastewater plants.

Plants	Flow Rate (m^3/d)	HRT *	MLSS * Concentration (mg/L)	Treatment Processes
Plant A (BCS *)	80	20–24 h	5500–7500	Inflow→removal of impurities→vibrating screen→retention tank→centrifuge★→flow control tank→BCS (ceramic ball)→dissolved ozone flotation → biological aerated filter→activated carbon→discharge into streams★
Plant B (liquid-phase flotation)	120	20–30 day	20,000–22,000	Inflow→removal of impurities→retention tank (aeration)→centrifuge★→flow control tank (aeration)→liquid-phase flotation [denitrification tank(anoxic), nitrification tank (aeration)]→up-flow filter medium (plastic medium)→sand filter →activated carbon→combined with sewage treatment plant★
Plant C (MBR *)	100	12–18 h	10,000–11,000	Inflow→removal of impurities→primary retention tank→centrifuge★→secondary retention tank→flotation unit→flow control tank→anoxic tank→primary aerobic tank→intermittent aerobic tank→secondary aerobic tank→drum screen→perforated panel→buffer tank→sieve screen→U/F membrane→treated water tank →combined with sewage treatment plant★
Plant D (BIOSUF *)	150	18–24 h	9500–12,000	Inflow→removal of impurities→primary retention tank→centrifuge★→secondary retention tank→primary denitrification tank→sedimentation tan →secondary denitrification tank→U/F membrane→combined water tank→combined with sewage treatment plant★
Plant E (B3 *)	70	12–14 h	6000–9000	inflow→removal of impurities→centrifuge★→retention tank→aerobic tank→sedimentation tank→physicochemical treatment (ferric chloride, polymer, NaOH) →sedimentation tank (dissolved air flotation tank)→combined with sewage treatment plant★

* ★: Sampling sites. * HRT: hydraulic retention time, MLSS: mixed liquor suspended solids, BCS: bio-ceramic sequencing batch reactor, MBR: membrane bioreactor, BIOSUF: bioreactor and ultrafiltration, B3: Biobest *Bacillus.*

2.3. Sample Pretreatment and Analysis

Samples were collected and cleaned for a liquid chromatography-tandem mass spectrometry (LC-MS/MS; Agilent 1200–6410 series, Santa Clara, CA, USA) assay (Figure 1). First, 500 µL of 0.1 mg/mL Na2-EDTA (Sigma-Aldrich, St. Louis, MO, USA) and 20 µL of 0.01 mg/mL sulfamethazine-6-13C (Cambridge isotope laboratories, Tewksbury, MA, USA), which is an isotopically labeled standard for sample clean-up, were added to a 500-mL centrifuged and filtered (glass microfiber filter (GF/B), 1 µm; Whatman, Maidstone, UK) sample, and 3.5 M sulfuric acid (Wako Pure Chemical, Osaka, Japan) was used to adjust the pH level to 3. Oasis HLB (200 mg, 6 cc; Waters, Milford, MA, USA) and Oasis MCX (150 mg, 6 cc; Waters, MA, USA) cartridges were mounted on the vacuum decompression unit and the sample was prepared by releasing 2 mL ultrapure water and 2 mL methanol (J.T. Baker, Phillipsburg, NJ, USA), and allowing 2 mL sulfuric acid to pass through. The HLB cartridge, being placed on top, was connected to the MCX cartridge, and the sample was released at a rate of 10 mL/min. The samples that passed through the cartridges were separated. The HLB cartridge was washed with 1 mL ultrapure water before being eluted with 8 mL methanol. The MCX cartridge was washed with 1 mL ultrapure water, and the two cartridges were reconnected for elution with 2 mL and, subsequently, 6 mL of methanol. Next, the HLB cartridge was removed and the MCX cartridge was re-eluted with 4 mL 5% ammonia water–methanol solution. Eluent concentrate was prepared using a nitrogen evaporator, and 100 µL of 0.01 mg/mL terbuthylazine (Sigma-Aldrich, MO, USA), which is a syringe reference material, was added. The concentrate was filled with 20 mM ammonium acetate (pH 9; Sigma-Aldrich, MO, USA) up to 2.0 mL, and the residue was dissolved. The sample was filtered using a 0.2 µm syringe filter (Sartorius, Göttingen, Germany) and prepared for analysis. Considering the properties of compounds, the pretreated samples were divided into two groups for analysis purposes: Group 1 for sulfamethazine and sulfathiazole; and Group 2 for chlortetracycline and oxytetracycline. A scan mode was applied to examine the mass spectrum and a precursor ion was then selected. In addition, product ions and characteristic ions were selected for the multiple reaction monitoring analysis (Table 4).

