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Effects of antibiotics on characteristics and microbial resistance of aerobic granules in sequencing batch reactors

Lin Liu[a,b,c,*], Valerie Gibson[a], Xu Huang[a], Chaoxiang Liu[a,*], Gefu Zhu[a]

[a]*Key Laboratory of Urban Pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China, Tel./Fax: +86 0592 6190533; email: lliu@iue.ac.cn (L. Liu), Tel./Fax: +86 0592 6190514; email: vee@iue.ac.cn (V. Gibson), Tel./Fax: +86 0592 6190533; email: xhuang@iue.ac.cn (X. Huang), Tel./Fax: +86 0592 6190781; email: cxliu0592@gmail.com (C. Liu), Tel./Fax: +86 0592 6190533; email: gfzhu@iue.ac.cn (G. Zhu)*
[b]*Ningbo Urban Environment Observation and Research Station-NUEORS, Chinese Academy of Sciences, Ningbo 315000, China*
[c]*State Environmental Protection Key Laboratory of Microorganism Application and Risk Control, Tsinghua University, Beijing 100084, China*

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ABSTRACT

The response of characteristics and microbial resistance of aerobic granules to sulfamethazine, oxytetracycline, and ciprofloxacin at ppb level in swine wastewater was investigated in this study. Results showed that no significant changes in physical strength, average granules size, and settling property of aerobic granules were observed with target antibiotics exposure ($p > 0.05$). However, compared to the control group, the protein/polysaccharide ratio and zeta potential of aerobic granules in treatment groups showed a higher level ($p < 0.05$). Meanwhile, the specific removal rates of organics and ammonia nitrogen in reactors with antibiotics addition all decreased greatly, and the inhibition rates of those were 21–29% and 35–42%, respectively. In terms of microbial resistance, the presence of target antibiotics would obviously increase the relative abundances of antibiotics resistance bacteria and antibiotics resistance genes in aerobic granules, ranging from 0.5 to 5 orders of magnitude; thereby, it concluded that antibiotics at ppb level without effect of other induction factors can actually reach an effective content capable of spreading antibiotic resistance in aerobic granular reactor.

Keywords: Aerobic granules; Swine wastewater; Antibiotics; Antibiotic resistance genes

1. Introduction

The daily discharge of swine wastewater was up to 6.8 million tons with high concentrations of chemical oxygen demand (COD) and nutrients, and it has been identified as a major source of diffuse pollution in China [1]. To date, although anaerobic digestion has been extensively and successfully applied in treating carbonaceous compounds of swine wastewater; the technological properties of anaerobic digestion determined low nitrogen removal efficiency [2]. Thus, the subsequent treatment for swine wastewater with nitrogen removal processes is indispensable. Aerobic granules are mainly used for the aerobic degradation of organics as well as nutrients removal under aerobic and anoxic conditions in sequencing batch reactor (SBR) [3]. At present, aerobic granulation technology has been

*Corresponding authors.

widely used to deal with high-strength wastewaters by virtue of its advantages, and has shown substantial feasibility for the advanced treatment of swine wastewater [2,4]. However, much of the existing research on granular reactors are limited to COD and nutrients removal, while there is little available information about the effects of residual antibiotics at ppb level in swine wastewater on the physicochemical properties and microbial resistance of aerobic granules [5–7].

