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Removal of veterinary antibiotics from anaerobically digested swine wastewater using an intermittently aerated sequencing batch reactor

Wei Zheng[1,2], *Zhenya Zhang*[1,*], *Rui Liu*[2], *Zhongfang Lei*[1]

1. *Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan*
2. *Zhejiang Provincial Key Laboratory of Water Science and Technology, Yangtze Delta Region Institute of Tsinghua University, Zhejiang 314016, China*

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ABSTRACT

A lab-scale intermittently aerated sequencing batch reactor (IASBR) was applied to treat anaerobically digested swine wastewater (ADSW) to explore the removal characteristics of veterinary antibiotics. The removal rates of 11 veterinary antibiotics in the reactor were investigated under different chemical organic demand (COD) volumetric loadings, solid retention times (SRT) and ratios of COD to total nitrogen (TN) or COD/TN. Both sludge sorption and biodegradation were found to be the major contributors to the removal of veterinary antibiotics. Mass balance analysis revealed that greater than 60% of antibiotics in the influent were biodegraded in the IASBR, whereas averagely 24% were adsorbed by sludge under the condition that sludge sorption gradually reached its equilibrium. Results showed that the removal of antibiotics was greatly influenced by chemical oxygen demand (COD) volumetric loadings, which could achieve up to 85.1% ± 1.4% at 0.17 ± 0.041 kg COD/m^{-3}/day, while dropped to 75.9% ± 1.3% and 49.3% ± 12.1% when COD volumetric loading increased to 0.65 ± 0.032 and 1.07 ± 0.073 kg COD/m^{-3}/day, respectively. Tetracyclines, the dominant antibiotics in ADSW, were removed by 87.9% in total at the lowest COD loading, of which 30.4% were contributed by sludge sorption and 57.5% by biodegradation, respectively. In contrast, sulfonamides were removed about 96.2%, almost by biodegradation. Long SRT seemed to have little obvious impact on antibiotics removal, while a shorter SRT of 30–40 day could reduce the accumulated amount of antibiotics and the balanced antibiotics sorption capacity of sludge. Influent COD/TN ratio was found not a key impact factor for veterinary antibiotics removal in this work.

Introduction

Veterinary antibiotics have been widely used in intensive livestock farming to reduce bacterial infection and promote livestock growth. However, their continuous misuse and resultant pollutions have triggered increasing concerns and serious environmental issues recently (Srinivasan et al., 2014; Luo et al., 2011). In fact, only a small fraction of the ingested antibiotics may be absorbed by the organisms, and greater than 85% of them run off through animal excretion and are finally discharged into water, sediment, soil, and other related environments (Bailey et al., 2016; Li et al., 2012; Wegst-Uhrich

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[*] *Corresponding author.*
E-mail: zhang.zhenya.fu@u.tsukuba.ac.jp (Zhenya Zhang).

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et al., 2014). Although the concentrations of residual antibiotics in livestock wastewater are generally around μg/L levels, they can still exert negative effects on drinking water safety and public health (Richardson and Ternes, 2011; Zhou et al., 2007). In addition, the discharge standards for veterinary antibiotics in livestock and poultry excreta are still lacking in China. More importantly, due to the dietary habits of Chinese people, pig farming is developing rapidly and pig farms have been reported to be the major contributors to residual antibiotics in the aquatic environment (Li et al., 2016).

Swine wastewater is the mixed wastewater of pig urine and piggery washing water with high chemical oxygen demand (COD) of 5000–20,000 mg/L, total nitrogen (TN) of 800–2000 mg/L and total phosphorous (TP) of 25–65 mg/L (Shin et al., 2005), which has become an important source of veterinary antibiotics in the environment in China (Zhao et al., 2010). Anaerobic digestion process is one of the widely applied technologies for large-scale swine farm wastewater treatment (Sakar et al., 2009). However, the anaerobically digested swine wastewater (ADSW) containing high concentrations of organic pollutants, nitrogen and antibiotics (Rajagopal et al., 2011) needs further polishing before final disposal. Traditional methods such as land spreading cannot cope with the huge volume of ADSW due to lack of sufficient farmland and increasingly stringent legislation for soil and water pollution control. Taking the efficiency and cost of treatment into consideration, biological processes are commonly used for ADSW treatment (An et al., 2007; Dosta et al., 2008; Daumer et al., 2007). Intermittently aerated sequencing batch reactors (IASBRs) have been used to treat swine wastewater achieving enhanced nitrogen and organic pollutant removals (Li et al., 2008; Zhang et al., 2012). The IASBR system can create repeated alternating aerobic and anoxic environments in each operation cycle to realize partial nitrification and denitrification under imprecise control conditions of dissolved oxygen (DO), pH, and temperature (Zhang et al., 2011). For example, Pan et al. (2014) achieved excellent COD, TN, and ammonium nitrogen (NH_4^+–N) removal rates about 89.8%, 76.5%, and 99.1%, respectively when using IASBR to treat swine wastewater with COD, TN, and NH_4^+–N concentrations of 11,540 ± 860, 4041 ± 59, and 3808 ± 98 mg/L, respectively. Still, the removal performance and characteristics of antibiotics remain poorly understood in IASBRs for swine wastewater treatment. On the other hand, some researchers reported veterinary antibiotic removals from swine wastewater by using other treatment processes. For instance, Ben et al. (2009) found that an effective degradation of the six selected antibiotics (five sulfonamides and one microcline) could be achieved under the optimum condition by using Fenton's reagent to treat swine wastewater pretreated with sequencing batch reactor (SBR), independent on the tested COD and suspended solid (SS) levels. Huang et al. (2015) tested the performance of vertical up-flow constructed wetlands (VUF-CWs) on swine wastewater containing tetracycline (TC) compounds, achieving 69.0%–99.9% of removal efficiencies. Restated, little information is available on the differences and characteristics of sludge sorption and biodegradation regarding to antibiotics removal in biological processes, especially IASBR for swine wastewater treatment.