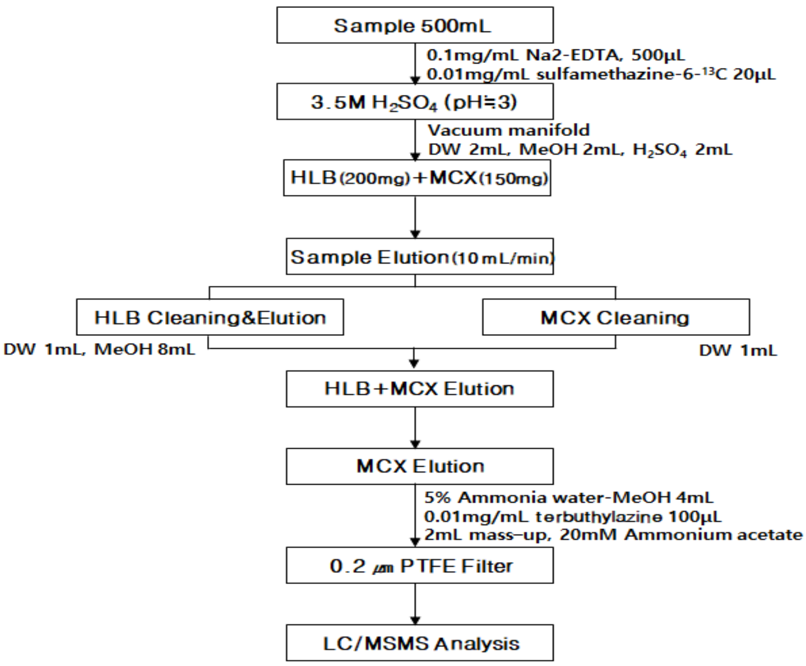

Figure 1. Sample pretreatment and analysis.

Table 4. Analytical conditions.

Parameters	Conditions
Column	UltraHT Pro C18, 50 × 2.0 mm I.d., 2 μm
Mobile phase (Group 1)	A: 20 mM ammonium acetate (pH 6.5) B: Acetonitrile
Mobile phase (Group 2)	A: 20 mM ammonium formate (0.3% formic acid) B: Acetonitrile
Column flow rate	0.3 mL/min
Injection volume	5 μL
Column temperature	40 °C
Ionization mode	ESI positive
Capillary voltage	4.5 kV
Gas temperature	350 °C
Gas flow	10 L/min (N2)
Nebulizer	45 psi

2.4. Laboratory-Scale Experimental Conditions

The removal efficiency of antibiotics for different HRTs was evaluated using three laboratory-scale A2O reactors. Table 5 shows the influent characteristics of basic items, such as laboratory-scale experimental conditions, along with chemical oxygen demand (COD), total organic carbon (TOC), total nitrogen (TN), total phosphorous (TP) and suspended solids. With the raw wastewater (swine and cattle) from the livestock wastewater treatment plant located in G city, Chungcheongbuk-do, this experiment was performed in each reactor under three HRT conditions, i.e., Lab-1 27.4 h, Lab-2 18.2 h, and Lab-3 13.7 h, for 90 days. During operation, nine samples in the influent and effluent were regularly collected at 10-d intervals for the analysis of organic matter, nitrogen and phosphorus, as well as veterinary antibiotics. The A2O process consisted of an anaerobic tank, anoxic tank and aerobic tank. To maintain anaerobic conditions during denitrification, all joints were sealed and oxygen inflow into the reactor was blocked so that nitrogen gas could circulate internally during the experiment. The aerobic tank was agitated by diffusion using a diffuser plate, and its dissolved oxygen level was maintained at 3–5 mg/L. The mixed sludge that was nitrified in the aerobic tank was transferred to the anoxic tank with an internal recirculation of 200%. With an external recirculation of 100%, solid–liquid separation was carried out in the final clarifier to ensure effluent water quality.

Table 5. Laboratory-scale experimental conditions.

Parameters	Specification		
Days of Operation (d)	90		
Temperature (°C)	16.5–23.2		
pH	5.8–7.6		
COD * (mg/L)	10,800–15,673		
TOC * (mg/L)	2291–3025		
TN * (mg/L)	6510–9550		
TP * (mg/L)	333–497		
SS * (ng/L)	9000–11,900		
	Lab-1	Lab-2	Lab-3
Flow rate (L/d)	24	36	48
HRT * (h)	27.4	18.2	13.7
MLSS * (mg/L)	3700–4800	3550–4600	3250–5050
Internal recirculation (%)	200	200	200
External recirculation (%)	100	100	100

* COD: chemical oxygen demand, TOC: total organic carbon, TN: total nitrogen, TP: total phosphorous, SS: suspended solids, HRT: hydraulic retention time, MLSS: mixed liquor suspended solids.

