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Occurrence, fate and mass loadings of antibiotics in two swine wastewater treatment systems

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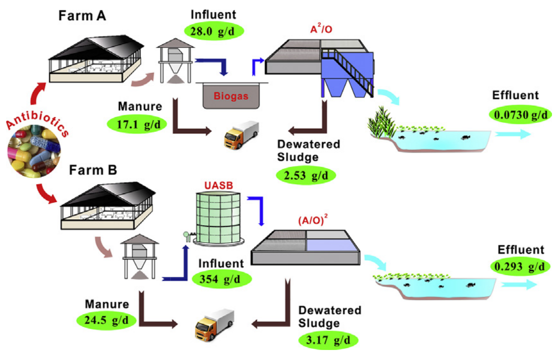
HIGHLIGHTS

- We investigated the occurrence and fate of 40 antibiotics in two swine farms.
- 25 target antibiotics were detected in the manure and wastewater treatment plants.
- The anaerobic digester and UASB played a key role in eliminating antibiotics.
- WWTPs could effectively remove the target antibiotics from the swine wastewater.

GRAPHICAL ABSTRACT



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ABSTRACT

Antibiotics are widely applied in livestock industry to prevent or treat animal diseases. However, those antibiotics are poorly metabolized in livestock animals, most of them being excreted via feces or urine. Hence we need to understand the removal of antibiotics in swine farm wastewater treatment systems. This study investigated occurrence and fate of various antibiotics in two full-scale swine farm wastewater treatment systems (Farm A: anaerobic digester-A^2/O-lagoon; Farm B: upflow anaerobic sludge blanket (UASB)-(A/O)^2-lagoon). The results showed the presence of 25 antibiotics out of 40 target antibiotics in the wastewater and sludge samples from the two farms. In Farm A, sulfamonomethoxine, sulfachlorpyridazine, oxytetracycline and lincomycin were predominant in the influent with concentrations up to 166 ± 3.64 μg/L, while in the dewatered sludge chlortetracycline, oxytetracycline, tetracycline and norfloxacin were the predominant target compounds with concentrations up to 29.2 ± 3.74 μg/g. In Farm B, high concentrations (up to 3630 ± 1040 μg/L) of sulfachlorpyridazine, sulfamonomethoxine and lincomycin were detected in the influent, and the predominant target antibiotics detected in the dewater sludge were similar to those in Farm A, with concentrations up to 28.6 ± 0.592 μg/g. The aqueous removal rates for the total antibiotics were >99.0% in the wastewater treatment plants of both farms. Among a series of treatment units, the anaerobic digester in Farm A and UASB in Farm B made a significant contribution to the elimination of the target antibiotics from the animal wastewater. The daily mass loadings of total antibiotics in the manure, influent, dewatered sludge and effluent were 17.1, 28.0, 2.53, and 0.0730 g/d for Farm A and 24.5, 354, 3.17, and 0.293 g/d for Farm B. The full-scale swine wastewater treatment facilities could

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effectively remove antibiotics from swine wastewater, but the dewatered sludge needs to be further treated before disposal on land.

1. Introduction

Antibiotics are widely applied to prevent and treat diseases and also used for growth promotion in livestock industry (Boxall et al., 2003; Guo et al., 2016). In China, the total antibiotic usage was estimated to be approximately 162,000 tons for 2013, and 52% of which was consumed by animals (Zhang et al., 2015). In United States, 50% of the 22,700 tons antibiotics prescribed annually are used for animals, and approximately 11,200 tons of antibiotics used for non-therapeutic purposes primarily to promote the growth of swine, poultry and cattle (Kümmerer, 2009). The extensive usage of antibiotics in livestock industry affected water quality, food safety, and development of antibiotic resistance (He et al., 2014, 2016). Though the all kinds of alternatives to antibiotics are identified and developed, such as phytogenic feed additives, probiotics, prebiotics, feed acidifiers, bacteriophages and antibodies, none of existing alternatives could meet the requisites to replace the efficiency and cost efficacy of antibiotics (Suresh et al., 2018). Therefore, antibiotics are still widely used in livestock animals in many countries.

Commercial swine production consumed the largest amount of antibiotics compared with other livestock (Sarmah et al., 2006; Zhang et al., 2015). Due to the effectiveness, broad-spectrum and favorable price, sulfonamides, tetracyclines, fluoroquinolones, macrolides and other antibiotics such as lincomycin, trimethoprim, bacitracin and ormetoprim were extensively used in swine industry (Hunter and Shaner, 2011; Zhang et al., 2015; Lou et al., 2018; Posyniak et al., 1999; Pyorala et al., 2014; Collinder et al., 2003; Baert et al., 2001). Nevertheless, as much as 30%–90% of parent compounds of antibiotics are excreted via feces or urine after application (Sarmah et al., 2006). Antibiotics residues was extensively detected in the wastewater, sludge and manure (Huang et al., 2017; Zhao et al., 2010; Jiang et al., 2013; Zhou et al., 2013a). Consequently, these antibiotic residues would end up in the receiving water and land via discharge of wastewater and disposal of manure or sludge. Therefore, it is essential to remove antibiotics in waste streams before their release into the receiving environments.

China is the biggest producer and consumer of pork meat in the world, and Chinese farmers use four times more antibiotics than their US counterparts to produce the same amount of meat (Cully, 2014; Zhang et al., 2015). Therefore swine farms have been known to be a major source for antibiotics entering into the environment. In China, most swine farms are only equipped with simple treatment technologies such as lagoon and anaerobic digester or even do not have any facilities for treatment and disposal of manure and wastewater (He et al., 2016; Tong et al., 2009; Zhou et al., 2013b). Furthermore, recent studies have demonstrated that lagoon and anaerobic digester cannot efficiently remove antibiotics from swine wastewater (Campagnolo et al., 2002; Varel et al., 2012; Zhou et al., 2013a). In recent years, some swine farms started to build more complex or advanced swine farm wastewater treatment systems, such as upflow anaerobic sludge blanket (UASB) and anaerobic-aerobic oxidation (Nuengjamnong and Rachdawong, 2016; Gobel et al., 2005). As a result, these advanced treatment technologies should be explored for the effectiveness in removing antibiotic residues in swine wastewater.

The objectives of this study were to investigate the occurrence and fate of antibiotics in two swine farms in South China which are equipped with full-scale wastewater treatment facilities. Forty antibiotics of different classes were analyzed in swine manure, wastewater, dewatered sludge. The mass loadings at different stages of waste treatment systems were estimated, and the efficiencies of the systems in removing antibiotics were also assessed. The results from this study can help us understand the effectiveness of the complete wastewater treatment systems applied in the swine farms.

2. Materials and methods

2.1. Materials and chemical standards

Due to the extensive use in swine industry (Hunter and Shaner, 2011; Zhang et al., 2015; Lou et al., 2018; Posyniak et al., 1999; Pyorala et al., 2014; Collinder et al., 2003; Baert et al., 2001), forty antibiotics of seven classes were selected as target compounds in this study: sulfonamides (SAs), tetracyclines (TCs), fluoroquinolones (FQs), macrolides (MLs), others (polypeptides, lincosamides and diaminopyrimidines). The basic physicochemical properties of target antibiotics are shown in Table S1 (Supporting information). The sources and preparation methods of the target standard antibiotics, the internal standard, reagent and materials can be referred to our previous study (Zhou et al., 2012).