Compared to the contamination of environment by organics and nutrients, veterinary antibiotics (VAs) in swine wastewater have recently attracted increasing attention because of their high residual concentrations and great capacity to trigger the increase of resistant bacterial strains [8]. Recent research has shown that tetracyclines, sulfonamides, and fluoroquinolones were the dominant VAs with high concentrations and frequencies of detection; further, the maximum concentrations of these have ranged from 23.8 to 685 μg/L in swine wastewater [9]. Further, tetracyclines and fluoroquinolones generally exhibited lower subsurface mobility because they possessed more polar/ion functional groups, but sulfonamides which are considered to be highly mobile compounds exhibited relatively low sorption affinity to solid phases [9]. To date, about more than 100 unique resistance genes have been found in the environment. Zhu et al. detected 149 unique antibiotics resistance genes (ARG) at three swine farms in China, and found that the top 63 ARGs were enriched 192-fold (median) up to 28,000-fold (maximum) compared with their respective antibiotic-free manure or soil controls [8]. Some surveys of the occurrence of ARGs in livestock wastewater indicated that tetracycline resistance (*tet*) genes and sulfonamides resistance (*sul*) genes, e.g. *tet*O, *tet*W, *sul*I, and *sul*II, were particularly abundant and suitable for monitoring by quantitative polymerase chain reaction [10,11]. Compared to other *tet* genes, ribosomal protection protein genes (e.g. *tet*W, *tet*M, and *tet*O) have a greater relative abundance in swine wastewater and in soils adjacent to representative swine farms [12,13]. Baker-Austin et al. also found that metals could provide a long-term coselective pressure for antibiotic resistance, while metals are usually added to swine feed for growth promotion and showed a higher content in swine wastewater [14]. Meanwhile, several studies indicated that horizontal gene transfer can cause the spread of antibiotic resistance in biological wastewater treatment plants, and the fate of antibiotics resistance bacteria (ARB) and ARG in flocs sludge may be affected by plant designs and operation [15]. However, it is still unclear whether antibiotics themselves, at ppb level, can actually reach an effective content towards maintaining or spreading antibiotic resistance in treatment plants, especially in aerobic granular reactor [16,17].

Thus, the study presented in this paper aimed to (1) estimate the response of performance and physicochemical properties of aerobic granules to sulfamethazine (SM2), oxytetracycline (OTC), and ciprofloxacin (CIP) at ppb level in swine wastewater; (2) investigate the correlation of antibiotics and corresponding ARB and ARG in aerobic granular reactor. It was expected that the results derived from this study would be useful for further understanding the behaviors of VAs and ARG in aerobic granular reactor.

2. Materials and methods

2.1. Experimental setup and SBR operation

Experiments were performed in the four SBR reactors (R1, R2, R3, and R4) with a working volume of 1 L. The influent was added from the top of the reactor with a volumetric exchange ratio of 80%. The reactors were continuously operated in successive cycles of 8 h, and each cycle consisted of 1 min influent feeding, 475 min aeration, 1 min settling, and 3 min effluent withdrawal. Air was introduced through a diffuser at reactor bottom by air pump at a flow rate of 0.4 m^2/h (superficial gas velocity of 1.8 cm/s). The pH was measured and maintained at 7.1–7.4 using real-time pH control systems. The oxygen saturation level was monitored, but not controlled. The reactors were operated at room temperatures (around 20–26℃), and were protected from light in order to prevent photolytic degradation of target antibiotics.

The real swine wastewater contain COD with a high content range, while the swine the wastewater would normally firstly be treated by an anaerobic digestion system which could receive the biogas. The COD content could be kept at a lower content in the effluent of the anaerobic digestion system, and the aerobic sludge reactor usually was the subsequent treatment system for swine wastewater with nitrogen removal processes. Thus, a synthetic swine wastewater from an anaerobic reactor prepared with distilled water was used in this study. The composition was as follows: the influent glucose content measured as COD was 1,000 mg/L, ammonia nitrogen (NH_4CI) 800 mg/L, and phosphate (KH_2PO_4) 60 mg/L, Ca^{2+} ($CaCl_2$) 10 mg/L, Mg^{2+} ($MgSO_4$) 10 mg/L, trace element solution 1.0 mL/L. The composition of the trace element solution was $FeCl_3$ 20 mg/L, $CuSO_4$ 50 mg/L, $MnSO_4·H_2O$ 50 mg/L, KCl 18 mg/L, $AlCl_3$ 15 mg/L, $ZnSO_4·7H_2O$ 30 mg/L. During the 130 operation days, R1 as control group without antibiotics addition in wastewater; SM2 spiked into the wastewater to produce concentration of 100 μg/L for R2, and OTC was added to R3 with 100 μg/L, while CIP was added to R4 with 100 μg/L. Mature aerobic granules, obtained from an SBR in our laboratory, were used as seed sludge with an initial concentration of 4,000 mg/L in mixed liquor suspended solids (SS). The seed granules were developed using the activated sludge from a local wastewater treatment plant by controlling the settling time of SBR [18] and the culture solution without any antibiotics addition during the sludge granulation.