3. Results and Discussion

3.1. Results of Quality Control

The quality control results of the target compounds are summarized in Table 6. Sulfamethazine and sulfathiazole were classified as Group 1, while oxytetracycline and chlortetracycline were classified as Group 2. To calculate the method detection limit (MDL), the reference material was added to the effluent at the minimum detectable concentration level, and the t value at the 99% confidence level was multiplied by the standard deviation of the concentrations detected from seven repetitions of the test. The results showed that MDLs were in the range of 0.010–0.034 μg/L; similar levels of MDLs were found in sulfamethazine and chlortetracycline, while the MDL of sulfathiazole was higher. Compound-specific concentration levels, at three levels of each compound, and in a range of 0.32–5.17 μg/L for sulfamethazine, 0.78–10.10 μg/L for sulfathiazole, 12.50–125.00 μg/L for oxytetracycline and 12.50–125.00 μg/L for chlortetracycline were tested for their recovery rate. The results showed that chlortetracycline had the highest recovery of 98.1% on average, followed by oxytetracycline with 97.0%, sulfathiazole with 89.2%, and sulfamethazine with 81.2%. Sulfamethazine had a relatively low recovery (70%) at lower concentration levels, but all compounds exhibited relatively high recovery rates at higher, as well as at low, concentration levels.

Table 6. Quality control results for different compounds.

Group	Compounds	MDL* (μg/L)	LOQ * (μg/L)	Conc. (μg/L)	Recovery (%)	RSD * (%)
1	Sulfamethazine	0.011	0.035	0.32	70.2	8.78
				0.89	86.6	6.08
				5.17	86.9	5.71
	Sulfathiazole	0.034	0.108	0.78	83.4	9.46
				1.96	91.8	13.57
				10.10	92.5	2.37
2	Oxytetracycline	0.024	0.076	12.50	94.9	10.51
				25.00	98.9	5.94
				125.00	97.1	3.74
	Chlortetracycline	0.010	0.032	12.50	96.7	7.18
				25.00	102.6	4.78
				125.00	94.9	7.85
Sulfamethazine-6-13C		-	-	5.20	95.8	6.32

* MDL: method detection limit, LOQ: limit of quantitation, RSD: relative standard deviation.

The relative standard deviation (RSD; n = 7), which measures precision, was calculated as 5.7–8.8% for sulfamethazine, 2.4–13.6% for sulfathiazole, 3.7–10.5% for oxytetracycline and 4.8–7.9% for chlortetracycline, indicating that the RSDs of all target compounds were less than 15%, and satisfied the precision criteria established by EPA [24,25]. The recovery rate of the surrogate used to identify the loss of target compounds during the pre-treatment process was 95.8% (94.1% in influent and 97.5% in effluent), and the precision (n = 7) was 6.32% (7.14% in influent and 5.50% in effluent). The analytical methods employed in this study were deemed accurate and reproducible in detecting the presence of antibiotics in livestock wastewater treatment plants.

3.2. Concentration and Removal Efficiency of Antibiotics in Livestock Wastewater Treatment Plants

3.2.1. Comparison of Influent and Effluent Concentrations with Previous Studies

With respect to influent, the compound-specific detection frequencies were 100.0% for sulfamethazine and sulfathiazole, and 27.8% and 5.6% for chlortetracycline and oxytetracycline, respectively, which are relatively high compared with other studies. The concentration levels of the detected compounds are shown in Table 7 [18,26–32]

Table 7. Influent and effluent concentrations in livestock wastewater treatment plants at home and abroad (unit: μg/L).