2.2. Site description and sample collection

Two swine farms equipped with full-scale wastewater treatment systems were selected and they are located in Yunfu City of Guangdong Province, South China. The basic information about the two swine farms is given in Table S2. The treatment technologies applied in the systems are anaerobic digester–A^2/O (anaerobic-anoxic-oxic)–lagoon in Farm A, and UASB–$(A/O)^2$ (anoxic/oxic-anoxic/oxic)–lagoon in Farm B. The technical flow chart and sampling points are shown in Fig. 1.

Manure, wastewater and sludge were collected for this study. Sampling campaigns were carried out on July 6–7 (Farm A and Farm B), August 23–24 (Farm B), September 9–10 (Farm A) and October 26–27, 2016 (Farm A and Farm B). Time integrated composite wastewater from the two wastewater treatment systems were collected in 1 L amber glass bottles in two consecutive days. All the water samples were adjusted to pH 2 by using 4 M H_2SO_4, and added with methanol (5% v/v) to inhibit microbial activity and then transported in coolers to laboratory. Solid samples (manure and dewatered sludge) were collected and stored in 200 mL glass bottles and added each with 2 g sodium azide to inhibit microbial activity. Then the wastewater samples were immediately stored at 4 °C before being analyzed. Meanwhile the dewatered sludge and manure samples were freeze-dried and then kept at −18 °C in the dark until extraction.

2.3. Sample extraction

Sample extraction and instrumental analysis applied in the present study followed our previous analytical methods (Zhou et al., 2012). Briefly, the aqueous samples (1000 mL) were filtered through 0.7 μm glass fiber filters, and added with 100 ng of internal standard mixture (100 μL each of 1 mg/L mixture solution). Subsequently, the aqueous samples were extracted by solid phase extraction (SPE) with Oasis HLB cartridges (500 mg, 6 mL), which were pre-conditioned consecutively by 10 mL methanol and 10 mL Milli-Q water. The target antibiotics were eluted from the cartridges each with 10 mL methanol. The elution was evaporated to near dryness under a gentle stream of nitrogen, and then the final extracts were re-dissolved in 1 mL methanol prior to the instrumental analysis. The concentrations of antibiotics in aqueous samples were expressed in ng/L.

The dewatered sludge and manure samples were extracted by using an ultrasonic extraction method (Zhou et al., 2012). Briefly, each solid

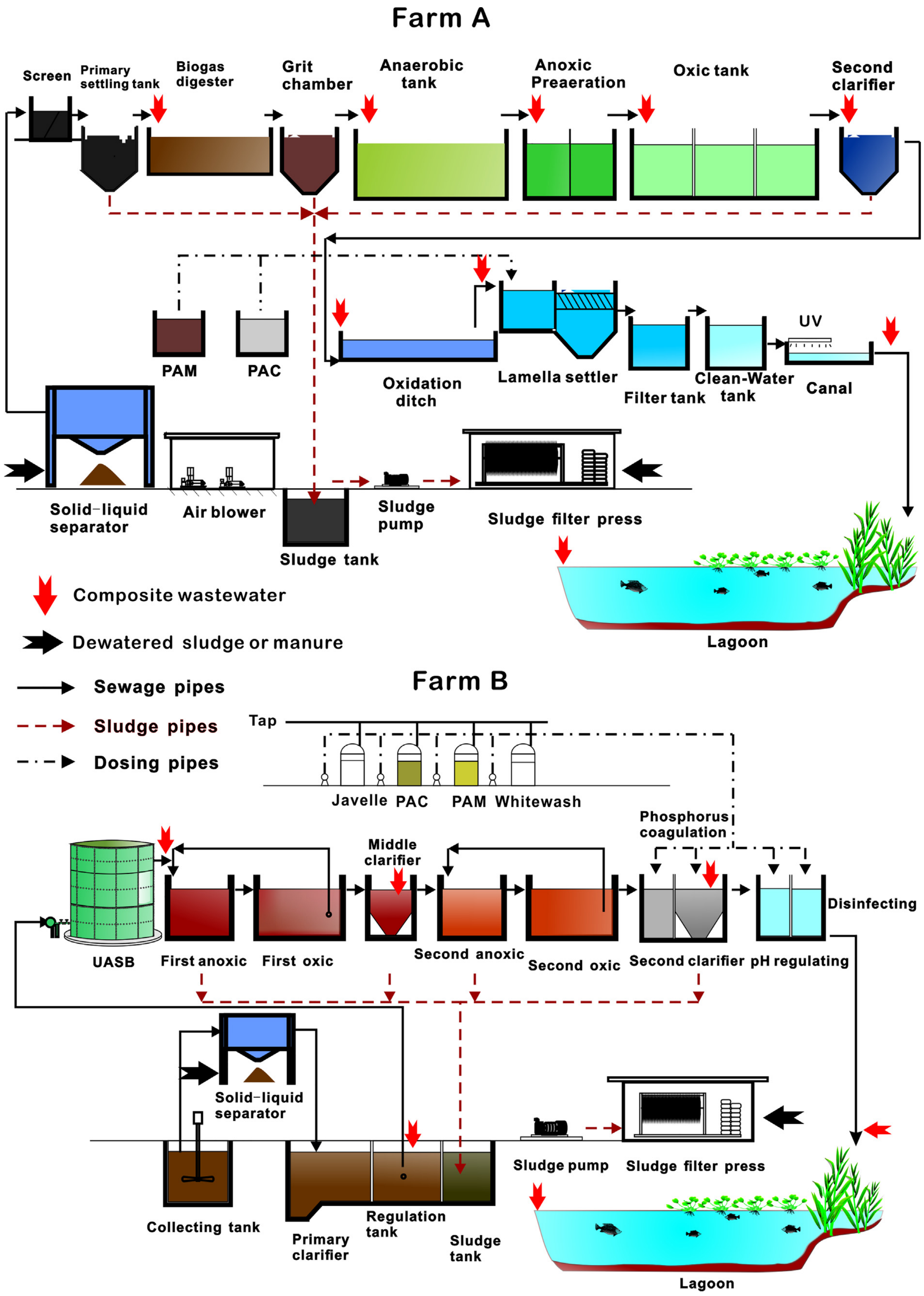

Fig. 1. The technical flow chart of sewage treatment system in Farm A and Farm B.

sample (0.5 g) was added into a glass centrifuge tube (30 mL), followed by addition of 100 ng of internal standard mixture. After being well mixed, the samples were stored in 4 °C overnight. On the following day, these samples were extracted each with 10 mL acetonitrile and 10 mL citric acid buffer ($pH = 3$), followed by mixing on a vortex for 1 min. Subsequently, all glass tubes were sonicated for 15 min and centrifuged at 1370*g* for 10 min. Each supernatant was transported into a 200 mL round-bottom flask. This extraction process was repeated twice and the supernatants from the three extractions were merged. The supernatants were subsequently evaporated at 55 °C to eliminate the organic solvent, and then diluted to 200 mL with Milli-Q water followed by addition of 0.2 g of Na_4EDTA in order to chelate with metal cations. The strong anion exchange (SAX) cartridges (500 mg, 6 mL) and HLB cartridges (200 mg, 6 mL) were assembled in tandem for the extraction of the target antibiotics. After being pre-conditioned with 10 mL methanol and 10 mL Milli-Q water. The SAX cartridges were used to remove the negatively charged humic and fulvic acids present in dewatered sludge and manure samples, meanwhile the target antibiotics which were neutral or positively charged at pH 3 were absorbed into the filling materials of HLB cartridges. The elution and reconstitution processes were the same as the method used in the extraction of aqueous samples. The suspended solid matter samples were also extracted by ultrasonic-assisted extraction with solvent (methane, 0.1% formic acid and $pH = 4$ sodium acetate) (Zhou et al., 2012). The concentrations of antibiotics in solid samples were expressed in ng/g dry weight.