In this study, a lab-scale IASBR was applied for ADSW treatment. The characteristics of sludge sorption and biodegradation for the removal of different veterinary antibiotics were investigated through mass balance analysis. The removal rates of 11 antibiotics by the IASBR were studied under different COD volumetric loadings, solid retention time (SRT) and COD/TN ratios. This study aimed to optimize the operation conditions of the IASBR for the removal of antibiotics, and to explore the relationship between antibiotics biodegradation and sludge sorption in biological treatment.

1. Materials and methods

1.1. *Wastewater and chemicals*

The wastewater, *i.e.*, ADSW used in study was collected from the effluent pipe of an anaerobic digester in a large-scale swine farm in Jiaxing City, China. The wastewater was treated by coagulation and flocculation to remove SS and stored at 4°C prior to use. Two batches of ADSW were sampled for this study: (1) Batch 1 ADSW was used for the experiments from day 1 to day 78, which had a COD of 1210 ± 291 mg/L, NH_4^+–N of 798 ± 188 mg/L, TN of 1439 ± 195 mg/L, pH of 7.8–8.1, and alkalinity of 4800–6200 mg/L (in terms of $CaCO_3$); and (2) Batch 2 was used for the experiments from day 79 to day 193, having a COD of 1060 ± 278 mg/L, NH_4^+–N of 852 ± 131 mg/L, TN of 1387 ± 187 mg/L, pH of 7.8–8.3, and alkalinity of 5200–6800 mg/L (in terms of $CaCO_3$). All the above indices were determined with standard methods (APHA, 1998).

The 11 veterinary antibiotics including tetracycline (TC), chlortetracycline (CTC), oxytetracycline (OTC), doxycycline (DC), sulfamethoxazole (SMX), sulfadimidine (SMD), ciprofloxacin (CIP), norfloxacin (NOR), enrofloxacin (ENR), tylosin (TYL) and roxithromycin (RTM) were detectable in the ADSW and selected for this study. All standard samples of these antibiotics were purchased from Dr. Ehrenstorfer GmbH (Germany). Simatone ordered from AccuStandard (USA) was used as the internal standard substance (Ben et al., 2008; Yang et al., 2011a,b). The surrogate standard substances including thiabendazole-d_4 (TB-D_4), sulfamethoxazole-d_4 (SMX-D_4), ciprofloxacin-d_8 (CFX-D_8), and erythromycin-^{13}C-D_3 (ETM-^{13}C-D_3) were bought from Toronto Research Chemicals (Canada). Five mg of each antibiotic standard was dissolved into 100 mL volumetric flask with methanol as solvent to prepare the standard mixture solution of antibiotics. The simatone–methanol solution of 10 mg/L was used as the internal standard solution. Accurately 0.1 mg of each surrogate standard was dissolved into 100 mL volumetric flask with methanol as solvent to prepare the surrogate standard mixture. All the above solutions were stored at 4°C, and all chemicals used were of analytically pure grade.

1.2. *Experimental setup and operation conditions*

The IASBR was composed of a stainless-steel cylinder of Φ25 cm × H 40 cm with an effective volume of 15 L. The operation of the IASBR was controlled by a programmable logic controller following the sequence of 10 min filling, 4 cycles with 40 min stirring (anoxic) and 60 min aeration (aerobic) alternatively, 60 min settling, and 10 min drainage. DO was around 0.5–2.0 mg/L during aeration, and the water temperature was kept at 30 ± 1°C during the whole operation.

Seed sludge was collected from a municipal wastewater treatment plant near Jiaxing City, China. The initial mixed liquor suspended solids (MLSS) concentration in the IASBR was 6.8 g/L. The IASBR was operated for three runs under different operation conditions (Table 1). In Run 1 (day 1 to day 64), the IASBR system was fed with the raw ADSW at a long hydraulic retention time (HRT) of 7 days. In Run 2 (day 65 to day 98) and Run 3 (day 99 to day 193), sodium acetate was added into the influent to increase the COD/TN ratio from 0.8 to 2.4 on average, and at shorter HRT of 5 and 3 days, respectively. Excess sludge was discharged only in Run 3 and the corresponding sludge retention time (SRT) was 30–40 days. The influent, effluent, and activated sludge in the IASBR were sampled on day 34, 54, 62, 70, 93, 136, and 184, respectively for detection of antibiotics.

1.3. *Quantification of veterinary antibiotics*

1.3.1. *Solid phase extraction (SPE) of antibiotics from wastewater*

After filtration through a 0.7 μm glass fiber membrane (Whatman, UK), 20 mL of the filtrated wastewater was diluted 10-fold by Milli-Q water prior to antibiotics extraction. Ethylendiaminetetraacetic acid disodium salt (EDTA-2Na) (0.2 g) was then added to the diluted wastewater, with pH adjusted to 4.0 using 10% HCl. Later, 200 mL of diluted wastewater was loaded into a pre-conditioned Oasis HLB column (6 cm^3/200 mg, Waters, USA) at a flow rate of 2–3 mL/min, followed by 10 min of vacuum drying. The extracted antibiotics in the Oasis HLB column were eluted with 5 mL of methanol, and then concentrated to approximately 0.5 mL under a stream of nitrogen gas. Finally, the solution was diluted to 2 mL with methanol after the addition of 20 μL of simatone standard solution and filtered through a 0.22 μm PTFE filter (Anpel, China) before antibiotics detection.