2.2. Analytical methods

COD, ammonia nitrogen, sludge volume index (SVI), SS, and mixed liquor volatile suspended solids (VSS) were analyzed according to the Standard Method [19]. Aerobic granules for physicochemical properties analysis were sampled and measured at the end of the second cycle on the fixed days of steady-state phase. Extraction and detection method of extracellular polymeric substances (EPS) in the granules wase used as described by Adav and Lee [20]. Physical strength of aerobic granules was expressed as the integrity coefficient (%) [21]. Granule samples were mixed thoroughly by a homogenizer, and the supernatant was sampled after 5 min settling for zeta potential measurement using Nano Series Zeta Potential Analyzers (MALVERN, ZEN 3600). Average size of aerobic granules was measured by a laser particle size analysis system (Malvern Mastersizer 2000).

Quantitative real-time PCR analysis was used to detect corresponding resistance genes: three *tet* genes (*tet*W, *tet*M and *tet*O) and two *sul* genes (*sul*I and *sul*II) in granular sludge, and the determination was based on the published method [22,23]. Genomic DNA was extracted from 0.5 g of sludge using E.Z.N.A® DNA kit (OMEGA Bio-Tek, USA) following the manufacturer's instructions. We determined the concentration of the extracted DNA using spectrophotometer analysis (NanoDrop ND-1000, NanoDrop Technologies, Wilmington, DE, USA). The selected target *tet* genes, *sul* genes, and 16S-rRNA genes were quantified using ABI 7500 Real-time PCR system (Applied Biosystems, USA) and SYBR Green qPCR mix (Takara Biotechnology, Dalian, China). Plasmids carrying target genes were used to generate calibration curves, and the concentration of genes was measured by a spectrophotometer. The copy numbers of the target gene were calculated directly from extracted plasmid DNA concentration, and the production of the standard curve was generated by 10-fold serial dilutions of a known copy number of the plasmid DNA. The PCR efficiencies (91.3–111.7%) were examined to test for inhibition. The R^2 values of all calibration curves were more than 0.99. Data analysis was carried out with iCycler software. Each reaction was performed in a 25 μL volume containing 12.5 μL of SYBR Premix Ex Taq (TaKaRa), 0.2 μM of each primer, and 2 μL of template DNA which was diluted 5-fold. The Q-PCR protocol for tet genes was as the following program: 1 min at 95℃, followed by 40 cycles of 10 s at 94℃, 45 s at the annealing temperatures, and the temperature at which the fluorescence was read during each cycle was varied according to different target genes (*tet*M 80℃; *tet*O 81℃; *tet*W 83℃). The Q-PCR protocol for *sul* genes was as the following program: 15 min at 95℃, followed by 50 cycles of 15 s at 95℃, 30 s at the annealing temperature (*sul*I 65℃; *sul*II 57.5℃), and 30 s at 72℃ followed by a final melt curve stage with temperature ramping from 60 to 95℃. All Q-PCR reactions were carried out in triplicate. Product specificity was confirmed by melting curve analysis and visualization in agarose gels.

The analysis method of total (MPN) and antibiotics resistant (rMPN) culturable bacteria were used as described by Heuer and Smalla [24]. Aerobic granules (0.5 g) were suspended in 9 mL of sterile saline by shaking overhead with glass beads for 30 min. Serial 10-fold dilutions in 1/10 TSB (Becton Dickinson, Sparks, MD) with 100 mg/L cycloheximide, and without or with standard content of individual antibiotic were aliquoted into microplates with eight replicates of 0.15 mL. The standard contents of SM2, OTC, and CIP were 512, 16, and 4 mg/L, respectively, and those were set according to Performance Standards for Antimicrobial Susceptibility Testing [25]. The most probable numbers of total and antibiotics-resistant culturable bacteria were calculated from the number of turbid wells per dilution after 2 and 4 d of incubation at 28℃.