2.4. Instrumentation

The 40 target antibiotic compounds in the extracts were analyzed by an Agilent 1200 series ultrahigh-performance liquid chromatograph coupled to an Agilent 6460 triple quadrupole mass spectrometer with electrospray ionization under positive ionization modes (UHPLC-ESI-MS-MS). The detailed operating conditions, method recoveries, limit of detection (LOD) and limit of quantification (LOQ) for each target compound can be referred to our previous report (Zhou et al., 2012). When the actual concentration is below the LOD or LOQ, LOQ/2 is used for calculation and statistical analysis. Strict QA/QC (quality assurance and quality control) were performed during the analysis.

2.5. Mass loading calculation

Aqueous phase removal rate for each antibiotic in the wastewater treatment systems was calculated by the following equation:

$$AR\% = [(C_{Influent} - C_{Effluent})/C_{Influent}] \times 100\% \quad (1)$$

where, AR% is the aqueous removal rate of each antibiotic; $C_{Influent}$ and $C_{effluent}$ are the aqueous concentrations of the target antibiotic in influent and effluent.

The mass loadings of target antibiotics in Farm A and Farm B were calculated based on concentrations in influent, effluent, sludge, and manure by the following equations:

$$W_{Influent} = C_{Influent} \times Q_I/10^3 \quad (2)$$

$$W_{Effluent} = C_{Effluent} \times Q_E/10^3 \quad (3)$$

$$W_{Sludge} = C_{Sludge} \times Q_S/10^3 \quad (4)$$

$$W_{Manure} = C_{Maunre} \times Q_M/10^3 \quad (5)$$

where $W_{Influent}$ (mg/d), $W_{Effluent}$ (mg/d), W_{Sludge} (mg/d), W_{Manure} (mg/d) are the daily mass loadings of each antibiotic in the influent, effluent, dewatered sludge and manure, respectively. $C_{Influent}$ (ng/L), $C_{Effluent}$ (ng/L), C_{Sludge} (ng/g) and C_{Manure} (ng/g) represent the concentrations of the antibiotic in the influent, effluent, dewatered sludge and manure, respectively. Q_I (m^3/d) and Q_E (m^3/d) are the average daily flow of influent and effluent, while Q_S (kg/d) and Q_M (kg/d) represent the average dry weight of dewatered sludge and swine manure in Farm A or Farm B per day, respectively.

3. Results

3.1. Concentrations of antibiotics in swine wastewater

Twenty-two target antibiotics were detected in all the aqueous samples of Farm A, including 5 sulfonamides, 5 tetracyclines, 8 fluoroquinolones, 2 macrolides, lincomycin and trimethoprim (Fig. 2). Among these detected antibiotics, sulfachloropyridazine, sulfamonomethoxine, oxytetracycline, and lincomycin were predominant in the anaerobic digester influent, while in the effluent sulfamonomethoxine, chlortetracycline, tetracycline, oxytetracycline, and lincomycin were the predominant compounds. Large variations in antibiotic concentrations were observed among the three sampling times (July 7, September 10, and October 27) (Tables S7–S9). For example, the concentrations for sulfachloropyridazine in the influent varied between 137 ± 52.7 μg/L on July 7 and 80.5 ± 30.1 μg/L on September 10. The highest concentrations in the anaerobic digester influent and lagoon effluent of Farm A were found for lincomycin (166 ± 3.64 μg/L) and oxytetracycline (1.40 ± 1.00 μg/L).

Similarly, twenty-two target antibiotics were quantified in all aqueous phase samples of Farm B, including 5 sulfonamides, 5 tetracyclines, 7 fluoroquinolones, 3 macrolides, lincomycin and trimethoprim (Fig. 3). Among these detected antibiotics, sulfachlorpyridazine, and oxytetracycline were predominant in the influent, while in the effluent the predominant compounds included sulfachlorpyridazine, sulfamonomethoxine, oxytetracycline, trimethoprim and lincomycin. Sulfachlorpyridazine and oxytetracycline showed the highest concentrations in the UASB influent (3.65 ± 1.04 mg/L; 918 ± 67.0 μg/L) and lagoon effluent (2.95 ± 0.241 μg/L; 1.82 ± 1.39 μg/L) (Tables S10–S12). The total antibiotic concentration in the influent of Farm B (0.988 mg/L–3.78 mg/L) was found higher than that in the influent of Farm A (0.0990 mg/L–0.326 mg/L).

3.2. Concentrations of antibiotics in manure and sludge

For the dewatered sludge, 20 compounds out of 40 target antibiotics were detected, including 5 sulfonamides, 5 tetracyclines, 7 fluoroquinolones, 1 macrolides, trimethoprim and lincomycin (Fig. 4 and Table S13). Among the detected antibiotics, the tetracyclines and fluoroquinolones were predominant in the dewatered sludge samples, with chlortetracycline and oxytetracycline accounting for >85.0%. The concentrations for chlortetracycline and oxytetracycline in the dewatered sludge ranged from 23.6 ± 4.92 μg/g to 31.1 ± 2.38 μg/g in Farm A and from 21.9 ± 1.36 μg/g to 28.6 ± 0.592 μg/g in Farm B, respectively. Meanwhile the total antibiotic concentrations in the dewater sludge of Farm A and Farm B were 77.1 ± 11.3 μg/g and 67.8 ± 4.65 μg/g in average, respectively.

As to the swine manure, 20 compounds, including 7 sulfonamides, 5 tetracyclines, 6 fluoroquinolones, trimethoprim and lincomycin, were also found with the concentrations up to 34.3 ± 3.45 μg/g in Farm A and up to 41.9 ± 3.67 μg/g in Farm B (Fig. 4 and Table S14). Chlortetracycline, tetracycline, oxytetracycline and lincomycin showed much higher concentrations than the other compounds. When compared to the dewatered sludge, the concentrations of the detected antibiotics in manure displayed larger variations among the three sampling times. The total antibiotic concentrations in the manure of Farm A and Farm B were 38.1 ± 11.9 μg/g and 37.8 ± 15.6 μg/g, respectively.