	Influent			**Effluent**			**Country**	**Reference**
	Frequency	**Concentration**		**Frequency**	**Concentration**			
		Mean	**Range**		**Mean**	**Range**		
sulfamethazine	18/18	251.24	1.64–1629	9/18	20.82	N.D.*–115	Korea	This study
	6/8	57.8	1.76–189	4/8	11.6	0.011–25.4	Korea	[18]
	2/20	0.002	0.005–0.007	0/20	N.D	N.D	Korea	[26]
	4/4	2.928	0.597–7.995	3/4	4.448	N.D.–7.300	Korea	[27]
	8/10	148.5	N.D.–658.5	6/10	0.3236	N.D.–1.856	Korea	[28]
	7/8	29.828	N.D.–69.69	1/8	4.656	N.D.–37.24	Korea	[29]
	5/6	-	0.022–0.963	5/6	-	0.007–0.035	China	[30]
	8/8	-	35.0–45.0	16/16	-	11.07–13.15	China	[31]
sulfathiazole	18/18	230.78	0.75–922	7/18	28.20	N.D.–159	Korea	This study
	8/8	153	7.44–403	5/8	72.2	0.028–170	Korea	[18]
	0/20	N.D	N.D	0/20	N.D	N.D	Korea	[26]
	4/4	N.D	0.179–11.76	4/4	6.329	0.179–16.63	Korea	[27]
	10/10	666.6	0.193–2294	9/10	0.6567	N.D.–4.08	Korea	[28]
	8/8	317.45	1.137–659.7	7/8	30.415	N.D.–241.7	Korea	[29]
chlortetracycline	5/18	483.71	N.D.–1491	5/18	11.47	0.73–33.52	Korea	This study
	2/8	20.7	1.37–40.0	2/8	1.05	0.067–2.03	Korea	[18]
	0/20	N.D	N.D	0/20	N.D	N.D	Korea	[26]
	4/4	0.644	0.017–2.407	2/4	0.002	N.D.–0.003	Korea	[27]
	2/10	4.680	N.D.–31.08	0/10	N.D	N.D	Korea	[28]
	5/8	754.97	N.D.–2960	4/8	16.328	N.D.–129.2	Korea	[29]
	5/6	-	N.D.–1.74	6/6	-	N.D.–0.056	China	[30]
	-	-	N.D.–4.32	-	-	-	Taiwan	[32]
oxytetracycline	1/18	25.70	N.D.–25.70	0/18	-	N.D	Korea	This study
	0/8	N.D	N.D	1/8	0.42	N.D.–3.38	Korea	[18]
	0/20	N.D	N.D	0/20	N.D	N.D	Korea	[26]
	4/4	2.291	0.038–8.50	2/4	1.834	N.D.–3.65	Korea	[27]
	7/10	3.5578	N.D.–24.74	3/10	0.0928	N.D.–0.69	Korea	[28]
	1/8	19.777	N.D.–158.2	1/8	0.0928	N.D.–0.28	Korea	[29]
	6/6	-	1.76–76.4	5/6	-	N.D.–1.82	China	[30]
	8/8	-	25.0–58.0	16/16	-	2.2–4.34	China	[31]
	-	-	N.D.–5.33				Taiwan	[32]

* N.D.: Not Detected.

Sulfamethazine exhibited the highest concentration (mean: 251.2 μg/L, range: 1.6–1629.2 μg/L), and was similar to that of sulfathiazole (mean: 230.8 μg/L, range: 0.8–922.2 μg/L). Chlortetracycline was detected with the highest average concentration (mean: 483.7 μg/L, range: N.D.–1490.5 μg/L), but mainly in the samples collected in June, while oxytetracycline (mean: 25.70 μg/L) was detected in only a single sample collected in October. This can be explained by the amount of antibiotics consumed in 2014 for veterinary clinics, self-treatment, and prevention of livestock diseases (cattle, swine and poultry), having reached 97,101 kg for sulfamethazine, 16,016 kg for sulfathiazole, 112,411 kg for oxytetracycline, and 53,225 kg for chlortetracycline [6]. Compared to previous studies, the highest mean and maximum concentrations of sulfamethazine detected in the influent were observed in this study. According to National Institute of Environmental Research (NIER; 2006), the mean and maximum detected concentrations of sulfamethazine were 666.6 μg/L and 2294.0 μg/L, respectively [28]; the highest mean (755.0 μg/L) and maximum (2960.0 μg/L) concentrations were reported by NIER (2007) [29], indicating that major pharmaceuticals have been detected at high concentrations from the influent in South Korea. However, the influent concentrations of oxytetracycline detected in other countries were relatively similar to those in South Korea. For example, in China, Zhang et al. (2018) and Liu et al. (2013) reported ranges of concentrations of detected oxytetracycline in influents of 1.76–76.4 μg/L [30] and 25.0–58.0 μg/L [31], respectively. The influent concentrations of oxytetracycline in Taiwan ranged from N.D. to 5.33 μg/L [32].

With respect to the effluent, the detection frequencies of sulfamethazine, sulfathiazole and oxytetracycline were reported as 60.0%, 90.0% and 30.0%, respectively, in NIER (2006) [28]. Being similar to each other, sulfathiazole (mean: 28.2 μg/L, range: N.D.–158.7 μg/L) and sulfamethazine (mean: 20.8 μg/L, range: N.D.–115.9 μg/L) were detected at the highest concentrations. They were followed by chlortetracycline (mean: 11.5 μg/L, range: 0.7–33.5 μg/L), and no oxytetracycline was detected. Compared to previous studies, the highest compound-specific detection concentration for the effluent was reported in this study, but the high concentration was only observed from a sample collected in October. NIER (2007) reported the highest mean concentrations of sulfathiazole and chlortetracycline detected in the effluent (30.4 μg/L, and 16.3 μg/L, respectively) [29], and Liu et al. (2013) reported relatively high concentrations (2.2–4.34 μg/L) of oxytetracycline [31]. A comparison was made between detection concentrations by country, and the results showed that sulfamethazine and chlortetracycline were detected at the highest concentrations in South Korea, and high concentration levels of oxytetracycline were detected in the influent of South Korea, as compared to China and Taiwan. Higher concentrations of antibiotics in the effluent of wastewater treatment plants were also observed in South Korea, which highlights the need to develop policies and regulations on the discharge of pharmaceuticals into the environment.