1.3.2. *SPE of antibiotics from activated sludge*

Briefly, 200 μL of the surrogate standard mixture containing TBD-D_4, SMX-D_4, CFX-D_8, and ETM-^{13}C-D_3 at 1 mg/L each was added to 0.2 g of 24 hr freeze-dried activated sludge. The sludge sample was subsequently immersed in 5 mL of extraction solution which was composed of methanol, 0.1 mol/L of EDTA-2Na and citrate buffer (pH 4) at a volumetric ratio of 3:1:2. The sludge sample and extraction solution mixture was vortexed for 1 min, ultrasonicated for 15 min, and centrifuged at 3500 r/min for 5 min. After the first extracted supernatant liquid was collected, the extraction process was repeated twice. The three extracts were pooled, diluted with Milli-Q water to 200 mL, combined with 0.2 g of EDTA-2Na, and adjusted to a pH of 4.0 with 10% HCl. The extract was finally loaded into two pre-conditioned, serially connected SAX-HLB columns (Waters, USA) to remove humus particles and concentrate the antibiotics. The elution of antibiotics from the HLB column and further treatments were the same as those for the wastewater samples described in Section 1.3.1.

Table 1 – Operation conditions of the intermittently aerated sequencing batch reactor (IASBR).

Parameters	Run 1	Run 2	Run 3
Stage	Day 1–day 64	Day 65–day 98	Day 99–day 193
HRT (day)	7	5	3
SRT (day)	62 *	98 *	30–40
Influent COD/TN	0.8 ± 0.2	2.4 ± 0.5	2.4 ± 0.4
Influent COD (mg/L)	1210 ± 291	3252 ± 159	3218 ± 219
Influent NH_4–N (mg/L)	798 ± 188	805 ± 169	832 ± 151
Influent TN (mg/L)	1439 ± 195	1392 ± 138	1321 ± 198
Sludge discharge	No	No	Intermittently

HRT: hydraulic retention time; SRT: solid retention time; COD: chemical oxygen demand; TN: total nitrogen; MLSS: mixed liquor suspended solid.

* Estimated according to the MLSS in the reactor and suspended solids concentration in the effluent.

1.3.3. *Analytical method for antibiotics with liquid chromatography–tandem mass spectrometry (LC–MS/MS)*

Antibiotics were determined using a liquid chromatography (LC) (Waters e2695, Waters, USA) coupled with a triple quadrupole-linear mass spectrometer (MS) (Waters TQ Detector, Waters, USA). Agilent Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm pore size) was used for separation of antibiotics. The injection volume was 10 μL with column temperature at 30°C. A combination of three mobile phases was used at a constant flow rate of 0.3 mL/min. Mobile phase A was composed of 99.9% water and 0.1% formic acid (*V*/*V*). Mobile phases B and C were methanol and acetonitrile, respectively. The separation of antibiotics was achieved with a gradient program described as follows: the mobile phase ratio of A:B: C was 90:4:6 at 0 min and maintained for 10 min, 90:0:10 at 11 min, 87:0:13 at 13 min, 78:0:22 at 15 min, 55:0:45 at 25 min, 0:0:100 at 26 min and maintained for 5 min, 90:4:6 at 33 min and maintained for 12 min for column equilibration. The MS system equipped with an electrospray ionization (ESI) source and operated in the positive ion mode. The optimal conditions for the MS system were determined as capillary temperature 120°C, desolvation temperature 350°C, capillary voltage 4.0 kV, and desolvation gas flow 550 L/hr. The multi-reaction monitoring (MRM) parameters for the 11 antibiotics, internal standard, and surrogate standards are listed in Table 2. The limits of detection (LOD) for the antibiotics in the wastewater and activated sludge were 4–71 ng/L and 0.4–7.1 μg/kg, respectively, with signal to noise ratios (S/N) of 3. The fortified recovery rates of the antibiotics in the wastewater ranged from 73% to 105.2% with standard deviations (SD) of 3.1% to 10.2% ($n = 3$), which were slightly higher than those in the activated sludge (57.4%–104.6%, SD = 1.9%–10.9%, $n = 3$). The recovery rates and LODs in this study were comparable to previous research works (Yang et al., 2010; Ben et al., 2008).

1.4. *Analysis of mass balance for antibiotics*

Samples from the IASBR during stable operation stage between day 34 to day 62 were used for mass balance analysis in terms of the 11 antibiotics. The influent was from batch 1 ADSW with total concentration of antibiotics of 44.88 ± 1.15 μg/L, and total concentration of antibiotics 6.69 ± 0.58 μg/L in the effluent. The mass balance between the liquid phase, sludge phase, and biodegradable portion of the antibiotics was calculated based on Eqs. (1) and (2):

$$M_{\text{inf}} - M_{\text{eff}} = M_S + M_d \tag{1}$$

$$Q \cdot c_{\text{inf}} - Q \cdot c_{\text{eff}} - Q \cdot c_S \cdot SS \cdot 10^{-3} = MLSS \cdot c_S \cdot V \cdot 10^{-3} + M_d \tag{2}$$

Table 2 – Multi-reaction monitoring parameters of liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the 16 compounds.

Target	Parent ion (*m*/*z*)	Quantitative ion (*m*/*z*)	Qualifier ion (*m*/*z*)	Cone voltage(V)	Collision voltage(V)
TC	444.9	427.3	409.7	30	20/20
OTC	461.1	443	425.8	40	25/25
CTC	479	443.8	461.9	40	25/25
DC	444.9	427.7	153.6	30	20/30
SMD	279	155.6	185.7	40	25/25
SMX	254	155.6	91.6	30	20/35
ENR	360	341.8	315.8	40	30/25
CIP	331.9	313.7	287.8	40	20/25
NOR	320	301.7	275.8	40	30/25
TYL	916.3	772	173.8	40	30/40
RTM	837.2	679.1	157.7	40	20/35
TBD-D_4	206	134.7	178.5	40	35/25
SMX-D_4	257.8	159.7	111.7	30	15/30
CFX-D_8	340.2	321.8	295.9	40	20/30
ETM-^{13}C-D_3	720.3	161.8	562.0	40	35/25
Simatone	198	127.6	123.6	40	25/25

TC: tetracycline; OTC: oxytetracycline; CTC: chlortetracycline; DC: doxycycline; SMD: sulfadimidine; SMX: sulfamethoxazole; ENR: enrofloxacin; CIP: ciprofloxacin; NOR: norfloxacin; TYL: tylosin; RTM: roxithromycin; TBD-D_4: thiabendazole-d_4; SMX-D_4: sulfamethoxazole-d_4; CFX-D_8: ciprofloxacin-d_8; ETM-^{13}C-D_3: erythromycin-^{13}C-D_3.