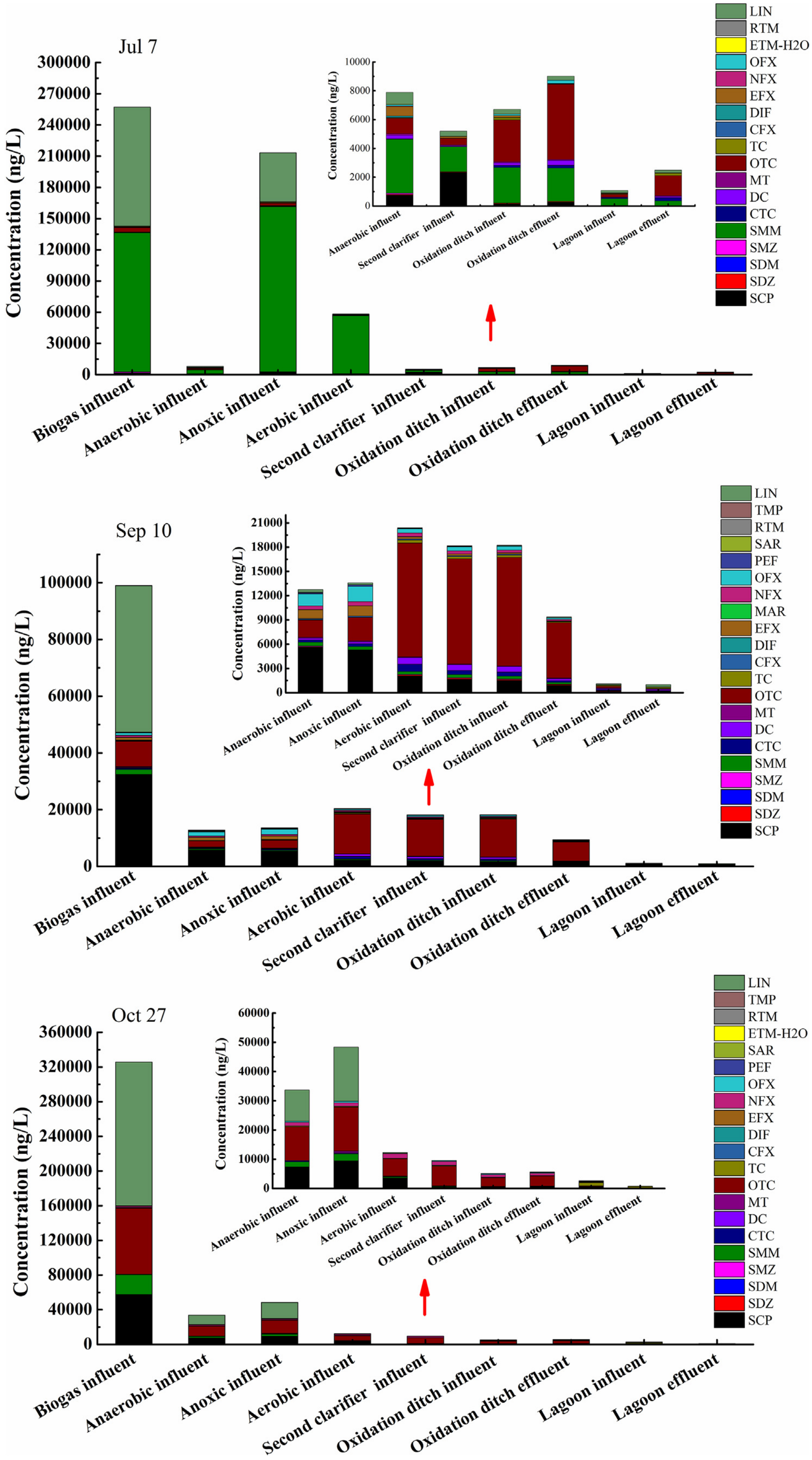

Fig. 2. The concentrations of antibiotics in liquid phase of each treatment unit in Farm A. SCP, Sulfachlorpyridazine; SDZ, sulfadiazine; SDM, sulfadimethoxine; SMZ, sulfamethazine; SMM, sulfamonomethoxine; CTC, chlortetracycline; DC, doxycycline; MT, methacycline; OTC, oxytetracycline; TC, tetracycline; CFX, ciprofloxacin; DIF, difloxacin; EFX, enrofloxacin; MAR, marbofloxacin; NFX, norfloxacin; OFX, ofloxacin; PEF, pefloxacin; SAR, sarafloxacin; ETM-H2O, Erythromycin-H2O; RTM, roxithromycin; TMP, trimethoprim; and LIN, lincomycin.

