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Removal of veterinary antibiotics from swine wastewater using anaerobic and aerobic biodegradation

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Abstract

High amounts of antibiotics, used in animal farms for the prevention of diseases, are released back into the natural environment with a possible risk of chronic toxicity to other organisms and the development of antibiotic-resistant genes. The antibiotics, including sulfonamides and β -lactams, detected in swine wastewater were between 99.2–339.3 $\mu\text{g/L}$. The typical on-site swine wastewater treatment process turned out to be inadequate, reflected by the high conventional contaminant and antibiotic residuals even under a long hydraulic residence time (HRT, around 2–3 months). The lab-scale combined anaerobic and aerobic biological process showed that anaerobic digestion was mainly responsible for chemical oxygen demand (COD) reduction and aerobic biodegradation contributed significantly to antibiotic removal, with overall removal efficiencies of 95% for COD and 92% for antibiotics under a short HRT of 3.3 days. The removal of selected antibiotics could be a combined result of biodegradation, the balance between adsorption and desorption, and the transformation between antibiotic metabolites and their parent antibiotics. The current work provides valuable insights into the appropriate selection and optimisation of biological processes for the treatment of typical wastewater with high COD and trace antibiotics.

Keywords: Antibiotics; Swine wastewater; Anaerobic digestion; Aerobic biodegradation

1 Introduction

Annual antibiotic consumption in China has reached 162,000 tons in 2013, accounting for ~50% of global usage [1]. Fifty-two percent of the antibiotics are used in animal farms for the prevention and treatment of diseases and to increase production [2-5]. The majority of antibiotics (30%–90%), in the form of raw substances or their metabolites, are released back into the natural environment through animal faeces and urine [6-8]. The amount of antibiotics released by animal waste reached 50,000 tons per year [4]. Sulfonamides (SAs) and β -lactams have been reported as the most widely used antibiotic classes in swine industry and the dominant antibiotic components detected in the swine wastewater [9-12]. The long-term presence of these substances, despite only trace ng/L and μ g/L levels in the aquatic environment, may cause chronic toxicity to other organisms and lead to the formation of antibiotic resistance genes (ARGs) [13]. ARGs occur frequently in the effluent of municipal wastewater treatment plants (WWTPs), soils, animal wastes, and even surface water [1, 14].

The treatment of antibiotics in animal wastewater is essential for protecting our ecosystem. Animal farms are commonly located far away from the municipal sewage system, which leads to the use of simple treatment units, such as anaerobic digestion (AD), constructed wetlands or lagoons, to treat the animal wastewater [5, 15, 16]. The storage of animal wastewater in a tank for up to several months is also common. These

treatment processes are easily performed and have relatively low operational and maintenance costs. Nevertheless, they have some significant drawbacks such as 1) a long hydraulic residence time (HRT, corresponding to large disposal facility); 2) climate-dependent treatment efficiency; and 3) a source of contaminants to the surrounding environment. Furthermore, these conventional on-site treatment processes, with the main focus on bulk contaminant removal (e.g. COD), often turn out to be inadequate for the elimination of trace antibiotics in swine wastewater [17]. Other treatment methods, like activated carbon adsorption, membrane filtration, advanced oxidation processes, have been used to remove antibiotics as well [18-22]. However, these advanced treatment methods require complicated pretreatment and post-treatment processes and their costs are relatively high. A cost-effective treatment technique is urgently needed to deal with the substantial amount of animal wastewater that is rich in refractory antibiotics, with the dual consideration of conventional pollutant and antibiotic removal.

A cost-effective biological treatment has been widely used in WWTPs, mainly targeting conventional pollutant removal. Nevertheless, there are only a limited number of existing studies that assess the biological treatment of wastewater, particularly swine wastewater with high COD and trace antibiotics, from the angle of antibiotic removal efficiency. The appropriate selection of biological processes and optimisation of operating conditions is

of great importance in ensuring effective antibiotic removal from high COD-containing wastewater. The targeted removal efficiency of total antibiotics in swine wastewater is > 60% when the final effluent is to be reused for irrigation, based on project requirements. An appropriate amount of residual nutrients in the effluent, like nitrogen and phosphorus, is required for plant growth. This study aims to 1) identify the categories and quantities of antibiotics in swine wastewater and evaluate their degradation performance by on-site swine wastewater treatment processes; 2) assess the effectiveness of antibiotic removal by the integrated anaerobic and aerobic biological processes, via altering HRT; and 3) evaluate the respective role of each treatment unit and resolve the possible contaminant degradation mechanisms. The target of antibiotic removal efficiency rather than effluent concentration was set to minimise antibiotic emissions since the discharge standards for individual antibiotics are still unavailable. This study may provide valuable insights into understanding the mechanisms of antibiotic removal by biological treatments and offer information useful for the design or modification of the treatment unit in farms. The scope of this work should be of great interest to both practical and research fields.