where M_{inf} (μg) is the total amount of antibiotics in the influent, M_{eff} (μg) is the total amount of antibiotics in the effluent, M_S (μg) is the total amount of antibiotics adsorbed in the sludge, M_d (μg) is the total amount of antibiotics biodegraded by microorganisms, c_{inf} (μg/L) is the concentration of antibiotics in the influent, c_{eff} (μg/L) is the concentration of antibiotics in the effluent, SS (g/L) is the concentration of SS in the effluent, c_S (μg/kg) is the concentration of antibiotics in the sludge, Q (L) is the total amount of treated wastewater, V (L) is the effective volume of the reactor, and MLSS (g/L) is the concentration of sludge in the reactor. Since the SS concentration in the effluent was below 5 mg/L which was considered to be negligible when compared to the influent, Eq. (2) could be simplified as Eq. (3):

$$Q \cdot c_{\mathrm{inf}} - Q \cdot c_{\mathrm{eff}} = \mathrm{MLSS} \cdot c_S \cdot V \cdot 10^{-3} + M_d \qquad (3)$$

And c_{inf} and c_{eff} were calculated based on the average antibiotic concentrations on day 34, 54, and 62, and the total volume of treated wastewater (60 L) on day 34 to 62, respectively. M_d was calculated by subtracting M_{eff} and M_S from M_{inf}. In consideration of the change of MLSS and sorption concentration of antibiotics on the sludge, the mass balance was calculated as Eq. (4):

$$Q \cdot c_{\mathrm{inf}} - Q \cdot c_{\mathrm{eff}} = (\mathrm{MLSS}_{62} \cdot c_{S-62} - \mathrm{MLSS}_{34} \cdot c_{S-34}) \cdot V \cdot 10^{-3} + M_d \qquad (4)$$

2. Results and discussion

2.1. Performance of veterinary antibiotics removal from ADSW

The removals of the 11 veterinary antibiotics by the IASBR are shown in Table 3. In Run 1, samples were collected three times, respectively on day 34, 54, and 62 for antibiotics removal determination. In Run 2 and Run 3, two times of determination (day 70 and 93 in Run 2, and day 136 and 184 in Run 3, respectively) were conducted. The concentrations of total detected veterinary antibiotics in Batch 1 ADSW on day 34, 54, 62, and 70 ranged from 42.93 to 46.54 μg/L, much higher than those in Batch 2 ADSW (day 93, 136 and 184, 30.16–31.06 μg/L). The components of antibiotics in the two batches ADSW were also quite different. The tetracyclines including TC, CTC, OTC, and DC averagely accounted for 69.7% ± 3.7% of total antibiotics in Batch 1 ADSW, which were the second most abundant antibiotics in Batch 2 (36.8% ± 1.3%). In Batch 1 ADSW, DC and TC were the two dominant tetracyclines, accounting for >90% of the total tetracyclines. In Batch 2 ADSW, the concentrations of DC and TC decreased substantially, whereas OTC and CTC remained stable resulting in their dominance among the tetracyclines. The sulfonamides, especially SMD, were the second most abundant classes of antibiotics in Batch 1 ADSW (19.3% ± 1.1% of total detected antibiotics), which became the most abundant antibiotics in Batch 2 ADSW amounting to 40.3% ± 2.4% of the total antibiotics. The quinolones including NOR, CIP, and ENR exhibited similar low concentrations of below 4.5 μg/L. Among the macrolides, TYL was detected at very low concentrations in the ADSW (0.04–0.98 μg/L), whereas RTM was not detectable in all the ADSW samples used in this study (<LOD). In fact, the characteristics and water quality of ADSW fluctuated greatly with the change of seasons, most probably attributable to the variation in feeds to pigs, characteristics of swine wastewater and efficiency of the anaerobic digestion facilities. Besides, lack of professional and precision management of swine farms also contributed a lot to the above changes, especially the characteristics of ADSW, thus the change of antibiotic concentrations in ADSW.

In Run 1 and Run 2, the IASBR achieved high total veterinary antibiotic removals of averagely 85.1% ± 1.4% and 75.9% ± 1.3%, respectively. The concentrations of total detected antibiotics in the effluents of Run 1 and Run 2 ranged from 5.94 to 10.88 μg/L. The tetracyclines and sulfonamides were significantly removed from the influent by the IASBR in Run 1 and Run 2, with removal rates of 81.1%–91.3% and 92.1%–98.5%, respectively. The remaining sulfonamides were only detectable at 0.1–1.0 μg/L. In Run 3, however, the removal of antibiotics decreased dramatically, only about 37.3% on day 184. Fig. 1 shows the relationship between the influent concentrations of antibiotics and their removal rates. The removal rates of tetracyclines and sulfonamides almost remained >80% when their influent concentrations were higher than 5 μg/L; however, their removal rates greatly fluctuated and were difficult to achieve stable levels greater than 80% when their influent concentrations were lower than 5.0 μg/L. The removal rates of quinolones and macrolides changed irregularly and seemed to have no direct relationship with their influent concentrations, among which three of quinolones and one of macrolides were all below 5 μg/L. Therefore, the antibiotics removal could maintain much more stable at relatively higher efficiency when influent ADSW contained high concentrations of antibiotics, which is to some extent in agreement with McAdam et al. (2011) who stated that the removal rates of trace organic pollutants were higher at higher influent concentrations than those at lower ones. The above results may also attribute to the refractory characteristics of antibiotics and their unfavorable competition against other abundant organics in ADSW.