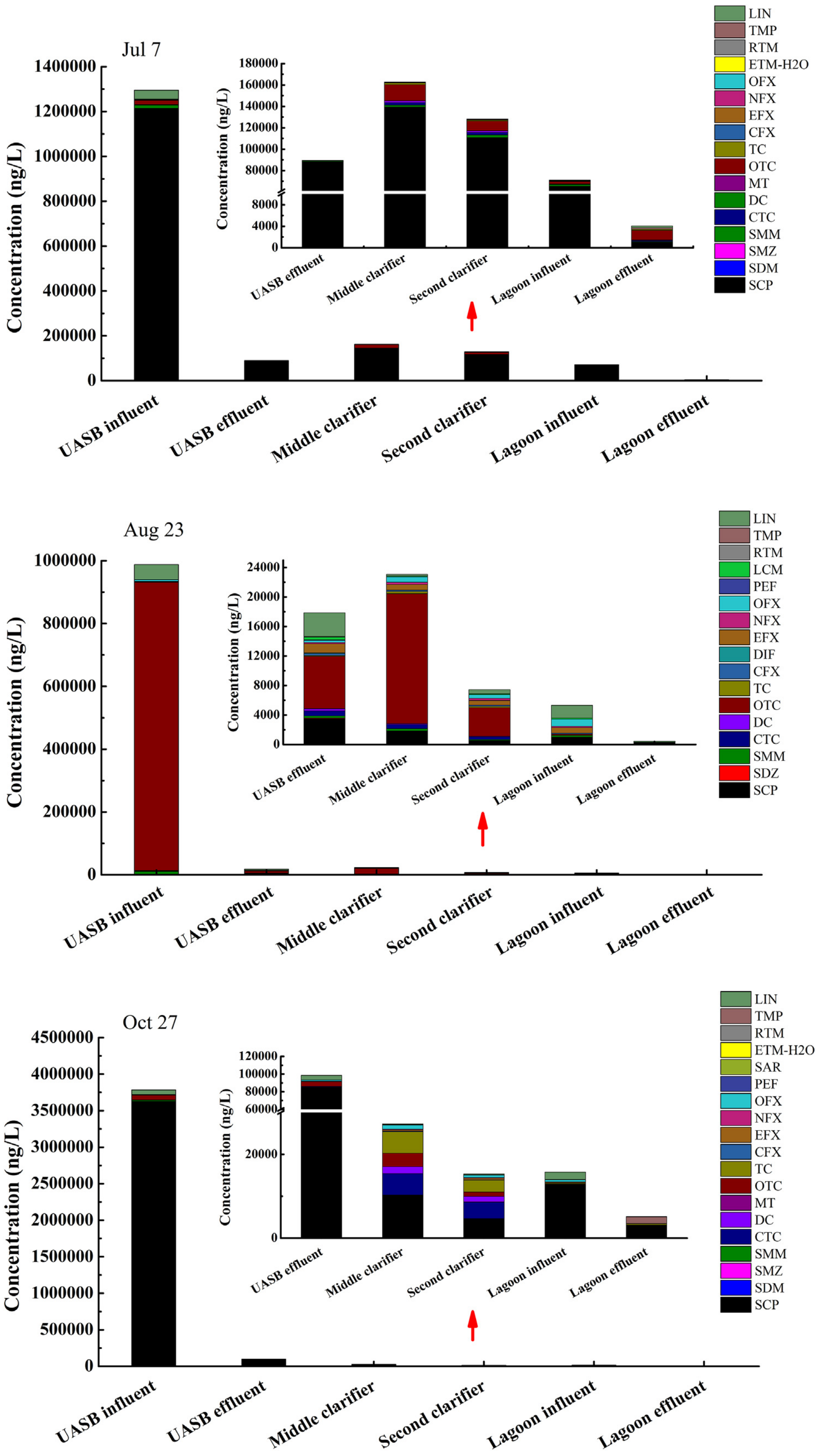

Fig. 3. The concentrations of antibiotics in liquid phase of each treatment unit in Farm B. SCP, Sulfachlorpyridazine; SDZ, sulfadiazine; SDM, sulfadimethoxine; SMZ, sulfamethazine; SMM, sulfamonomethoxine; CTC, chlortetracycline; DC, doxycycline; MT, methacycline; OTC, oxytetracycline; TC, tetracycline; CFX, ciprofloxacin; DIF, difloxacin; EFX, enrofloxacin; NFX, norfloxacin; OFX, ofloxacin; PEF, pefloxacin; SAR, sarafloxacin; ETM-H2O, Erythromycin-H2O; LCM, leucomycin; RTM, roxithromycin; TMP, trimethoprim; and LIN, lincomycin.

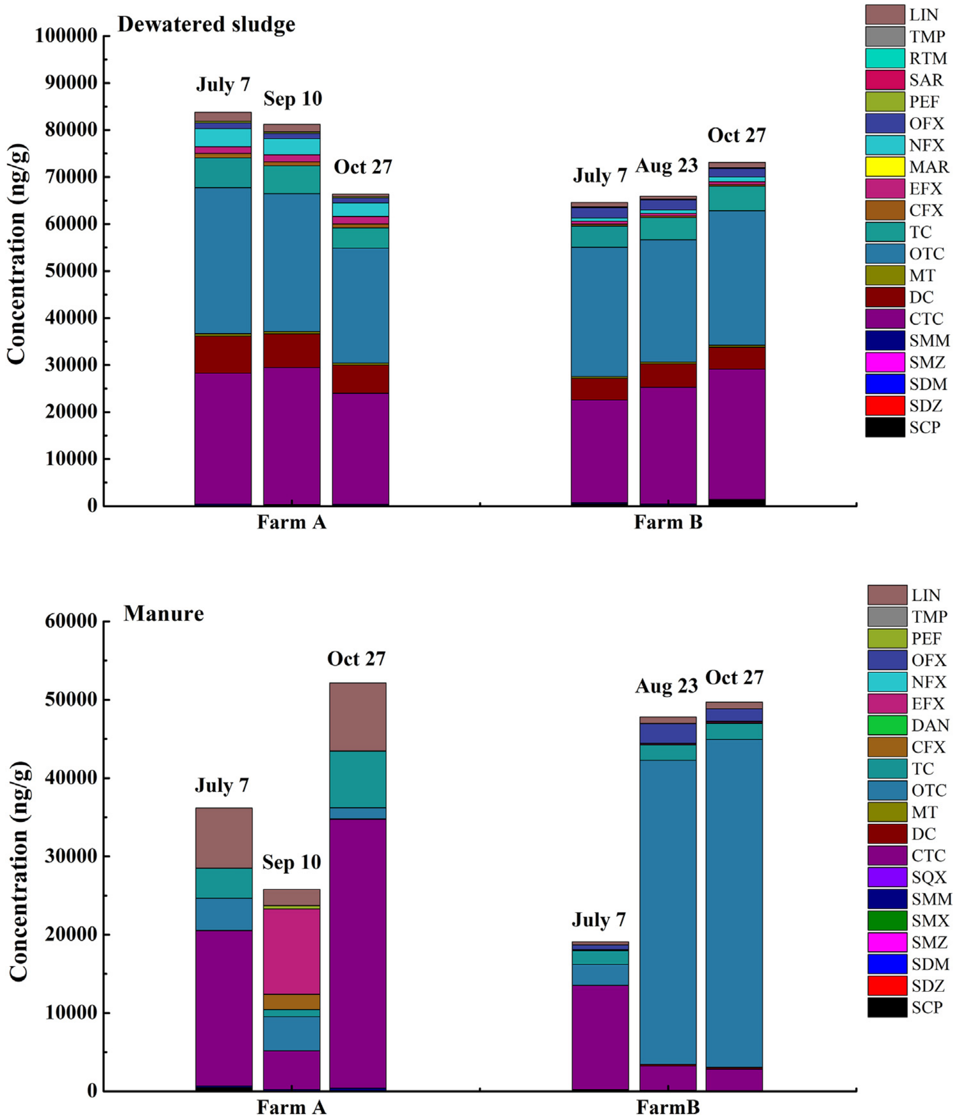

Fig. 4. The concentrations of antibiotics in dewatered sludge and swine manure in Farm A and Farm B. SCP, Sulfachlorpyridazine; SDZ, sulfadiazine; SDM, sulfadimethoxine; SMZ, sulfamethazine; SMX, sulfamethoxazole; SMM, sulfamonomethoxine; SQX, sulfaquinoxaline; CTC, chlortetracycline; DC, doxycycline; MT, methacycline; OTC, oxytetracycline; TC, tetracycline; CFX, ciprofloxacin; DAN, danofloxacin; EFX, enrofloxacin; NFX, norfloxacin; OFX, ofloxacin; PEF, pefloxacin; SAR, sarafloxacin; RTM, roxithromycin; TMP, trimethoprim; and LIN, lincomycin.

3.3. Removal efficiency of antibiotics in the wastewater treatment systems

Aqueous phase removal rates for the detected antibiotics in Farm A and Farm B varied widely between −763% and 100%, and between −947% and 100%, respectively (Table 1; Tables S15–S16). For individual SAs, the removal rates ranged from 12.8% to 100%, but the total removal rates for the SAs in Farm A and Farm B were 99.7 ± 0.241% and 99.3 ± 1.05%, respectively. Similar phenomena were also found for other groups of antibiotics such as tetracyclines, fluoroquinolones and macrolides. High removal efficiencies were found for the total antibiotics in both farms, with the percentages of 99.3 ± 0.427% in Farm A and 99.8 ± 0.137% in Farm B.

The aqueous removals in each treatment unit of both farms are given in Tables S15–S16. Among the functional units, the anaerobic digester in Farm A showed effective elimination for the total antibiotics (91.2 ± 5.11%), and for the sulfonamides (89.0 ± 7.42%), tetracyclines (76.2 ± 7.11%) and others (97.4 ± 3.30%). The A^2/O unit gave poorer performance in removing antibiotics (21.1%) than the anaerobic digester unit. In addition, the precipitation and filtration combined with UV produced good removal of antibiotics (76.4 ± 20.2%). Lagoon treatment showed further elimination for the sulfonamides (48.6 ± 27.0%) and fluoroquinolones (49.3 ± 26.2%). The UASB in Farm B produced high removal for the five classes of detected antibiotics, with the rates ranged from 52.0 ± 38.3% (macrolide) to 95.1 ± 4.20% (Others), and followed by the lagoon treatment, as well as other processes including the $(A/O)^2$ and precipitation/filtration/chlorination.