2 Materials and methods

2.1 Chemicals and materials

2.1.1 General chemicals

Hydrochloric acid, sodium hydroxide, sodium thiosulfate, Nessler's reagent, potassium

sodium tartrate tetrahydrate, sulfamic acid, p-aminobenzene sulfonic acid, ammonium molybdate, amino sulfonic acid, potassium antimony tartrate, potassium persulfate and ascorbic acid were used for the determination of ammonia nitrogen ($\text{NH}_3\text{-N}$), nitrate nitrogen ($\text{NO}_3^-\text{-N}$), nitrite nitrogen ($\text{NO}_2^-\text{-N}$), total phosphorus (TP) and phosphate ($\text{PO}_4^{3-}\text{-P}$). Ammonium chloride, disodium hydrogen phosphate, glucose and urea were used for the synthetic wastewater preparation (see Supporting Information S1). Sodium carbonate was used for pH adjustment during biodegradation. Detailed chemical information is listed in Supporting Information S2. Deionized (DI) water was used for all stock solution preparation unless otherwise specified.

Nineteen antibiotics, sulfathiazole (STH), sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfachlorpyridazine (SCP), sulfamethoxypyridazine (SMP), sulfadiazine (SD), sulfamerazine (SMR), sulfamethizole (STZ), trimethoprim (TMP), sulfamethazine (SMT), sulfamonomethoxine (SMM), amoxicillin (AMX), cefpirome sulfate (CPS), procaine penicillin G (PPG), ampicillin (AMP), ceftiofur (CEF), benzylpenicillin potassium (PCG), cloxacillin sodium (CLX) and benzathine penicillin G (BPG), were analytical grades with > 95% purity. The first eleven compounds are SAs and the rest are β -lactams. Sulfamethoxazole- D_4 (SMX- D_4) and benzylpenicillin- D_5 potassium salt (PCG- D_5) were used as internal standards. The detailed information for the antibiotics and internal standards is listed in Supporting Information S3. Sulfuric acid, EDTA-2Na,

formic acid, methanol, and ethyl acetate were used for the preservation and pretreatment of antibiotic samples. A stock solution containing a mixture of 0.1 g/L of each antibiotic was prepared by dissolving the pure chemicals in a mixture of methanol and MilliQ water (Purelab Ultra Analytic) with a volume ratio of 1:1. The stock solution was stored at -20 °C for no more than one month, otherwise, it was re-prepared to eliminate the effect of chemical instability. The antibiotic standards for calibration (0.004–10 µg/L) were obtained by diluting the stock solution with a mixture of methanol and MilliQ water (1:1, v/v) and stored at 4 °C until analysis (within 14 days).

2.1.2 Swine wastewater

The swine wastewater was collected from a medium-scale swine farm in Shanghai, China, with an annual production of 15,000 pigs. The SAs and β -lactams (with SMM and SMZ, in particular), as the most widely used antibiotics, were dosed to the swine farm via feed addition and injection. The on-site swine wastewater treatment process is shown in Figure 1. Solid waste is separated by a solid-liquid separation unit and an additional primary sedimentation unit and used as fertilizer. One-third of the wastewater undergoes AD treatment with an HRT of 53 days (calculated by using the effective reactor volume divided by the influent/effluent volume) and its effluent enters a storage tank. The rest of the wastewater enters the storage tank directly, due to the limited space of the AD tank. The wastewater stays in the storage tank for 90 days, after which the

effluent, after an appropriate mixing with natural water, is used for irrigation. The swine wastewater in the three sampling points (i.e., AD influent, AD effluent, and storage tank effluent, as shown in Figure 1) was collected in triplicate on August 27 and October 17, 2018, in order to understand the on-site treatment efficiency and the wastewater characteristics at the different times. The collected wastewater samples by 1 L amber glass bottles were pH adjusted to 3.0 by 4 M H_2SO_4 , dosed with methanol (5% v/v) to inhibit microbial activity [12, 23], and stored at 4 °C until antibiotic analysis. The swine wastewater (from AD influent) used for the lab-scale biological experiments was collected separately and was not adjusted.

2.1.3 Sludge for anaerobic and aerobic degradation

The anaerobic sludge was collected from an anaerobic reactor in a chemical company in Jiangsu province since on-site anaerobic sludge was unavailable. The aerobic-activated sludge was provided by Shanghai Changqiao municipal WWTP. The sludge was stored at 4 °C until usage. The mixed liquor suspended solids (MLSS) for anaerobic and aerobic degradation were adjusted to ~16 and ~3 g/L respectively.