Statistical analyses were conducted using SPSS Version 16.0 (SPSS Inc., USA) for comparison of characteristics and microbial resistance of aerobic granules in the four systems, and comparisons were considered to have statistically significant difference for $p < 0.05$.

3. Results

3.1. Performance and physicochemical properties

The total operational period of our experiment was 130 d. The four aerobic granular reactors reached steady-state phase on 36th day, namely no significant differences of COD and NH_4^+-N removal rate, sludge retention time, and so on were observed in individual SBR. Therefore, datum of performance and physicochemical properties of aerobic granules in Figs. 1–3 were averaged to produce a single value for each reactor (Table 1) after 50 d of operating.

Compared to the control reactor (R1), no significant changes in physical strength, granules size, and SVI_5 (the measurement time was 5 min) of aerobic granules in R2–R4 were observed ($p > 0.05$). The results indicated that aerobic granular reactor could keep sludge granulation and settling property at ppb concentrations of SM2, OTC, and CIP in swine wastewater. However, the presence of target VAs showed effect on the surface potentials, the EPS composition, and pollution removal rate of aerobic granules in reactors ($p < 0.05$). The zeta potential value of aerobic granules in R1 was −10.6 mV, while the surface charges of aerobic granules in R2–R4 were examined to increase to −13.4 to 19.9 mV, namely aerobic granules without antibiotics exposure were less negatively charged.

The major substances in EPS are the protein (PN) and polysaccharide (PS), and both were measured in this study (Fig. 2). The total content of EPS in the four systems remained at a similar level (about 126.92–131.59 mg/g VSS), but the ratio of PN to PS of the granules in R2–R4 showed a higher value than that in R1. Specific removal rates of COD and NH_4^+-N of aerobic granules were determined (Fig. 3), in order to minimize the variance caused by different sludge concentrations in the four systems. Overall treatment performance of organics and ammonia nitrogen were substantially affected by target VAs, and the specific removal rates of COD and NH_4^+-N in reactors with VAs addition all decreased greatly and dropped to 0.3–0.33 g COD/g VSS-d and 0.15–0.17 g NH_4^+-N/g VSS-d, respectively, yet no differences were detected among R2 to R4 with different VAs treatment ($p > 0.05$).

3.2. Response of microbial resistance

Approach in conceptualizing the fate and development of target antibiotics resistance in the four systems were the consideration of corresponding ARG and ARB as the primary indicators, and the relative abundances of ARG and ARB on 50th day and 120th day are shown in Figs. 4 and 5, respectively. In this study, the comparison and subsequent discussion were with respect to the relative abundances of ARG (target ARG/16S-rRNA genes) and ARB (rMPN/MPN). As shown in the normalized ARG data, two *sul*