3.4. Mass loadings of antibiotics

Mass loadings of the detected antibiotics in the two swine farms are given in Tables S17–S19. Obviously, Farm B had higher daily mass loadings in influent and effluent than Farm A. In the influent, the daily mass loadings of the total antibiotics were 12.2–40.1 g/d in Farm A and 173–662 g/d in Farm B. The average daily mass loadings for the predominant antibiotics sulfachlorpyridazine, sulfamonomethoxine, oxytetracycline and lincomycin were 3.74, 6.51, 3.69, and 13.6 g/d in Farm A, and 282, 2.48, 58.7, and 8.88 g/d in Farm B, respectively. In the effluent, the daily mass loadings of the total antibiotics were 41.2–127 mg/d in Farm A and 40.0–469 mg/d in Farm B. For primary target antibiotics oxytetracycline, tetracycline and lincomycin in the effluent of Farm A, their daily mass loadings were 24.9, 12.2 and 14.3 mg/d, respectively; while in Farm B, the daily mass loadings of sulfachlorpyridazine,

Table 1
Aqueous removal rates (%) of the antibiotics detected in the two swine farm wastewater treatment systems.

Compounds	Abbreviation	Removal rate (%)					
		Swine farm A			Swine farm B		
		July 2016	September 2016	October 2016	July 2016	August 2016	October 2016
Sulfonamides	TAs						
Sulfachlorpyridazine	SCP	98.9	99.8	100	99.9	89.6	99.9
Sulfadiazine	SDZ	93.7	NA[a]	97.9	ND[b]	NA	ND
Sulfadimethoxine	SDM	92.0	12.8	49.0	84.4	ND	18.1
Sulfamethazine	SMZ	99.2	49.8	54.9	79.8	ND	54.1
Sulfamonomethoxine	SMM	99.8	97.5	99.8	99.2	99.4	99.8
Tetracyclines	TCs						
Chlortetracycline	CTC	NA	78.7	74.0	91.5	99.3	70.5
Doxycycline	DC	66.5	36.8	68.2	52.9	99.3	62.0
Methacycline	MT	58.9	NA	70.6	81.9	ND	99.1
Oxytetracycline	OTC	69.4	99.5	100	90.6	100	100
Tetracycline	TC	−3.40	17.8	−164	88.6	99.8	−101
Fluoroquinolones	FQs						
Ciprofloxacin	CFX	93.1	97.9	82.0	86.6	96.6	91.1
Difloxacin	DIF	76.4	NA	−763	ND	95.1	ND
Enrofloxacin	EFX	98.4	99.6	96.0	98.4	98.7	84.8
Marbofloxacin	MAR	ND	64.9	ND	ND	ND	ND
Norfloxacin	NFX	95.0	99.5	99.7	92.4	97.8	85.7
Ofloxacin	OFX	96.3	99.6	91.8	99.7	99.9	99.5
Pefloxacin	PEF	ND	97.8	90.4	ND	NA	93.8
Sarafloxacin	SAR	ND	97.4	95.9	ND	ND	93.3
Macrolides	MLs						
Erythromycin-H_2O	ETM-H_2O	87.4	ND	78.4	88.6	ND	81.7
Leucomycin	LCM	ND	ND	ND	ND	92.5	ND
Roxithromycin	RTM	89.3	20.2	2.20	85.0	94.0	39.6
Others							
Trimethoprim	TMP	ND	70.5	58.5	−947	NA	−120
Lincomycin	LIN	99.8	99.4	100	99.2	99.8	99.9

[a] Not available (some of antibiotics not detected in influent but detected in some unit treatment process).

[b] Not detected in the aqueous phase of the wastewater samples.

oxytetracycline, tetracycline, trimethoprim and lincomycin were 125, 56.8, 15.2, 57.8 and 14.3 mg/d, respectively.

In the dewatered sludge, the daily antibiotic mass loadings were 2.17–1.75 g/d in Farm A and 3.02–3.42 g/d in Farm B (Tables S17–S18). For the predominant antibiotics tetracyclines in the dewatered sludge, their average daily mass loadings ranged from 15.3 mg/d to 929 mg/d in Farm A and from 18.1 mg/d to 1.28 g/d in Farm B. For the fluoroquinolones ciprofloxacin, enrofloxacin, norfloxacin and ofloxacin in the sludge, their average daily mass loadings were 28.6, 49.4, 111, 37.9 mg/d in Farm A, and 16.2, 28.4, 38.1, and 95.1 mg/d in Farm B, respectively.

In the swine manure, the daily mass loadings of the total detected antibiotics ranged from 11.6 g/d to 23.4 g/d in Farm A and from 12.4 g/d to 32.3 g/d in Farm B (Table S19). For the predominant antibiotics in Farm A, the average daily mass loadings of sulfamonomethoxine, chlortetracycline, oxytetracycline, tetracycline, ciprofloxacin, enrofloxacin, lincomycin were 0.121, 8.82, 1.46, 1.78, 0.292, and 1.63 and 2.74 g/d, respectively. Meanwhile, in Farm B, the average daily mass loadings of chlortetracycline, oxytetracycline, tetracycline, ofloxacin and lincomycin were 4.42, 1.71, 1.25, 0.938, and 0.425 g/d. According to Tables S20–S21, the average daily mass loadings of the total detected antibiotics excreted via manure and urine were 45.1 g/d in Farm A and 379 g/d in Farm B. In Farm A, 37.9% of target antibiotics were excreted via manure and 62.1% excreted via urine by swine. In Farm B, 6.50% of target antibiotics were excreted via manure and 93.5% excreted via urine by swine. The notable difference between the two farms was mainly attributed to the extremely high concentration of sulfachlorpyridazine in urine from the Farm B. Additionally, as the predominant antibiotics in both swine farms, sulfachlorpyridazine, sulfamonomethoxine, oxytetracycline and lincomycin were mainly excreted via urine by swine, while chlortetracycline mainly carried by the swine manure.

Based on daily mass loadings, higher excretion mass would be expected for Farm B than Farm A. According to Tables S22–S23, the daily masses of target antibiotics excreted via manure and urine by each sow were 22.6 mg/d/sow in Farm A and 131 mg/d/sow in Farm B. The yearly excretion masses of the target antibiotics in Farm A and Farm B were 16.5 kg/yr and 138 kg/yr, respectively (Fig. 5). Furthermore, the direct yearly environmental input masses of the target antibiotics via the effluent discharge were 26.6 g/yr in Farm A and 107 g/yr in Farm B.

4. Discussion

4.1. Occurrence and mass loading of antibiotics in swine farms

Twenty-five out of the 40 target antibiotics were detected in swine wastewater, dewatered sludge and manure of the two swine wastewater treatment systems. For the aqueous samples, sulfachlorpyridazine and sulfamonomethoxine were the most predominant sulfonamide compounds.