2.2 Anaerobic and aerobic experiments

The AD experiment was conducted in a glass container with a volume of 1 L, sealed by a rubber plug. A syringe was installed in the plug for sample collection and a headspace

tube was connected with another container for the release of pressure caused by gas production (Figure 2). The AD reactor was placed in an oscillator (SPH-100C) working at a shaking speed of 150 r/min and a temperature of 35 °C. The AD system operated for 21 h for anaerobic reaction and 2.5 h for sedimentation. The time for feeding and drainage was 0.5 h. The feeding of swine wastewater (collected from AD influent, pH adjusted to 8.0 ± 0.5) was performed via a sampling probe, after which nitrogen was introduced for five minutes to maintain the anaerobic condition with a dissolved oxygen (DO) concentration of less than 0.2 mg/L. The treated swine wastewater was discharged via a siphon. The organic load was enhanced gradually by increasing the feeding volume from 0.1 to 0.3 L (equivalent to a reduction of HRT from 10 to 3.3 days) with a total running time of 210 days (Table 1), to provide an adaptation process for the sludge, which was originally used for chemical wastewater treatment, and also to evaluate the effect of HRT on degradation performance. The wastewater after AD treatment was collected for COD, nitrogen and phosphorus analysis every two days. The antibiotic samples (in duplicate) were collected for analysis across nine independent days during the AD stabilisation period (after the 120th day).

The aerobic degradation was performed in a sequencing batch reactor (SBR), made of a plexiglass container with an effective volume of 5 L. Uniform aeration by an air pump (ACO-006) with a DO range between 2.0–4.0 mg/L was performed via an aeration tray

(HP-60) installed at the bottom of the reactor. The solution pH was adjusted to 7.0 ± 0.5 to sustain the appropriate condition for aerobic biodegradation. The SBR was operated as 21 h for aerobic reaction, 1 h for sedimentation and 1.5 h for resting, under ambient temperature (~ 25 °C). The time for feeding and drainage was 0.5 h. The synthetic wastewater was fed as a supplement due to the unavailability of real wastewater during the period of 1–28 days. During 29–55 days, 0.3 L swine wastewater from sampling one was used for feeding. Considering the rather high organic content (i.e., COD) in SBR effluent, even under low feeding volume (see more discussion in Section 3.3), the effluent from the laboratory AD reactor was fed to the SBR reactor for the subsequent running process (56–245 days). The feeding volume was increased from 0.3 to 1.5 L (corresponding to a reduction in HRT from 16.7 to 3.3 days, Table 1) to investigate the effect of HRT on degradation efficiency. The total running time for the SBR was 245 days. The wastewater after aerobic degradation was collected for COD, nitrogen and phosphorus analysis every two days. The antibiotic samples (in duplicate) were collected in three independent days in each period (the 128th, 136th, and 141st days for the period with an HRT of 10 days; the 171st, 181st, and 202nd days for the period with an HRT of 5 days; and the 212nd, 231st, and 243rd days for the period with an HRT of 3.3 days).

2.3 Analytical methods

2.3.1 General parameters

The concentrations of $\text{NH}_3\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$ were determined by Nessler's reagent spectrophotometry (HJ 535-2009), the spectrophotometric method (GB 7493-87) and ultraviolet spectrophotometry (HJ/T 346-2007), respectively. Samples for COD and TN determination were digested via Digital Reactor Block 200 and analysed by DR 3900 spectrophotometer (Hach, USA). TP was measured by the ammonium molybdate spectrophotometric method (GB 11893-89). The calcium and magnesium elements were determined by atomic absorption spectroscopy (AAS, novAA400, JENA). The DO and pH were monitored by portable DO (HACH, HQ40d) and pH meters (FE20 Plus), respectively. The MLSS was determined using the gravimetric method [24].

2.3.2 Antibiotic quantification

The sample for antibiotic determination was centrifuged in a glass centrifuge tube at 3000 r/min for five minutes and filtered by a 0.7 μm glass fibre membrane (Whatman GF/F). The 50 mL filtrated sample was diluted 10 times with MilliQ water to enhance the subsequent extraction efficiency. The increase of sample volume was equivalent to the increase of contact time between antibiotics and adsorbents. The 0.2 g EDTA-2Na was added to the diluted sample to coordinate the multivalent cations (e.g., calcium) which might affect the antibiotic determination [25]. The pH was adjusted to 3.0 by 2.5 M H_2SO_4 . The SMX-D₄ and PCG-D₅ of 100 ng each were added into the sample as internal