3.2.2. Seasonal Influent Characteristics

The antibiotics investigated in this study are not used for promoting livestock growth because, in 2011, the Korean government banned the inclusion of antibiotics in livestock feed as growth promoters [21]. Specific pharmaceuticals are used to prevent seasonal epidemics depending on the pathogen. This study conducted an analysis of influent samples to determine seasonal usage behaviors for pharmaceuticals (Figure 2). The seasonal concentrations detected in influents were investigated for different pharmaceuticals, and the results showed that no seasonal concentration differences were found in sulfamethazine and sulfathiazole. The mean seasonal concentrations of sulfamethazine detected in influents were 221.3 μg/L in spring, 332.8 μg/L in summer, 203.0 μg/L in fall and 239.5 μg/L in winter, and for sulfathiazole they were 188.5 μg/L in spring, 236.2 μg/L in summer, 255.5 μg/L in fall and 252.1 μg/L in winter, ranging from 180 μg/L to 330 μg/Lt regardless of the season. No chlortetracycline concentrations were detected in spring and winter, and the mean concentrations were 504.5 μg/L and 379.7 μg/L in summer and fall, respectively. Oxytetracycline was detected only one time in fall. The seasonal characteristics of the chlortetracycline inflow rate can be attributed to the effects of its increased consumption for preventing *E. coli* and *Salmonella*, which are susceptible to chlortetracycline, during summertime, when incidence of these diseases is highest.

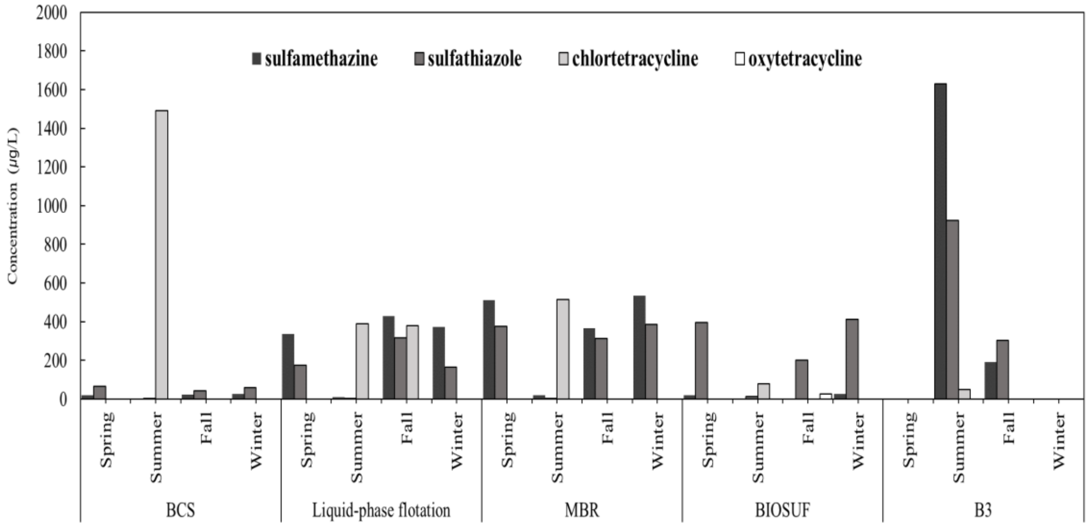

Figure 2. Seasonal antibiotic concentrations detected in influents from targeted livestock wastewater treatment plants (no data in spring and winter at B3). BCS: bio-ceramic sequencing batch reactor, MBR: membrane bioreactor, BIOSUF: bioreactor and ultrafiltration, and B3: Biobest *Bacillus*.

The regional concentrations of antibiotics were also investigated. The use of antibiotics varied depending on the type of livestock being raised near the wastewater treatment plants, as different types of antibiotics are prescribed for different livestock species. The mean concentrations, by region, of sulfamethazine and sulfathiazole were 910.8 μg/L and 613.1 μg/L, respectively, at Plant E, 287.1 μg/L and 165.5 μg/L, respectively, at Plant B, 358.2 μg/L and 269.7 μg/L, respectively, at Plant C, 17.0 μg/L and 42.0 μg/L, respectively, at Plant A, and 12.9 μg/L and 254.7μg/L, respectively, at Plant D; Plant E had the highest concentration. For chlortetracycline, Plant A had the highest mean concentration of 1490.5 μg/L, followed by Plant C (514.8 μg/L), Plant B (384.3 μg/L), Plant D (80.5 μg/L) and Plant E (47.9 μg/L). Oxytetracycline was only detected in Plant D at a mean concentration of 25.7 μg/L, which can be attributed to the effects of fish farms and fishing areas near Plant D's public wastewater treatment facility, when considering that oxytetracycline is widely used for the treatment of fish diseases [33].