This is different to the survey by Zhou et al. (2013b), in which sulfachlorpyridazine and sulfamonomethoxine were detected with relatively low concentrations or even not detected in the wastewater samples. This phenomenon resulted from the different compositions of swine among the surveyed farms. In the present study the two swine farms mainly bred sows, while piglets, growing and finishing pigs were dominant in Zhou et al. (2013b). Different breeding types resulted in different antibiotics feeding strategies. In the dewatered sludge samples, the sulfonamides were detected with relatively low concentrations. This is consistent to the previous studies (Gobel et al., 2005; Zhou et al., 2013c) as sulfonamides show little tendency to adsorb onto the sludge. Sulfachlorpyridazine and sulfamonomethoxine were detected at relatively high concentrations in the swine manure. Zhao et al. (2010) also found relatively high detection rates and concentrations for these two antibiotics in the swine manure samples from 8 Chinese provinces. This indicates the wide usage of the two sulfonamides in swine industry.

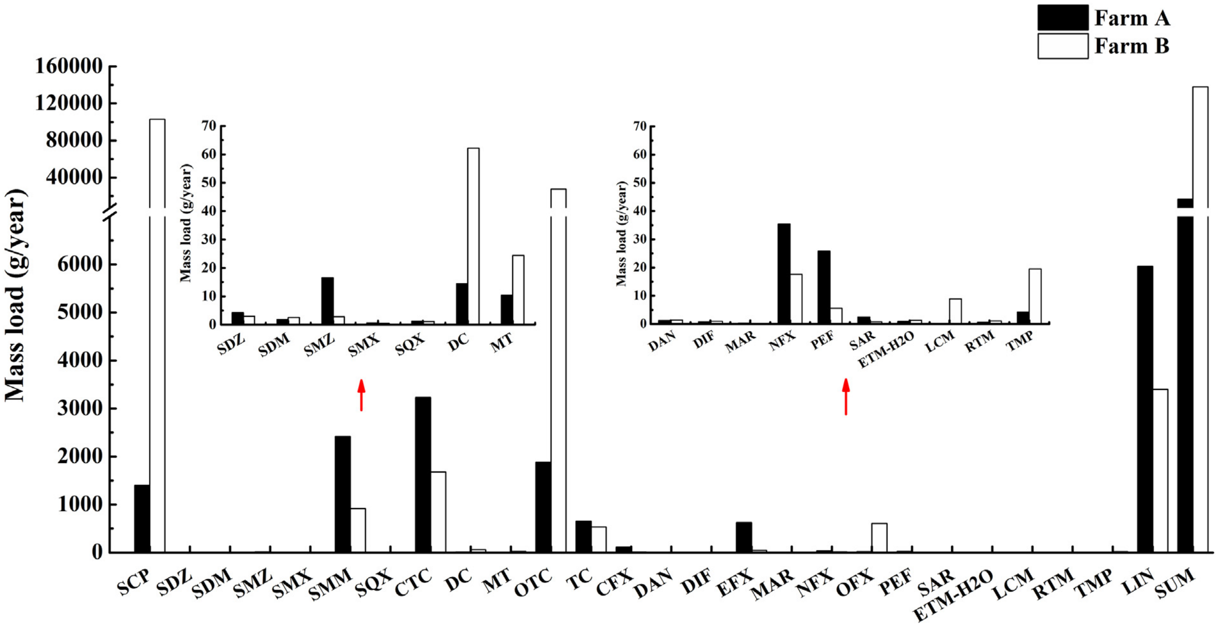

Fig. 5. The excretion mass loadings (g/year) of target antibiotics in the two swine farms via manure and urine. SCP, Sulfachlorpyridazine; SDZ, sulfadiazine; SDM, sulfadimethoxine; SMZ, sulfamethazine; SMX, sulfamethoxazole; SMM, sulfamonomethoxine; SQX, sulfaquinoxaline; CTC, chlortetracycline; DC, doxycycline; MT, methacycline; OTC, oxytetracycline; TC, tetracycline; CFX, ciprofloxacin; DAN, danofloxacin; DIF, difloxacin; EFX, enrofloxacin; MAR, marbofloxacin; NFX, norfloxacin; OFX, ofloxacin; PEF, pefloxacin; SAR, sarafloxacin; ETM-H2O, Erythromycin-H2O; LCM, leucomycin; RTM, roxithromycin; TMP, trimethoprim; and LIN, lincomycin.

For tetracyclines, all five tetracyclines (chlortetracycline, doxycycline, methacycline, oxytetracycline and tetracycline) were detected in wastewater and dewatered sludge samples of the two swine farms. Oxytetracycline was the predominant compound for aqueous samples, while chlortetracycline and oxytetracycline were found with higher concentrations than the other antibiotics in dewatered sludge and swine manure samples. In a previous study by Chen et al. (2012), individual tetracyclines were also found in the influent at high concentrations up to 10^5 ng/L. Similar results were found in other previous studies (Tong et al., 2009; Zhao et al., 2010). High concentrations of tetracyclines such as oxytetracycline in the swine wastewater found in the present and previous studies are attributed to their frequent use in veterinary medicine and high water solubility (Oleszczuk et al., 2009; Zhang et al., 2015; Sarmah et al., 2006). In addition, the predominance of tetracyclines in dewatered sludge and manure resulted from extensive usage in feed and their intense absorption ability because of strong interaction with clay, organic matter and metal oxides (Aristilde et al., 2010; Kong et al., 2012). The higher abundance of tetracyclines than sulfonamides in manure could be attributed to the difference in use strategy. Tetracyclines were mainly added into feeds to feed swine (Zhou et al., 2013a), while sulfonamides were mainly injected into swine to treat diseases (Abeshouse and Tankin, 1946).

Nine fluoroquinolones were detected in all the samples from the two swine farms. Enrofloxacin, norfloxacin and ofloxacin were predominant in both wastewater and dewatered sludge. Similar results have been reported in previous studies (Tong et al., 2009; Zhao et al., 2010; Zhou et al., 2013b).

Three macrolides, including erythromycin-H_2O, leucomycin and roxithromycin, were detected with relatively low concentrations in wastewater and dewatered sludge samples, while no macrolides were detected in the swine manures. This is consistent with a previous study by Zhou et al. (2013b). Since macrolides are mainly used in human (Murata et al., 2011; Zhang et al., 2015), implying their low detection in animal farms.

For other classes of antibiotics, two antibiotics trimethoprim and lincomycin were detected in all samples from the two swine farms. Trimethoprim was found with low concentrations, while lincomycin was found notably high. Similar results were found in previous studies (Zhou et al., 2013a, 2013b). Lincomycin was also detected in liquid swine manure stored in a lagoon (Kuchata and Cessna, 2009). Lincomycin is commonly used as the antimicrobial to prevent and control post-weaning diarrhea in weanling pigs (Kuchata and Cessna, 2009).

The present study found significant decrease in antibiotic concentrations after wastewater treatment systems. But the antibiotics in the dewatered sludge of both swine farms (A and B) were still very high with average values of 77.1 mg/kg and 67.8 mg/kg, respectively. The average total antibiotic concentrations in swine manures of Farm A and Farm B were also high with average values of 38.1 mg/kg and 37.8 mg/kg. The results implied that the dewatered sludge and swine manures should not be directly applied into agricultural land without any treatment due to potential risks posed by these antibiotics. Other further treatments such as composting could be applied for treating the dewatered sludge and swine manure (Sarmah et al., 2006).