standards. The solid-phase extraction cartridges (CNW Poly-Sery HLB, 500 mg, 6 mL) were preconditioned in an order with 5 mL ethyl acetate, 5 mL methanol and 5 mL MilliQ water (pH 3.0). The sample was subsequently introduced to the cartridge at a flow rate of 3-5 mL/min. The inner wall of sample container was washed with 10 mL MilliQ water (pH 3.0), which was introduced into the cartridge followed by 30 minutes of vacuum pumping. The impurity in the cartridge was washed by 5 mL MilliQ water with 5% methanol and 5 mL MilliQ water (pH 3.0), followed by 10 minutes of vacuum drying. The antibiotics retained on the cartridges were eluted with methanol alone (5 mL) and subsequently a mixture of methanol and ethyl acetate (1:1, v/v, 5 mL). The eluate was evaporated by a gentle stream of nitrogen at 15 L/min in a water bath at 35 °C. The 1 mL mixture of methanol and MilliQ water (1:1, v/v) was added slowly along the tube wall, followed by 5 minutes of ultrasonication via an ultrasonic cleaner (KQ-100V, 40KHz) to enhance antibiotic dissolving. The extract was further filtered via a 0.22 µm PTFE filter (Anpel, China) and was ready for antibiotic analysis.

The antibiotics were analysed using a liquid chromatography-tandem mass spectrometry (LC-MS/MS, LC-30A, LCMS-8050, Shimadzu) coupled with a Shim-pack GISS C18 column (100 mmL× 2.1 mmI.D., 1.9 µm, P/N: 227-30048-02, Shimadzu). The 2 µL sample was injected with the freshly prepared mobile phase as a combination of solution A (MilliQ water with 0.1% formic acid) and B (methanol) at a total flow rate of 0.4

mL/min. The mobile phase program, which took a total of 10 minutes to run, was controlled as follows: 1) hold 10% solution B (the rest 90% was solution A) for 0.2 minutes, 2) increase gradually to 25% until 1 minute, 3) increase gradually to 30% until 4 minutes, 4) increase gradually to 80% until 4.5 minutes and hold for 2.5 minutes, and 5) hold 10% for 3 minutes for column equilibration. The column temperature was kept at 40 °C. The MS system was equipped with an electrospray ionisation source and operated in a positive ion mode; except for the determination of BPG. The method development and validation parameters with retention time, precursor and product ions, linearity, limit of detection (LOD) and limit of quantification (LOQ) are listed in detail in Supporting Information S4.

2.4 Statistical methods

One-way analysis of variance (ANOVA) was used to compare the impact of different wastewater treatment methods and antibiotic concentrations during different periods. The specified level of type I error α is equal to 0.05, indicating that $p \leq 0.05$ means the difference is significant.

3 Results and discussion

3.1 On-site swine wastewater treatment

The characteristics of the swine wastewater collected at the three different locations are

shown in Table 2. The swine wastewater after primary sedimentation still had substantial organic and nutrient content, with COD, TN and TP concentrations of 5683, 865 and 52 mg/L, respectively. The $\text{NH}_3\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ as the main components, accounted for 97% and 85% of TN and TP, respectively. After AD treatment, a significant proportion of these substances was removed, with removal efficiencies of 81% and 62% for COD and TP, respectively (calculated by the concentrations of samples collected in AD influent and effluent, as shown in Table 2). Only 21% of TN was removed since the anaerobic conditions were unsuitable for nitrification (as a major mechanism for the removal of the abundant $\text{NH}_3\text{-N}$). The removal of TN and TP might be attributed to the formation of struvite, calcium phosphate and other potential precipitations as a result of the abundant presence of calcium (95 mg/L) and magnesium (57 mg/L) in swine wastewater. The significant decrease of calcium and magnesium concentrations to 26 and 34 mg/L, respectively, in the AD effluent further verified our assumption. In addition, we observed a significant number of crystals deposited on the wall of the container (see Supporting Information S5). The formation of phosphine may contribute to phosphorus removal under AD conditions as well [26, 27]. The regular cleaning of pipelines and devices is highly recommended to prevent deposition and scaling issues. Treatment efficiencies of 84% for COD, 17% for TN and 35% for TP for the storage tank were calculated based on the data from the three sampling points. For example, based on COD concentrations (i.e., 5683, 1097 and 657 mg/L for AD influent,

AD effluent and storage tank effluent, respectively, see Table 2) and the split-flow (as only one-third of wastewater underwent AD treatment, see Figure 1), the COD removal efficiency was calculated as $1 - \frac{657}{5683 \times \frac{2}{3} + 1097 \times \frac{1}{3}} = 84\%$. The residual COD was within the range of 560–752 mg/L, which was significantly higher than the maximum contaminant level (MCL= 200 mg/L) permitted by Irrigation Water Quality Standards (GB 5048-2005). Further treatment is therefore needed to ensure the discharge is within acceptable levels.