2.2. Sorption of veterinary antibiotics onto sludge

Antibiotics adsorbed on the sludge were detected, as shown in Table 4. No sludge was intentionally discharged during Run 1 and Run 2. The concentrations of the 11 veterinary antibiotics in the sludge increased with the operation of IASBR in Run 1, from 5.02 mg/kg on day 34 to 13.78 mg/kg on day 62. The total amount of antibiotics in the sludge increased from 413.3 μg on day 34 (MLSS of 5.5 g/L) to 1055.7 μg on day 62 (MLSS of 5.1 g/L), resulting in an increase in the sludge antibiotics content from day 34 to day 62. The sludge concentration in IASBR increased from 5.1 g/L on day 62 to 6.9 g/L on day 93 in the presence of sufficient external carbon sources. However, the increase of sludge concentration didn't help to increase antibiotics sorption amount onto sludge particles, resulting in almost similar total

Table 3 – Variations in antibiotic concentrations in the influent and effluent of the IASBR (Unit: μg/L).

ADSW batch	Run	Operation		DC*	TC	OTC	CTC	SMD	SMX	ENR	CIP	NOR	TYL	RTM	Total tetracyclines	Total sulfonamides	Total quinolones	Total macrolides	Total antibiotics
1	1	Day 34	Inf.	19.43	11.36	1.59	1.08	6.38	1.34	1.02	1.04	1.07	0.13	–**	33.46 ± 3.85	7.72 ± 1.88	3.13 ± 2.18	0.13 ± 0.06	44.44 ± 3.48
			Eff.	2.94	1.71	0.29	0.21	0.12	0.03	0.19	0.36	1.24	0.04	–	5.15 ± 2.56	0.15 ± 0.08	1.79 ± 0.56	0.04 ± 0.02	7.13 ± 2.34
		Day 54	Inf.	17.62	9.22	1.05	1.93	8.60	0.25	1.39	2.84	3.53	0.11	–	29.82 ± 2.98	8.85 ± 2.02	7.76 ± 2.25	0.11 ± 0.07	46.54 ± 2.14
			Eff.	1.04	0.62	0.36	0.58	0.23	0.20	0.38	0.55	1.88	0.10	–	2.60 ± 1.27	0.43 ± 0.12	2.81 ± 1.12	0.10 ± 0.05	5.94 ± 1.17
		Day 62	Inf.	15.44	11.19	1.71	2.92	8.88	0.22	0.56	0.77	1.86	0.11	–	31.26 ± 3.68	9.1 ± 2.56	3.19 ± 1.88	0.11 ± 0.08	43.66 ± 3.19
			Eff.	1.14	1.06	0.37	1.04	0.23	0.21	0.39	0.33	1.61	0.07	–	3.61 ± 1.22	0.44 ± 0.28	1.33 ± 0.64	0.07 ± 0.04	7.01 ± 1.05
	2	Day 70	Inf.	16.5	9.51	0.84	2.31	8.29	0.21	1.54	1.25	2.01	0.47	–	29.16 ± 2.33	8.5 ± 1.44	4.8 ± 1.67	0.47 ± 0.22	42.93 ± 1.98
			Eff.	3.21	1.77	0.02	0.39	0.25	–	0.51	0.46	0.66	0.34	–	5.39 ± 1.45	0.25 ± 0.12	1.62 ± 1.15	0.34 ± 0.12	10.88 ± 1.21
2		Day 93	Inf.	3.78	1.28	3.01	3.35	12.16	0.41	2.26	2.11	1.14	0.98	–	11.42 ± 1.98	12.57 ± 2.12	5.51 ± 2.15	0.98 ± 0.11	30.48 ± 1.87
			Eff.	0.79	0.73	0.31	0.33	0.81	0.18	0.35	1.65	0.48	0.31	–	2.16 ± 1.65	0.99 ± 0.43	2.48 ± 1.92	0.31 ± 0.08	6.94 ± 1.15
	3	Day 136	Inf.	3.56	1.01	2.82	3.45	11.21	0.19	1.01	2.91	4.25	0.65	–	10.84 ± 1.22	11.4 ± 2.87	8.17 ± 3.76	0.65 ± 0.28	31.06 ± 1.67
			Eff.	0.47	0.46	0.94	1.34	4.36	–	0.67	1.33	2.37	0.07	–	3.21 ± 1.31	4.36 ± 1.22	4.37 ± 1.88	0.07 ± 0.11	12.01 ± 1.43
		Day 184	Inf.	3.87	1.51	2.97	3.13	12.72	0.23	1.23	1.91	2.36	0.23	–	11.48 ± 3.45	12.95 ± 2.12	5.5 ± 2.97	0.23 ± 0.08	30.16 ± 2.51
			Eff.	0.9	0.67	1.6	2.91	7.44	0.21	1.2	3.9	–	0.07	–	6.08 ± 2.28	7.65 ± 1.27	5.1 ± 1.21	0.07 ± 0.12	18.9 ± 2.11

IASBR: intermittently aerated sequencing batch reactor; ADSW: anaerobically digested swine wastewater; DC: doxycycline; TC: tetracycline; OTC: oxytetracycline; CTC: chlortetracycline; SMD: sulfadimidine; SMX: sulfamethoxazole; ENR: enrofloxacin; CIP: ciprofloxacin; NOR: norfloxacin; TYL: tylosin; RTM: roxithromycin.

* Average value of triplicate tests for each antibiotic class;

** "–" means not detected.

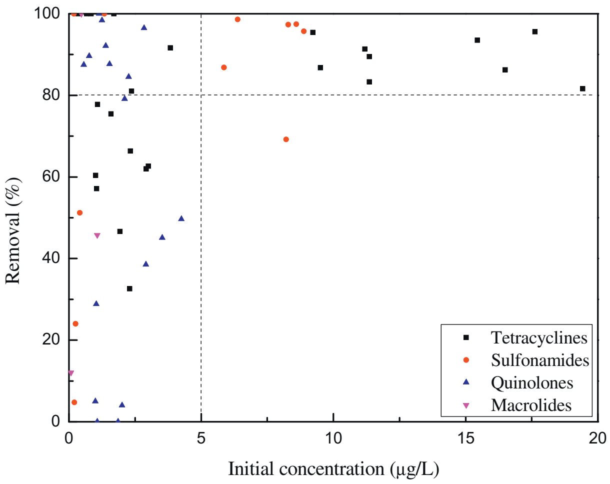

Fig. 1 – Variations in removal rates of antibiotics under different initial antibiotics concentrations in the influent.