Among all the antibiotics analyzed in the present study, sulfachlorpyridazine, sulfamonomethoxine, oxytetracycline and lincomycin were the predominant compounds in both liquid and solid samples. This suggests that these compounds should be listed as the main monitoring compounds in swine farms. Furthermore, large variations in antibiotic concentrations were observed among the different sampling times. That could be attributed to different antibiotic use strategies and epidemic situation according to their breeding stages in swine farms.

The mass loadings of target antibiotics excreted via manure and urine in Farm B (379 g/d) was higher than that in Farm A (45.1 g/d) (Tables S20 and S21). The daily excretion of target antibiotics per swine via manure and urine in Farm A and Farm B were 22.6 mg/d/sow and 131 mg/d/sow (Table S22–S23). The results from Farm A is similar to those (20.0 mg/d/sow and 48.3 mg/d/sow) reported by Zhou et al. (2013a, 2013b). The higher concentrations in waste samples and daily excretion from Farm B than from Farm A could be attributed to their differences in farming scale, feed, growth phase, breeding period, antibiotic use and injection strategy.

4.2. Evaluation of antibiotic removal in wastewater treatment systems

The present study showed effective elimination of antibiotics in the swine wastewater treatment systems used in both farms. Zhou et al.

(2013a) found that simple lagoon-anaerobic digester treatment systems could not effectively remove the antibiotics in swine waste. The aqueous removal rates (>99% total antibiotics) in the two farms of the present study were even better than those in municipal wastewater treatment plants (Watkinson et al., 2007). This suggests that the wastewater treatment systems in Farm A and Farm B are capable to prevent the antibiotics disseminating into surrounding environments via effluent discharge.

During treatment, antibiotics in wastewater could be transformed by photolysis, hydrolysis, and biotransformation, or removed from the aqueous phase by adsorption to sludge (Le-Minh et al., 2010). According to Wijekoon et al. (2015) and Phan et al. (2018), as a type of trace organic chemicals, the fate of antibiotics in waste treatment process depends on their hydrophobicity and molecular structure. For the sulfonamides and other class, the removal rates were above 98.1% (Tables S13–S14). The sulfonamides showed low sorption onto sludge due to their hydrophilic nature (Table S1), but they were found with high removal rates in the anaerobic digester and UASB. Sulfonamides are resistant to hydrolysis, but easily biodegradable in anaerobic conditions due to the presence of nitrogen and sulphur in their molecular structures (Kümmerer, 2009; Gobel et al., 2005; Wijekoon et al., 2015). Consequently, biodegradation was regarded as their main removal mechanism for sulfonamides in the wastewater treatment systems of Farm A and Farm B. For the tetracyclines and fluoroquinolones, their removal rates in the WWTPs of the present study ranged from 61.9% to 100%. Tetracyclines could interact strongly with clay, natural organic matter and metal oxides by cation exchange, surface complexation/cation, bridging hydrophobic partitioning, and electron donor-acceptor interactions (Aristilde et al., 2010; Kong et al., 2012). The predominance of tetracyclines in the dewatered sludge indicated that sorption was an important removal mechanism. In addition, hydrolysis and biodegradation could be another two important removal mechanisms for tetracyclines in swine wastewater treatment systems (Kümmerer, 2009; Wijekoon et al., 2015). Meanwhile the resistance to hydrolysis and accumulation in dewatered sludge for fluoroquinolones indicated that the predominant removal mechanism was adsorption to sludge and/or flocs (Batt et al., 2007; Golet et al., 2003; Lindberg et al., 2006; Zorita et al., 2009; Kümmerer, 2009). Although macrolides were detected with low concentrations, the aqueous removals were up to 52.4% and 84.0% for Farm A and Farm B, respectively. Biodegradation could play an important role in the removal of macrolides in wastewater treatment plants because of the presence of plenty of electron donating groups (EDGs) (e.g., $—CH_3$, —OH, $—OCH_3$), which render them more susceptible to biodegradation (Zhou et al., 2013c; Phan et al., 2018). In the present study the instability in the removal of macrolides in aerobic units of both Farm A and Farm B may be ascribed to the low concentrations and big variations of these antibiotics in the influent. For the others, lincomycin was predominant in the swine wastewater, and its removal was above 99.2% (Table 1). This is consistent with the results in a previous study by Zhou et al. (2013c), with its aqueous removal rates up to 91.0% in the conventional WWTPs. It should be noted in the present study that the high removal of lincomycin was found in the anaerobic digester for Farm A and UASB for Farm B, which could be attributed to the microbial degradation due to the presence of nitrogen, sulphur and EDGs (e.g., $—CH_3$ and —OH) in its molecular structure (Zhou et al., 2013c; Wang et al., 2018; Wijekoon et al., 2015; Phan et al., 2018).

The aqueous removals of antibiotics in every treatment unit of the swine farms A and B are given in Tables S15–S16. In the wastewater treatment system of Farm A, the order of removal for the total detected antibiotics was: anaerobic digester (91.2%) > Precipitation/Filtration/UV (76.4%) > A^2/O (21.1%) > Second clarifier (5.69%) > Oxidation ditch (1.30%) > Lagoon (−16.2%). Meanwhile, the removal order for Farm B was: UASB (96.2%) > Lagoon (84.5%) > $(A/O)^2$ (33.2%) > Precipitation/Filtration/Chlorination (23.4%) (Fig. 2). This demonstrated that the anaerobic digester and UASB units made a significant contribution to the elimination of antibiotics. This result is very different to that in a previous study by Zhou et al. (2013a), in which the wastewater treatment system (lagoons and anaerobic digester) was found to be ineffective in the elimination of antibiotics due to the short hydraulic retention time (HRT) and excess input of wastewater. In the present study, the HRT of anaerobic digester was up to 5 d; accordingly, antibiotics have enough time to be degraded and adsorbed by the microbe and particles. UASB has the advantage of its granular sludge, which could protect susceptive microorganisms from toxic substrate and make the reactor highly resistant to antibiotics (Oktem et al., 2008). As a consequence, it shows excellent performance in removing antibiotics among all the anaerobic processes (Oktem et al., 2008).

5. Conclusion

The present study surveyed 7 classes of 40 antibiotics in the full-scale swine wastewater treatment systems of two typical swine farms in South China. Totally 25 out of 40 antibiotics were detected in all the samples including wastewater, sludge and manure. Sulfamonomethoxine, sulfachlorpyridazine, oxytetracycline and lincomycin were the predominant antibiotics in the influent. Chlortetracycline and oxytetracycline were the primary antibiotics detected in the dewatered sludge, while chlortetracycline, tetracycline, oxytetracycline and lincomycin were predominant in the swine manure. The two full-scale wastewater treatment systems showed effective elimination of the total antibiotics in swine wastewater, with the anaerobic digester from Farm A and UASB from Farm B making significant contributions. The daily excretion masses of target antibiotics via manure and urine were estimated to be 45.1 g/d for Farm A and 379 g/d for Farm B. Furthermore, manure and dewatered sludge could be a potential source for antibiotic release into the environment, and need to be further treated before disposal on land.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.05.230.

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