The total amount of antibiotics in AD influent on August 23, 2018 was 99.2 µg/L, of which 98% were SMM (Figure 3a). The SMM concentration of 97.6 µg/L was within the range of 23–134 µg/L reported by other researchers [12]. The high concentration of SMM is a result of its universal use, due to its high efficacy, easy dissolution, and low acetylation rate. The other SAs were present with ng/L levels, following a decreasing order of SMZ, STH, SD, SCP, SMP and SMX 276, 134, 126, 85, 53 and 18 ng/L, respectively. The β-lactams also existed at trace levels: CLX, BPG, PCG, AMP, AMX, CEF and PPG had levels of 339, 190, 156, 75, 60, 51 and 35 ng/L, respectively. Other researchers similarly found that β-lactams have relatively low concentrations or are even undetectable due to their hydrolysable character [28, 29]. The other antibiotics, SMR, STZ, SMT, TMP and CPS, were not detectable (< 4 ng/L). AD treatment reduced the total amount of antibiotics to 11.3 µg/L, of which SMM was still the predominant

compound. After 90 days of natural degradation in the storage tank, 73% of the total antibiotics were further removed. At this point, SMZ exceeded SMM (14.2 vs. 4.3 $\mu\text{g/L}$) becoming the most abundant antibiotic.

The overall removal efficiency of antibiotics was 81% after the on-site swine wastewater treatment (Figure 3b). Individual antibiotics, including SMM, CLX, PCG, CEF, SMT, SD, BPG, SMP and STH, were better eliminated by the storage tank than AD. Notably, the concentrations of some antibiotics, particularly SAs (e.g., SMZ and SMX), increased after AD or storage tank treatment (corresponding to negative removal efficiencies). The possible reason is that the analytically-camouflaged antibiotic metabolites are conversed back to their detectable parent antibiotics, triggered by a complicated evolutionary process [2, 30, 31]. For example, SMZ in the storage tank effluent was significantly higher than that in AD influent (14.2 vs. 0.3 $\mu\text{g/L}$, $p \leq 0.05$, ANOVA), which might be a result of the transformation of glucuronide of N-4-acetylated sulfamethazine (a metabolite of SMZ) to their parent SMZ in the liquid phase [32]. SMZ in animal bodies tends to bind with carbohydrates and reverses to its original form in a period after excretion due to the rapid biodegradation of carbohydrates in the natural environment [33]. Similarly, 53–68% of SMX was excreted from the animals in the form of transformed SMX [34], which may transfer back to SMX molecules during the wastewater treatment process [35]. Another possible reason

is that the antibiotics originally absorbed on the surface of the suspended solids (with total suspended solids of 5.45 g/L) in swine wastewater dissolved back to the aqueous phases during the reaction process [10].

The total antibiotic concentration on October 17 was significantly higher compared to that on August 23 (339.3 v.s. 99.2 $\mu\text{g/L}$, $p \leq 0.05$, ANOVA, Figure 3c). More types of antibiotics, including TMP, STZ and SMR, occurred in the AD influent. The concentrations of some antibiotics (SMZ, SCP, AMX, CLX, and SMX) in the AD influent more than doubled. However, AMP and CEF were no longer detectable. The different types and amounts of antibiotics used to deal with different swine diseases during the various periods could be partially responsible for this diversity. The drug usage records in the farm show that the average antibiotic dosage in October was doubled that of August over the past four years. The antibiotics in the storage tank effluent were dominated by SMM (12.9 $\mu\text{g/L}$) and SMZ (7.6 $\mu\text{g/L}$). The total residual antibiotic concentrations after the on-site swine wastewater treatment were comparable in across the two sampling dates (~ 20 $\mu\text{g/L}$). For most individual antibiotics, AD showed better removal efficiencies than the storage tank in October, in contrast to the observation in August. The lower temperature in mid-October (< 15 $^{\circ}\text{C}$) may restrain the treatment efficiency of the outdoor storage tank, while its effect on the sealed AD unit might be negligible. The fact that the residual antibiotic and COD concentrations

were still high after the on-site wastewater treatment process, even under an HRT as high as 2–3 months, triggered the subsequent exploration of a more effective process for swine wastewater treatment.

3.2 Performance of anaerobic digestion

The COD removal efficiency did not show any significant difference with the gradual decrease of HRT from 10 to 3.3 days in the AD unit (Figure 4a). The variance for the effluent COD concentration seemed to be caused solely by the influent COD concentration. For example, the sharp increase of influent COD from ~1750 to ~4000 mg/L at around the 150th day resulted in the effluent concentration increasing from ~1500 to ~3000 mg/L. The average COD removal efficiency was 43%. AD showed a poor TN degradation performance throughout the entire reaction process (Figure 4b). The majority of TN in AD effluent was still $\text{NH}_3\text{-N}$ (93%) and nitrogen oxides (the summation of $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$) were less than 20 mg/L. The TP removal efficiency was 29%, possibly caused by precipitation and evaporation (e.g., struvite and phosphine).