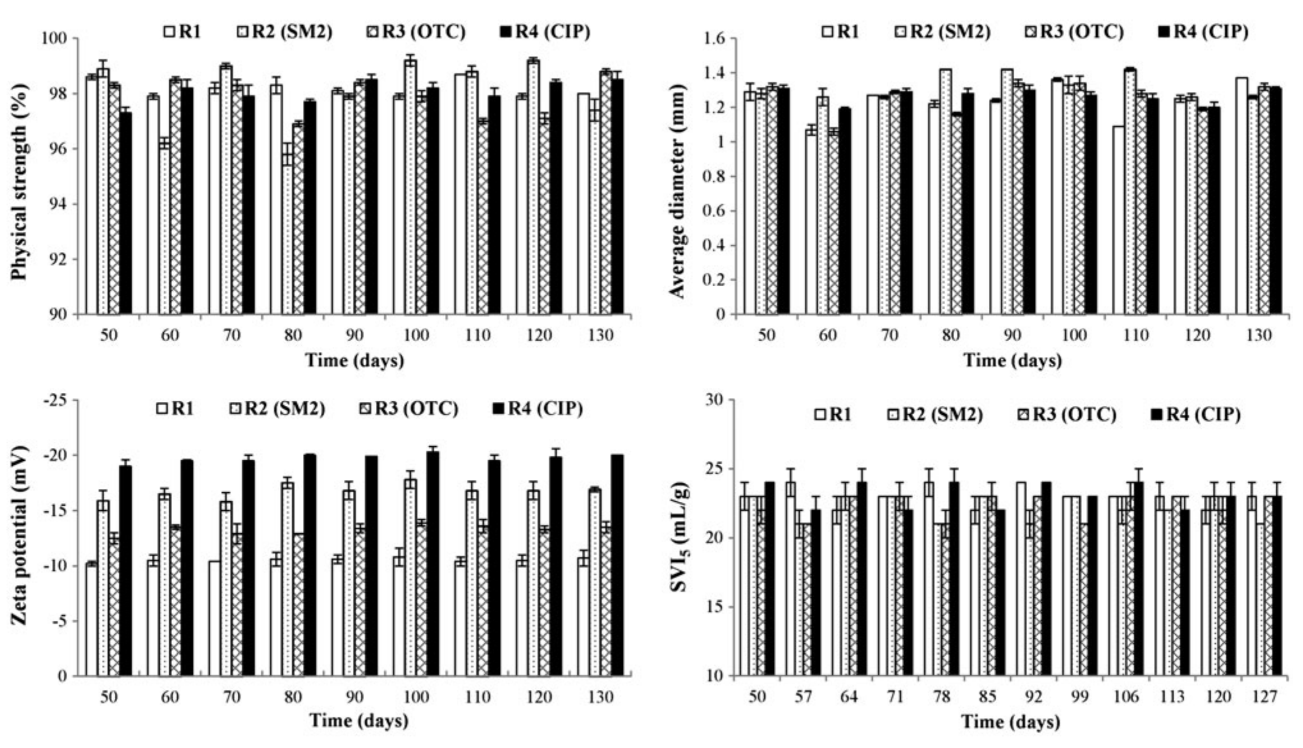

Fig. 1. Physical strength, average diameter, zeta potential, and SVI_5 of the aerobic granules in R1–R4. Value is the mean ± SD of triplicate.

Table 1
Mean properties data of aerobic granules with different amendments at steady-state phase (50–130th day). All values include standard error of the measurements, with 8–12 samples

	R1	R2 (SM2)	R3 (OTC)	R4 (CIP)
Physical strength (%)	98.2 ± 0.5	97.1 ± 2.1	97.7 ± 0.8	98.1 ± 0.4
Average diameter (mm)	1.22 ± 0.15	1.34 ± 0.08	1.20 ± 0.14	1.25 ± 0.06
Zeta potential (mV)	−10.6 ± 0.2	−16.8 ± 1	−13.4 ± 0.5	−19.9 ± 0.4
PN in EPS (mg/g VSS)	79.2 ± 4.3	88.5 ± 5.3	86.9 ± 2.1	92.6 ± 4.4
PS in EPS (mg/g VSS)	50.5 ± 1.8	38.4 ± 1.5	40.7 ± 1.5	39 ± 1.1
PN/PS ratio	1.57 ± 0.03	2.31 ± 0.05	2.14 ± 0.03	2.38 ± 0.05
SVI_5 (mL/g)	23 ± 1	22 ± 1	22 ± 1	23 ± 1
Specific rate (g NH_4^+-N/g VSS-d)	0.26 ± 0.04	0.16 ± 0.03	0.17 ± 0.02	0.15 ± 0.02
Specific rate (g COD/g VSS-d)	0.42 ± 0.02	0.32 ± 0.02	0.33 ± 0.03	0.3 ± 0.05