Table 4 – Variations in antibiotic concentrations in the activated sludge of the IASBR (Unit: mg/kg).

ADSW batch	Run	Operation	DC*	TC	OTC	CTC	SMD	SMX	ENR	CIP	NOR	TYL	RTM	Total tetracyclines	Total sulfonamides	Total quinolones	Total macrolides	Total antibiotics
1	1	Day 34	2.541	1.517	0.150	0.673	0.002	0.002	0.015	0.093	0.021	0.004	–**	4.881 ± 1.882	0.004 ± 0.002	0.128 ± 0.066	0.004 ± 0.001	5.017 ± 1.632
		Day 54	4.396	3.054	0.154	1.656	0.001	0.007	0.113	0.155	0.115	0.007	–	9.260 ± 2.543	0.008 ± 0.002	0.384 ± 0.092	0.007 ± 0.003	9.659 ± 2.432
		Day 62	6.838	3.858	0.265	1.823	0.053	0.032	0.185	0.257	0.437	0.028	–	12.784 ± 3.335	0.085 ± 0.039	0.880 ± 0.115	0.028 ± 0.011	13.777 ± 2.967
	2	Day 70	7.634	2.448	0.270	2.850	0.044	0.016	0.115	0.119	0.226	0.034	–	13.202 ± 3.165	0.060 ± 0.041	0.460 ± 0.124	0.034 ± 0.018	13.755 ± 2.798
2		Day 93	7.808	1.047	0.406	3.294	0.031	0.019	0.276	0.285	0.193	0.039	–	12.554 ± 2.023	0.049 ± 0.034	0.754 ± 0.135	0.039 ± 0.021	13.397 ± 1.961
	3	Day 136	1.506	0.259	1.240	6.466	0.172	0.019	0.219	0.280	0.251	0.007	–	9.471 ± 1.729	0.190 ± 0.068	0.750 ± 0.087	0.007 ± 0.002	10.418 ± 1.534
		Day 184	1.423	0.215	1.350	6.337	0.168	0.018	0.207	0.270	0.242	0.007	–	9.325 ± 1.432	0.187 ± 0.065	0.718 ± 0.103	0.007 ± 0.005	10.237 ± 1.454

IASBR: intermittently aerated sequencing batch reactor; ADSW: anaerobically digested swine wastewater; DC: doxycycline; TC: tetracycline; OTC: oxytetracycline; CTC: chlortetracycline; SMD: sulfadimidine; SMX: sulfamethoxazole; ENR: enrofloxacin; CIP: ciprofloxacin; NOR: norfloxacin; TYL: tylosin; RTM: roxithromycin.

* Average value of triplicate tests for each antibiotic class;

** "–" means not detected.

antibiotic contents of 13–14 mg/kg in sludge in Run 2 as that on day 62 in Run 1. The total amount of antibiotics accumulated in sludge slightly increased from 1306.2 μg on day 70 to 1386.9 μg on day 93, and most of the antibiotics in the influent ADSW were steadily removed through biodegradation instead of sludge sorption. Therefore, it is reasonable to suppose that the sorption of veterinary antibiotics on sludge reached their equilibrium state in Run 2. In addition, biodegradation may play a dominant role in antibiotics removal when the sorption of veterinary antibiotics onto sludge reaches the sorption capacity of sludge before being discharged. Excess sludge was intermittently discharged in Run 3, and the concentration of total antibiotics in the sludge declined remarkably to 10.42 mg/kg on day 136, and again reached a similar concentration of 10.24 mg/kg on day 184. Meanwhile, the total amount of antibiotics in the sludge slightly decreased from 1279.2 μg on day 136 to 1193.4 μg on day 184. These results imply that sludge sorption was an important pathway for the removal of veterinary antibiotics in ADSW and a balanced sorption capacity of sludge could be achieved at a long SRT during stable operation, while a shorter SRT could decrease the balanced sorption capacity of the sludge. The existence of a balanced sorption capacity could be attributable to the establishment of antibiotics adsorption–desorption equilibrium in the sludge (Morissette et al., 2015; Yang et al., 2011a,b). More specifically, the tetracyclines were the most abundant in the sludge in accordance with the influent ADSW composition, amounting to 94% ± 2.1% of the total adsorbed antibiotics. The macrolides were detected at the lowest concentrations, which is in consistence with their low concentrations in ADSW. In contrast, the sulfonamides, the second abundant antibiotics in ADSW, were detected at low concentrations in the sludge (4.2 to 0.2 mg/kg).

2.3. Removal mechanisms of veterinary antibiotics

Generally, both sludge sorption and biodegradation are the main removal pathways for antibiotics in biological processes rather than other possible pathways like volatilization or hydrolysis (Dorival-García et al., 2013; Li and Zhang, 2010). The IASBR system achieved excellent antibiotics removal when treating the raw ADSW without any external carbon sources from day 34 to day 64. The role of sludge sorption for antibiotics removal can be seen more clearly before the bioreactor reached the balanced sorption capacity of sludge. Therefore, mass balance analysis based on data from days 34 to 62 was conducted to explore the removal routes of veterinary antibiotics in this IASBR system. As shown in Table 5, the results reveal that 15.1% of the antibiotics were

Table 5 – Mass balance analysis on antibiotics during operation from day 34 to day 62.