Only 3% of the total antibiotics were removed by the AD unit on the 120th day (Figure 5). A low degradation efficiency was also observed in other intermittent sampling days (Supporting Information S6). Recent studies have demonstrated that AD is limited in its

ability to removal antibiotics from wastewater [10, 12, 36, 37]. Antibiotics, particularly SAs, containing aromatic rings and double bond functional groups are robust and have high resistance to biodegradation [2]. However, some individual trace SAs still had relatively high removal efficiency, for instance, 85% for SMP, 97% for SCP and 88% for SMX. The presence of nitrogen and sulphur in the molecular structures of some SAs might enhance anaerobic degradation [10, 38]. The different reduction potential may also affect degradation performance. For instance, SMX needs a lower reduction potential than SMZ in the initiation of an ipso-hydroxylation with subsequent fragmentation, which is a common degradation pathway for SAs [38, 39]. The β -lactams seemed more anaerobic degradable than SAs, reflected in total removal efficiencies of 69% for β -lactams and 2% for SAs. The removal efficiency of individual β -lactams followed a decreasing order of CLX (97%) > CEF (83%) $\approx$ AMP (83%) > BPG (77%) > AMX (57%) > PCG (17%) > PPG (0.5%). The negative removal efficiencies for SD, SMZ, and STH were the same as those observed in the on-site AD treatment (Section 3).

Following the increase of feedwater antibiotic concentration to $\sim 340 \mu\text{g/L}$ on the 172nd day (the feedwater was collected on October 17, 2018), effluent concentration increased within 10 days (see Supporting Information S6). A notable antibiotic reduction was subsequently observed, indicating the potential enhancement of antibiotic degradation by extending the AD operation time. The higher influent antibiotic concentration

corresponding to better degradation efficiency, may be attributed to its comparative advantage over the conventional organic substances for biodegradation [36].

The lab-scale AD effluent (with 1172 mg/L COD, 848 mg/L $\text{NH}_3\text{-N}$ and 19 mg/L TP) had a comparable organic and nutrient content to the on-site AD effluent, although HRT was shortened from 53 to 3.3 days. However, the overall antibiotic degradation in this lab-scale AD unit was much poorer compared to the on-site AD treatment (3% vs. 89%). It seems that the extension of AD operation time may potentially enhance degradation performance, while extremely long-term inoculation seems impractical in a farm [36]. A fast and efficient treatment method is needed further to achieve better water quality.

3.3 Performance of aerobic degradation

Conventional parameters including COD, nitrogen and phosphorus were continuously monitored to assess the SBR performance. The COD removal efficiency increased gradually from ~50% to 80% in the first 28 days with synthetic wastewater as feedwater (Figure 6a). The easily degradable glucose, as the main organic component of synthetic wastewater (Supporting Information S1), resulted in a high COD removal efficiency. The COD in the effluent reached as high as ~600 mg/L once the swine wastewater was available (29–55 days), although only 0.3 L was fed to the reactor. In consideration of the presence of the AD unit in the on-site treatment system, the lab-scale AD effluent

was subsequently fed to the SBR with a gradual increase of feedwater volume from 0.3 to 1.5 L. The effluent COD concentrations remained within ~200–400 mg/L despite the significant fluctuations of feedwater COD concentrations (~200–800 mg/L). The sludge seemed to be well inoculated, reflected in increased COD removal efficiencies from 18% to 58% and the gradual growing of MLSS from 3.5 to 7.2 g/L (Table 1).

The TN removal was not obvious in the entire SBR running process, except between the 29th and 55th days (Figure 6b). The denitrification effect should be responsible for the TN decline. It was not uncommon to observe the solution DO as low as 0.5 mg/L, especially within two hours after wastewater feeding. This short-term anoxic environment may allow the survival of heterotrophic denitrifying bacteria, with the aid of easily degradable glucose residuals in the reactor. Upon the change of the feedwater type and volume, a small portion of $\text{NH}_3\text{-N}$ remained in the reactor for a period and subsequently almost completely converted to $\text{NO}_3^-\text{-N}$ under aerobic conditions (29–55 days, Figure 6c). The $\text{NO}_2^-\text{-N}$ up to ~300 mg/L was accumulated between the 29th and 55th days. The sharp pH decline during the reaction, from 7.8 to 5.5, caused by the nitrification process, might be responsible for the deteriorated $\text{NO}_2^-\text{-N}$ transformation, since an excess of hydrogen ions may inhibit the growth of nitrite-oxidizing bacteria (NOB) [40]. The pH was adjusted to 7.0 ± 0.5 by sodium carbonate in the subsequent running process and $\text{NO}_2^-\text{-N}$ was no longer accumulated. In addition, the competition

for oxygen utilisation between NOB for nitrification and microbes for COD degradation may contribute to NO_2^- -N accumulation, since the influent COD was the highest between the 29th and 55th days than in other periods [40]. The NO_2^- -N accumulation may also be associated with trace antibiotics [41].