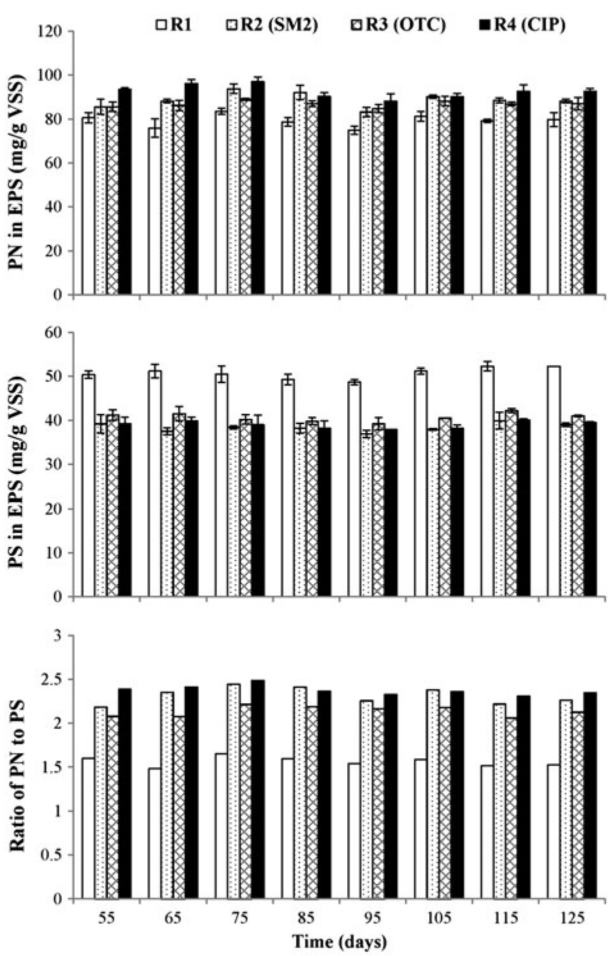

Fig. 2. EPS content and the ratio of protein to polysaccharides of the aerobic granules in the four systems. Value is the mean ± SD of triplicate.

genes and three *tet* genes were detected in aerobic granules from R1 to R3 with or without VAs treatment. Compared to the control reactor (R1), the level of two *sul* genes in R2, and *tet*W and *tet*M in R3 showed obvious increases with SM2 and OTC addition, ranging from 0.5 to 1 order of magnitude. However, the relative abundances of *tet*O in R1 and R3 retained a similar level (about 1.1×10^{-4} to 2.41×10^{-4}), which were unaffected by OTC amendment or operating time ($p > 0.05$). Meanwhile, the level of *sul* genes (10^0–10^{-2}) were apparently higher than that of *tet* genes (10^{-3}–10^{-5}), and *tet*M always be a predominant *tet* genes (1.17×10^{-3} to 1.35×10^{-4}) in aerobic granules from R1 to R3. On two sampling dates, no significant differences of *sul* genes and *tet* genes in reactors with VAs addition were observed ($p > 0.05$), i.e. the ARGs level did not persistently increase. In terms of culturable bacteria, the relative level of ARB followed the same trend as that of *sul* genes, *tet*W and *tet*M. The higher resistance of bacteria to SM2, and the equivalent resistant response of microbes at different phases (50th day and 120th day) were detected in aerobic granules. At the end period of this experiment, the relative abundances of ARB in control and treatment groups were 2.96×10^{-6} and 7.52×10^{-3} to SM2, 5.8×10^{-10} and 1.0×10^{-4} to OTC, 6.89×10^{-10} and 5.19×10^{-5} to CIP, respectively.

4. Discussion

Although some researchers studied the effect of VAs on the properties and performance of aerobic granules in SBR, a higher addition level (above ppm) was chosen; further, a decrease in granules size was observed accompanied by an increase in the solid content in the effluent [5,6]. However, in this experiment, the presence of SM2, OTC, and CIP did not lead to the breakup of granules into small fractions, and the settling ability of aerobic granules in the four systems was similar. Thus, it concludes that individual