Substances	M_{inf} (μg)	M_{eff} (μg)	M_s (μg)	M_d (μg)	M_{eff}/ M_{inf} (%)	M_s/ M_{inf} (%)	M_dM_{inf} (%)
Total antibiotics	2694.0	402.0	642.4	1619.6	15.1	24.1	60.8
Tetracyclines	1890.0	228.1	575.0	1086.9	12.1	30.4	57.5
Sulfonamides	516.0	19.8	6.2	490	3.8	1.2	95.0
Quinolones	282.0	150.0	58.9	73.1	53.2	20.9	25.9
Macrolides	6.6	4.1	1.8	0.7	62.1	27.3	10.6

remained in the effluent, with 60.8% being biodegraded and 24.1% adsorbed by sludge. This observation indicates that during a long and stable operation of the IASBR system biodegradation plays a more important role in antibiotics removal than sludge sorption. Specifically, 95% of the influent sulfonamides were biodegraded, indicating their easy biodegradability. Li and Zhang (2010) reported that two sulfonamides (SMX and sulfadiazine) were predominantly removed through biodegradation in an activated sludge process. Both Wu et al. (2009) and Yu et al. (2011) noticed the weak sorption of SMX and SMD in a biological system, and results from the latter work indicate that SMX possesses much stronger biodegradability than SMD. In this study, 96.8% of influent SMD was removed by biodegradation, much higher than SMX (71.9%). This observation is probably attributable to the huge difference in their initial concentrations in the influent ADSW. The poor sorption of sulfonamides can be explained by the acid–base equilibrium processes involved. The amphoteric sulfonamides with functional groups are in anionic form at neutral and basic pH, resulting in a low adsorption to the activated sludge (Ben et al., 2014; Yang et al., 2011a,b). On the other hand, only 57.5% of influent tetracyclines could be biodegraded with 30.4% left in the sludge, suggesting that unlike sulfonamides this class of antibiotics was more difficult to biodegrade, thus sludge sorption would be a more important removal route. Both Li and Zhang (2010) and Prado et al. (2009) claimed that TC exhibited good adsorbability and low biodegradability, which could be mainly removed by adsorption in biological processes. However, in this study, greater than 60% of influent TC, OTC and DC were removed through biodegradation. CTC was an exception, nearly all of which was adsorbed on the sludge. These results may be brought about by the starvation conditions under lower organic loading in Run 1, which promoted microorganisms to utilize antibiotics at trace levels and lower biodegradability. As observed by Shi et al. (2011), the removal of tetracyclines can be described as a quick sorption and then a slow biodegradation. In this study, neither biodegradation nor sorption could achieve excellent removal of quinolones, leaving 41.6% in the effluent. In addition, 25.9% of influent quinolones were removed by biodegradation, slightly higher than that by sorption, which is quite different from the findings of Dorival-García et al. (2013) who claimed that sorption by sludge played a dominant role in the elimination of 6 commonly found quinolones (CIP, moxifloxacin, NOR, ofloxacin, pipemidic acid, and piromidic acid) from wastewaters. Additionally, no comment could be made on macrolides due to the fact that their influent concentrations were too low in this study.

2.4. Effect of COD volumetric loading on antibiotics removal from ADSW

Organic loading usually has a profound influence on organics removal in bioreactors, including trace organic pollutants such as antibiotics, pharmaceuticals and endocrine disrupting chemicals (EDCs) (Carranza-Diaz et al., 2014; McAdam et al., 2011). In Run 1, the COD volumetric loading was 0.17 ± 0.041 kg COD/m^3/day with sludge concentrations (MLSS) of 5.1–6.8 g/L. The insufficient carbon source may limit the growth of microorganisms, leading to decreased sludge concentration thus relatively low and fluctuant COD removal rates (averagely 60.3% ± 15.7%). When the COD volumetric loading was increased to 0.65 ± 0.032 kg COD/m^3/day in Run 2 and 1.07 ± 0.073 kg COD/m^3/day in Run 3, correspondingly, average COD removal rates were increased to 88.3% ± 4.7% and 90.8% ± 5.5%, respectively (Table 6). The effluent CODs during Run 1, Run 2 and Run 3 were averagely 454, 396 and 296 mg/L, respectively. This observation together with the increase in MLSS from Run 1 to Run 2 and Run 3 (Table 6) indicates that external carbon sources or increasing organic loading favored the growth of microorganisms, thus enhanced the removal of organic pollutants.

However, antibiotics removal showed a different trend with the change of COD volumetric loading. The removal rates of the 11 veterinary antibiotics slightly decreased as the COD volumetric loading increased from Run 1 to Run 2, and abruptly dropped to 49.3% ± 12.1% when COD loading was further increased to 1.07 ± 0.073 kg COD/m^3/day in Run 3. This phenomenon implies that COD volumetric loading could significantly impact the antibiotics removal, and higher organic loading may have strongly negative effect on the competition of antibiotics over easily biodegradable carbon source (sodium acetate in this study) during biological wastewater treatment. This observation to some extent agrees with Conkle et al. (2010). In Run 1, MLSS was determined to gradually decrease from initial 6.8 to 5.1 g/L on day 62. The obvious decrease in MLSS in the reactor possibly reflected that a large proportion of the microorganisms might be in an endogenous respiration phase because of the lower organic loading applied and lack of readily biodegradable substances. Although veterinary antibiotics were determined at trace levels in terms of μg/L in the influent, the microorganisms might utilize them as much as possible for survival, resulting in somewhat amelioration of competition between antibiotics and other organics. In Run 2, the increase in sludge concentration was most probably resulted from the sufficient supply of readily biodegradable carbon source (sodium acetate in this study) and less need for antibiotics functioned as carbon source, thus decreased antibiotics removal rates noticeably. Moreover, when the reactor was operated at a much higher COD volumetric loading (Run 3), the microorganisms seemed to prefer to utilize the abundant readily biodegradable organics in the influent for their survival and metabolisms. Therefore, other organics than antibiotics achieved an overwhelming removal in terms of biodegradation, resulting in the

Table 6 – Average COD, antibiotics, NH_4^+–N and TN removal rates during Run 1, Run 2, and Run 3.