3.2.3. Removal Efficiency by Treatment Process

Removal efficiencies of veterinary antibiotics in livestock wastewater treatment plants are shown in Table 8. The results showed that sulfamethazine and sulfathiazole had the highest removal efficiencies, of 99.7% and 100%, respectively, at Plant A, indicating that these compounds contained in wastewater were fully treated in most cases. Furthermore, 99.5% and 99.4% removal efficiencies of sulfamethazine and sulfathiazole, respectively, were reported for Plant B, 97.4% and 97.8%, respectively, for Plant C, and 90.0% and 99.8%, respectively, for Plant D, indicating a high removal efficiency in most treatment plants. In contrast, low treatment efficiencies were observed in fall and summer at Plants C and D, respectively. For Plant E, low treatment efficiencies of 39.7% and 47.8% were observed in fall for sulfamethazine and sulfathiazole, respectively, which is attributable to temporary destabilizations in treatment processes, such as sludge bulking in a bioreactor caused by high pollutant loads, with a strong, greenish yellow color.

Table 8. Removal efficiencies of livestock wastewater treatment plants.

Treatment Plant	Influent	Sulfamethazine	Sulfathiazole	Chlortetracycline	Oxytetracycline
Plant A	Spring	99.0	100.0	-	-
	Summer	100.0	100.0	100.0	-
	Fall	100.0	100.0	-	-
	Winter	100.0	100.0	-	-
Plant B	Spring	98.0	97.6	-	-
	Summer	100.0	100.0	98.3	-
	Fall	100.0	100.0	99.2	-
	Winter	99.8	100.0	-	-
Plant C	Spring	98.7	98.9	-	-
	Summer	100.0	100.0	93.5	-
	Fall	91.1	92.3	-	-
	Winter	99.8	100.0	-	-
Plant D	Spring	100.0	100.0	-	-
	Summer	60.2	99.6	82.8	-
	Fall	100.0	99.5	-	100.0
	Winter	100.0	100.0	-	-
Plant E	Summer	98.6	99.4	100.0	-
	Fall	39.7	47.8	-	-

As chlortetracycline and oxytetracycline had low detection frequencies, it was difficult to determine their seasonal differences, but both compounds exhibited a high treatment efficiency, exceeding 90%. However, for chlortetracycline, the summertime treatment efficiency at Plant D was 82.8%, which was relatively low. A comparison was made between the four treatment plants, except for Plant E, as it was experiencing temporary problems with treatment processes. The results showed that most of the plants had an at least 90% treatment efficiency, revealing no major challenges in reducing the amount of veterinary antibiotics in wastewater. However, the monitoring results showed that insufficient operation and management of a treatment plant may cause its treatment efficiency to fall below 50%, thereby suggesting the need for maintenance improvements.

3.3. Evaluation of Removal Performance According to HRT in Livestock Wastewater Treatment Using a Laboratory-Scale Reactor

3.3.1. Removal of Organic Matter, Nitrogen, and Phosphorus

Of the indicators of organic matter, the influent CODcr (testing with the dichromate method) and TOC concentrations were 13945.4 mg/L and 2701.7 mg/L, respectively (Figure 3a). The removal efficiency of CODcr in the Lab-3 (HRT 14 h) was 97.8%, followed by the Lab-2 (HRT 18 h) efficiency of 95.4%, and the Lab-1 (HRT 27 h) efficiency of 89.3%. Similarly, the highest TOC removal efficiency of 98.3% was observed at an HRT of 14 h, followed by 95.6% at an HRT of 18 h, and 91.9% at an HRT of 27 h, thereby suggesting an efficient removal of TOC under all conditions. The mean influent concentration of TN was 7531.2 mg/L, ranging from 6510.0 to 9550.0 mg/L, with a relatively large variation, whereas high levels of removal efficiency were observed, with all exceeding 93%, under the three conditions (Figure 3b). The influent concentrations of TP and PO_4-P were 402.3 mg/L and 305.0 mg/L, respectively, in the ranges of 333.0–497.1 mg/L and 216.6–381.1 mg/L, respectively. The removal efficiency of TP was 85.2% at an HRT of 27 h, 90.7% at HRT = 18 h, 94.5% at HRT = 14 h, and the removal efficiency of PO_4-P was 81.7% at HRT = 27 h, 87.7% at HRT = 18 h, and 92.6% at HRT = 14 h. For organic matter and nitrogen, no significant variations were observed between different HRTs, and the removal efficiencies of TP and PO_4-P increased by approximately 10% for the shortest operation, at a HRT of 14 h, compared to the longest operation, at a HRT of 27 h, overall exhibiting a removal efficiency of at least 80%.