The total amount of antibiotics in the SBR effluent were 3.1, 18.9 and 27.7 $\mu\text{g/L}$ for the running periods, with HRTs of 10, 5 and 3.3 days, respectively (Figure 7a). Four SAs were detectable during the period with an HRT of 10 days, following a decreasing order: SMM (2.1 $\mu\text{g/L}$) > SD (290 ng/L) > SCP (75 ng/L) > STH (9 ng/L). CEF (3 ng/L) was the only detectable β -lactam. With the increase of feedwater volume from 0.5 to 1.5 L, all of the individual antibiotic concentrations increased, except SMX which decreased. The gradual growth of MLSS (from 4.1 to 7.2 g/L , Table 1) might contribute to the substantial SMX removal because of its high adsorption capacity in the activated sludge [36, 42]. The effluent concentrations of SMZ, AMX, SMR and TMP during the period with an HRT of 3.3 days were several times higher than the previous periods, demonstrating that an increase in organic load may have a negative impact on antibiotic removal [43, 44].

The overall removal efficiency of the antibiotics by aerobic treatment was 93%, significantly higher than that of AD treatment with a removal efficiency of 3% ($p \leq 0.05$, ANOVA). Other researchers have similarly found that the majority of antibiotics are

removed (71–85%) under aerobic conditions, while only a small portion (of 7–23%) are removed under anoxic conditions [18]. Figure 7b shows the antibiotic removal efficiencies in the three independent sampling dates for each period. The average removal efficiencies decreased from 97% to 89% when the HRT decreased from 10 to 3.3 days, while the COD removal efficiencies showed the opposite trend (Figure 6a). The competitive advantage of COD over antibiotics was more obvious when the influent COD concentration was higher between the 204^h and 245th days. The microorganisms tend to utilise the easily degradable conventional organic substances, rather than the refractory antibiotics, to maintain their survival and metabolism [36, 43]. The SAs seemed more degradable than β -lactams under aerobic conditions. SAs are more adaptable to the biodegradation environment and are involved in co-metabolic processes [44-46].

3.4 Implications

This study assessed antibiotic degradation performance in a full-scale on-site swine wastewater treatment process. The long HRT (corresponding to a larger disposal facility), and high COD and antibiotic concentrations in the effluent triggered the investigation of an alternative treatment process, the combined anaerobic and aerobic biological process, at lab-scale. Overall removal efficiencies of 92% for antibiotics (higher than our target of 60%, mainly attributed by aerobic biodegradation) and 95% for COD (mainly attributed

to AD) were achieved with an HRT of 3.3 days after integrated anaerobic and aerobic treatment. The substantial removal of COD by the anaerobic treatment unit could reduce the organic load (corresponding to lower treatment cost) and thereby enhance the competitive role of antibiotics over COD for degradation (corresponding to higher antibiotic removal efficiency) for the downstream aerobic treatment unit. The system effluent, with an adequate amount of nutrients (TN and TP), is supposed to be reused for irrigation purpose. This combined process is, therefore, more cost-effective than the commonly used single anaerobic or aerobic unit. The scope of this work related to the evaluation of the combined anaerobic and aerobic biological process, and the optimisation of operation conditions (i.e., HRT) should be of great interest to both research and practical fields. Nevertheless, the best operation for anaerobic and aerobic processes should not be limited to HRT control. Our further study will focus on the optimisation of other operation conditions (e.g., sludge residence time, solution pH, aeration time) in order to achieve better performance. Besides, the identification of microbes responsible for the degradation of specific antibiotics is crucial for the effective control of the biological process.

4 Conclusions

In this study, we investigated the occurrence of nineteen antibiotics in swine wastewater. The total antibiotic concentrations fluctuated from 99.2 to 339.3 $\mu\text{g/L}$ (of which 98%

were SMM), possibly because the varied incidence and types of swine disease in different periods resulted in the use of different types and amount of antibiotics. The long HRT and high residual contaminants demonstrated that the on-site treatment process (AD followed by the storage tank) was insufficient or unsuitable for swine wastewater treatment. Therefore, lab-scale combined anaerobic and aerobic processes for swine wastewater treatment were systematically investigated. The AD unit demonstrated higher COD than antibiotic removal (43% v.s. 3%). The aerobic treatment contributed significantly to antibiotic degradation. The overall removal efficiencies reached 95% for COD and 92% for antibiotics during the integrated biological process operated under an HRT as low as 3.3 days. The treated wastewater, with an appropriate mixing with natural water, could be reused for irrigation.