Item	Run 1	Run 2	Run 3
MLSS (g/L)	5.1–6.8	5.3–7.1	7.4–8.5
Influent COD volumetric loading (kg COD/m^3/day)	0.17 ± 0.041	0.65 ± 0.032	1.07 ± 0.073
COD removal rates (%)	60.3 ± 15.7	88.3 ± 4.7	90.8 ± 5.5
Antibiotics removal rates (%)	85.1 ± 1.4	75.9 ± 1.3	49.3 ± 12.1
NH_4^+–N removal rates (%)	62.5 ± 14.8	89.1 ± 8.8	51.2 ± 28.6
TN removal rates (%)	35.8 ± 18.2	85.9 ± 7.4	54.1 ± 19.5

COD: chemical oxygen demand; TN: total nitrogen; MLSS: mixed liquor suspended solid.

remarkable decline of antibiotics removal in Run 3. As shown in Fig. 2, all the four antibiotic classes exhibited decreased removal rates at higher COD volumetric loadings. Results also show that the sulfonamides exhibited higher removal rates than tetracyclines in Run 1 and Run 2, but lower in Run 3. These observations suggest that the removal of sulfonamides is more likely to be affected by organic loadings than that of tetracyclines, attributable to the higher adsorption capacity of tetracyclines onto sludge. Namely, regarding to veterinary antibiotic removal COD volumetric loading may pose even greater influence on biodegradation than sludge.

2.5. *Effect of SRT on antibiotics removal from ADSW*

SRT might also impact the antibiotic removal by IASBR systems as in other bioreactors. A too short SRT will lead to greatly declined sludge concentrations and thereby a significant decrease in sorption and degradation of antibiotics and other organic pollutants (Xia et al., 2012). No sludge was intentionally discharged in Run 1 and Run 2 due to the lower organic loadings applied, and their MLSS was maintained at 5.1–7.1 g/L. In Run 3, the organic loading was increased and SRT was shortened to 30–40 days with MLSS ranged between 7.4 and 8.5 g/L. As shown in Table 6, sludge discharge did not significantly affect the removals of generally organic pollutants indicated by COD using the IASBR system. On the other hand, the accumulated antibiotics were found to slightly decrease from 13.4 mg/kg on day 93 (Run 2) to 10.43 mg/kg on day 136 (Run 3), and maintained at a stable level of 10.24 mg/kg on day 184 (Table 3). Therefore, the applied shorter SRT might not be the reason for the poor antibiotics removal in Run 3. As known, SRT could definitely influence the balanced sorption capacity of sludge, and a shorter SRT could reduce the accumulation of antibiotics in sludge. In this study, due to the fact that both HRT and SRT were changed in Run 2 and Run 3, it is difficult to interpret the real impact of SRT on antibiotics removal, which needs further investigation in the followed-up research.

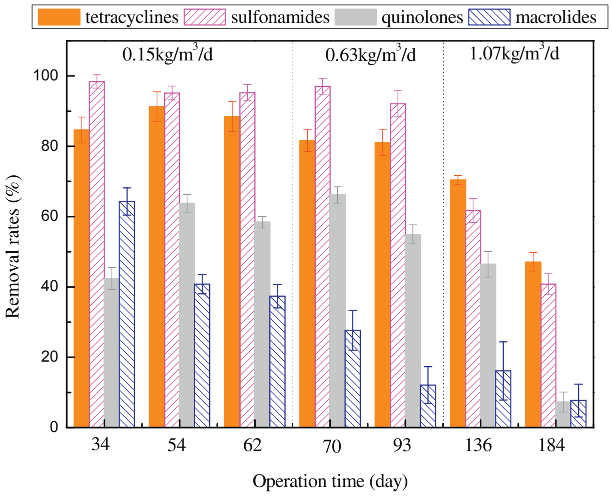

Fig. 2 – Variations in removal rates of antibiotics under different influent COD volumetric loadings.

2.6. *Effect of COD/TN ratio on antibiotics removal from ADSW*

The COD/TN ratio in the influent was found to significantly influence nitrogen removal. As shown in Table 6, TN removal was poor and fluctuated (35.8% ± 18.2%) under operation at a low COD/TN ratio of 0.8 ± 0.2 in Run 1, and nitrite was detected to accumulate at 751.9 ± 98.3 mg/L due to lack of sufficient carbon source for denitrification. This high concentration of nitrite might also exert a negative effect on the growth of nitrifying and denitrifying bacteria with resultant low TN removal. In Run 2, along with the addition of sodium acetate, the COD/TN ratio in the influent increased to 2.4 ± 0.5 averagely. As a result, the TN removal increased to 85.9% ± 7.4%, even though the reactor was operated under a shorter HRT and higher nitrogen volumetric loading conditions (compared to Run 1). However, due to a much shorter HRT applied in Run 3, the further increased nitrogen loading might impact nitrogen removal negatively. On the contrary, antibiotics removal appears not to be significantly influenced by COD/TN ratio, as Run 1 and Run 2 achieved high removal rates for total antibiotics (Table 6).

3. Conclusions

The IASBR is an efficiently biological treatment system for simultaneous removal of COD and veterinary antibiotics from ADSW. The removal of veterinary antibiotics was significantly decreased under higher organic volumetric loading or shorter HRT. A shorter SRT could reduce the accumulation of antibiotics and the balanced antibiotics sorption capacity of sludge. The COD/TN ratio in influent was a key factor for nitrogen removal, but not for veterinary antibiotics in ADSW treatment by using IASBR. The influent sulfonamides underwent obvious biodegradation in the bioreactor. The removal of both tetracyclines and quinolones was contributed by biodegradation and sludge sorption. Although the IASBR could achieve excellent removal rates (>80%) for all studied veterinary antibiotics from ADSW under lower COD volumetric loading, nearly 24% of the 11 antibiotics were found to adsorb onto the activated sludge and were not completely decomposed. The environmental risk caused by excessive discharge of veterinary antibiotics and the safe disposal of sludge containing adsorbed antibiotics should be monitored and controlled.

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