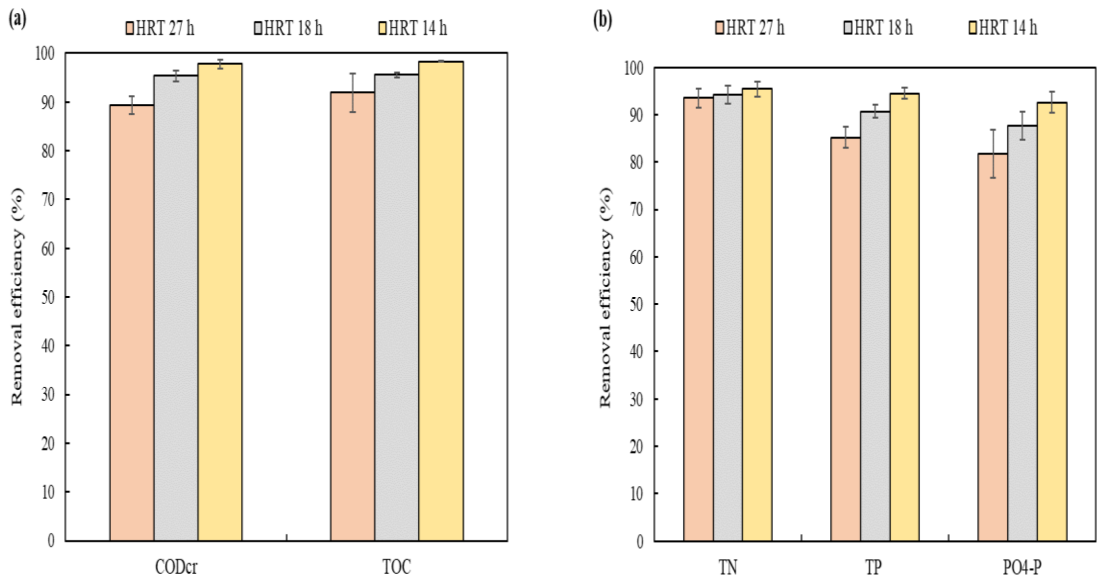

Figure 3. Removal efficiency of (**a**) organic matter and (**b**) nitrogen and phosphorus by HRT (*n* = 9).

3.3.2. Removal of Antibiotics

The efficiency of the removal of antibiotics by HRT is shown in Figure 4. The influent concentration of sulfamethazine ranged from 3.4 to 262.7 µg/L, with a mean concentration of 106.6 µg/L. Sulfamethazine was detected in all samples of influents, showing a large variation in concentration. The highest removal efficiency of sulfamethazine was observed at HRT = 27 h (99.4%), followed by 97.8% at HRT = 18 h, and 78.9% at HRT = 14 h. The observation of a ≥20% higher removal efficiency at HRT = 27 h than at HRT = 14 h suggests that a longer operation of the A2O process is considered more favorable for sulfamethazine removal efficiency. Oliveira et al. (2017) compared the removal efficiencies for sulfamethazine, between different HRTs in a horizontal-flow anaerobic immobilized biomass reactor, and revealed a higher removal efficiency at HRT = 24 h (63%) than at 8 h (22%) and 16 h (36%) [34]. The removal efficiency of sulfamethazine reported by Oliveira et al. (2017) was lower than that reported in this study, but both studies showed similar increasing trends in the removal efficiency of sulfamethazine with increased HRTs. Previous studies suggest that sulfonamides are removed mainly by quick adsorption onto the sludge, and subsequent biodegradation in the biological treatment process [35,36]. Thus, the biodegradability in a longer HRT might improve the removal of sulfamethazine. The removal efficiency of sulfonamides in engineered systems can be increased by optimizing their operational parameters [37].