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Declarations of interest: none

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Journal Pre-proof

Table 1. The parametric control for the anaerobic digestion and sequencing batch reactor processes

Anaerobic digestion					Sequencing batch reactor					
Feedwater r type	Swine wastewater				Synthetic wastewater	Swine wastewater	Anaerobic digestion effluent			
					r	r				
Time (d)	65				1–28	29–55	142 204			
	1–	27–	45	–			56–	94–	–	–
	26	44	–	21			93	141	–	–
			64	0					203	245
Feeding volume (L)	0.	0.12	0.2	0.3	1.5	0.3	0.3	0.5	1.0	1.5
	1	5								
HRT (d)	10	8	5	3.3	3.3	16.7	16. 7	10	5	3.3
MLSS (g/L)							3.5	4.1	4.8–	5.6–
	16.3				3.0–3.5		–	–		
					3.5–4.0		5.0	6.2	6.8	7.2

Note: HRT means hydraulic residence time; MLSS means mixed liquor suspended solids.

Table 2. The characteristics of synthetic and swine wastewater ^a

Parameter s	Unit	Synthetic wastewater	Swine wastewater		
			Anaerobic digestion influent	Anaerobic digestion effluent	Storage tank effluent
pH	--	6.8	7.8	7.7	7.7
COD	mg/L	1210	5683 (4970– 7010)	1097 (850– 1245)	657 (560–752)
NH ₃ -N	mg/L	300	836 (734–892)	661 (520–842)	642 (555–705)
NO ₃ ⁻ -N	mg/L	--	36 (22–62)	10 (8–11)	11 (5–16)
NO ₂ ⁻ -N	mg/L	--	14(12–14)	10 (9–10)	11 (6–16)
TN	mg/L	500	865 (780–945)	685 (540–883)	669 (575–743)
PO ₄ ³⁻ -P	mg/L	44	44 (42–48)	20 (17–23)	25 (22–28)
TP	mg/L	44	52 (43–56)	20 (18–24)	27 (26–28)

Note: ^a Mean (minimum-maximum), n=3.

Fig. 1. The flow chart of on-site swine waste treatment system.

Fig. 2. The schematic diagram of anaerobic digestion and sequencing batch reactor

Fig. 3. The concentrations and removal efficiencies of antibiotics in the on-site swine wastewater treatment plant on August 27, 2018 (a, b) and October 17, 2018 (c, d). The “Total” refers to the overall removal efficiencies of antibiotics by anaerobic digestion and storage tank. The error bars were based on three independent samples. Abbreviations: STH: sulfathiazole; SMZ: sulfamethazine; SMX: sulfamethoxazole; SCP: sulfachlorpyridazine; SMP: sulfamethoxypyridazine; AMX: amoxicillin; BPG: benzathine penicillin G; SD: sulfadiazine; CPS: cefpirome sulfate; SMR: sulfamerazine; TMP: trimethoprim; STZ: sulfamethizole; PPG: procaine penicillin G; AMP: ampicillin; SMT: sulfamethazine; CEF: ceftiofur; PCG: benzylpenicillin potassium; CLX: cloxacillin sodium; SMM: sulfamonomethoxine.

Fig. 4. The concentrations of COD (a), TN (b) and TP (c) during the anaerobic digestion process. The influent referred to the solution in the anaerobic digestion reactor after the addition of feedwater. The hydraulic residence time of each period has been shown in the figures.

Fig. 5. The concentrations (a) and removal efficiencies (b) of antibiotics for the anaerobic digestion process on the 120th day. The error bars were based on two independent samples.

Fig. 6. The concentrations of COD (a), nitrogen (b, c), and phosphorus (d) during the aerobic degradation process. The influent referred to the solution in sequencing batch reactor after the addition of feedwater. The hydraulic residence time of each period has been shown in the figures.

Fig.7. The concentrations (a) and removal efficiencies (b) of antibiotics under different hydraulic residence times for the aerobic degradation process. The concentrations were for samples collected in the 141st, 202nd and 243rd day during the periods with HRTs of 10, 5 and 3.3 days, respectively. The “Total” refers to the removal efficiencies of all antibiotics including sulfonamides and β-lactams. The hydraulic residence time of each period has been shown. The error bars were based on two independent samples.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract

Highlights

- The total antibiotic concentrations in swine wastewater were 99.2~339.3 µg/L.
- The combined anaerobic and aerobic treatment removed 95% COD and 92% antibiotics.
- The fate of antibiotics should be a combined result of biodegradation, adsorption and transformation.
- Higher HRT for aerobic process resulted in better antibiotic degradation performance.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7