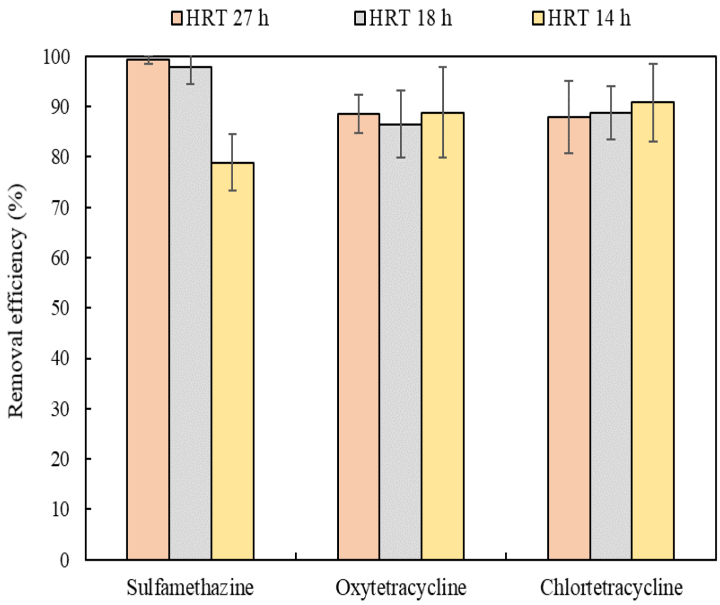

Figure 4. Removal efficiency of antibiotics according to HRT (n = 9). Sulfathiazole was not detected in any of the sampling campaigns.

The influent concentration of oxytetracycline ranged from 87.5 to 201.3 µg/L, and the mean concentration was 128.0 µg/L. The effluent concentrations of oxytetracycline were observed in the range of 8.4–23.2 µg/L at HRT = 27 h, 9.8–20.5 µg/L at HRT = 18 h, and 5.7–19.6 µg/L at HRT = 14 h. The removal efficiency of oxytetracycline was 88.6% at HRT = 27 h, 86.5% at HRT = 18 h, and 88.8% at HRT = 14 h, exhibiting similar removal levels under the three conditions. This indicates that there is no significant relationship between HRT variation and the removal efficiency of oxytetracycline in the A2O process. The influent concentration of chlortetracycline was 340.0 µg/L, ranging from 46.6 to 633.8 µg/L, which exhibited the largest variation of the antibiotics investigated in this study. Chlortetracycline was detected in both influent and effluent under the three conditions. The removal efficiency of chlortetracycline was 87.9% at HRT = 27 h, 88.7% at HRT = 18 h, and 90.8% at HRT = 14 h, and no significant variation was observed in all conditions. HRT, as in the case of oxytetracycline, had no significant effect on the removal of chlortetracycline in the A2O process. Previous studies also reported that the tetracycline antibiotics can be removed, to a great extent, via biodegradation and sludge adsorption in wastewater treatment plants [38,39]. In the study of Hou et al. (2019), the removal efficiency of antibiotics was evaluated by combining the laboratory-scale up-flow anaerobic sludge bed (UASB) and anaerobic–aerobic tank (A/O) techniques, in an effort to treat pharmaceutical wastewater [40]. The results showed that the removal efficiency of chlortetracycline was approximately 90% in the UASB, and about 80% in the A/O, while the removal efficiency of oxytetracycline was about 70% in both UASB and A/O. The removal efficiencies of chlortetracycline and oxytetracycline observed in the wastewater treatment plants of China were 62% and 63%, respectively, in the process that combines the granular sludge bed and membrane bioreactor, and 78% and 72%, respectively, in the process combining the sequencing batch reactor and bio-contact oxidation tank [41]. Overall, the removal efficiencies of the two pharmaceuticals reported in Hou et al. (2016) [41] were somewhat lower than those reported in this study, when comparing the removal efficiency between the two compounds in different treatment processes. In addition to HRT, many other operating conditions, and environments, of biological treatment processes, such as influent concentration and SRT, temperature and reactor sequencing, affect the removal of such compounds [42,43].

4. Conclusions

In this study, four types of veterinary antibiotics were investigated for their concentration, seasonal influent characteristics, and removal efficiency of different treatment processes in livestock wastewater treatment plants. Chlortetracycline was detected at the highest concentration in influents, followed by sulfamethazine, sulfathiazole and oxytetracycline. Sulfathiazole was detected at the highest effluent concentration, followed by sulfamethazine and chlortetracycline. A high removal efficiency (>90%), was observed in the four treatment plants. To evaluate the combined treatment measures for livestock wastewater in the A2O process, a laboratory-scale reactor was operated under three different HRT conditions. The highest removal efficiency of TP and PO_4-P was obtained at HRT = 14 h in the shortest operation, but the overall removal efficiency exceeded 80% under all conditions. For organic matter and nitrogen, no significant differences were observed in removal efficiency, even with varied HRTs. The removal efficiency of tetracycline antibiotics (oxytetracycline and chlortetracycline) ranged from 86.5% to 88.8% and from 87.9% to 90.8%, respectively, exhibiting no significant differences in varied HRT conditions. The removal efficiency of sulfamethazine was at least 20% higher at HRT = 27 h than at HRT = 14 h. This indicates that sulfamethazine was efficiently removed in the A2O process with increased HRT. The experimental results obtained from the laboratory-scale reactor are expected to be useful in removing the antibiotics, as well as the conventional contaminants, contained in livestock wastewater, which can be more effectively treated by combining the livestock wastewater treatment plants and conventional sewage treatment plants.

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