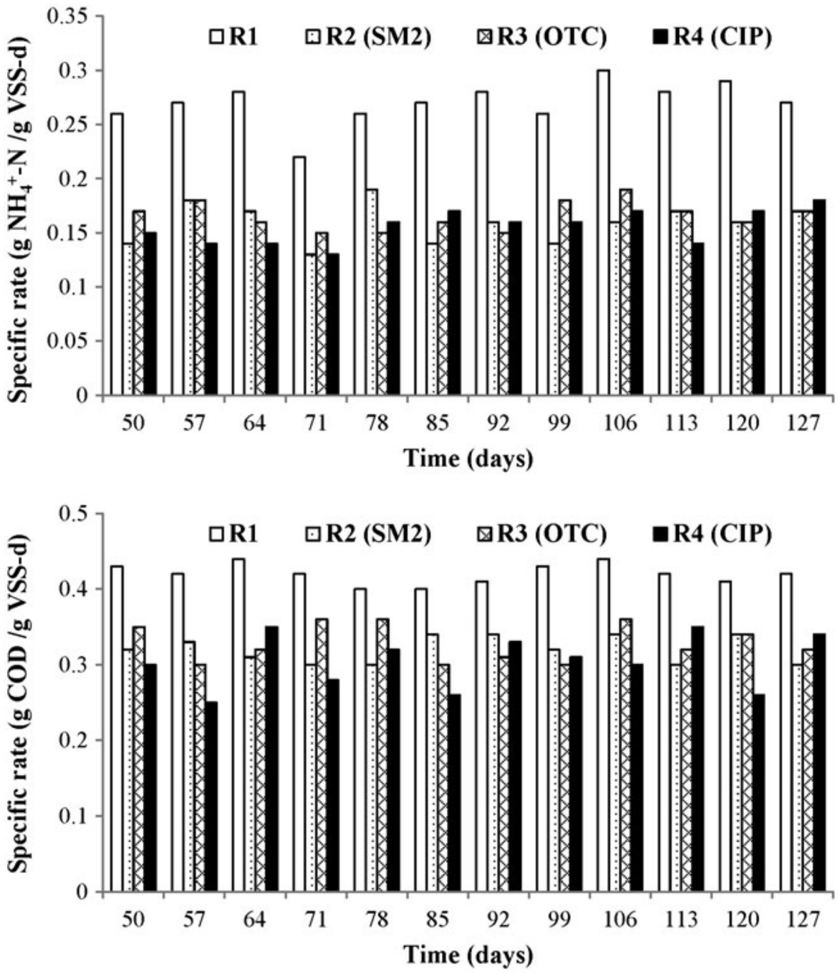

Fig. 3. Specific removal rates of NH_4^+-N and COD in the four systems.

antibiotics at ppb level in swine wastewater cannot actually reach an effective content capable of suppressing the physical structure of aerobic granules. In addition, a high nitrogen/COD ratio could be related to a more compact structure of aerobic granules since selection of slow growing organisms, and the compact granular structure is a valid defensive strategy that protects sludge against the inhibiting effects of toxic substances [3,26]. Aerobic granules from R1 to R4 had a similar clear and regular outer shape, and had enough physical strength (above 97%) to maintain the stability of their granular form during operation. These results implied that the composition of organics and nitrogen in swine wastewater would also be beneficial for weakening the negative effect of VAs on the physical properties of aerobic granules.

Exposure to the selected antibiotics at ppb level affected the granules in terms of biochemical properties, causing a decrease in the specific removal rates of COD and NH_4^+-N in the systems, and also changed the EPS composition and zeta potential of aerobic granules. Compared to the specific rates of COD and NH_4^+-N in R1, the inhibition rates range for treatments R2–R4 were 21–29% and 35–42%, respectively. Antibiotics, in general, have their selective effects on groups of microbes which could be as broad as fungi or bacteria, and subsequently interfere with the microbial ecological functions in various environmental systems in subinhibitory-level concentrations [27]. Shi et al. also found a similar result, and further indicated that the COD and NH_4^+-N removal rate of nitrifying granules would be notably affected with tetracycline addition, yet the performance of the conventional granules system did not change a lot [6]. While the aerobic granules in this study also enriched with nitrifying bacteria because of a high nitrogen/COD ratio in wastewater. Thus, as anticipated, the difference of COD and NH_4^+-N removal rate was detected among

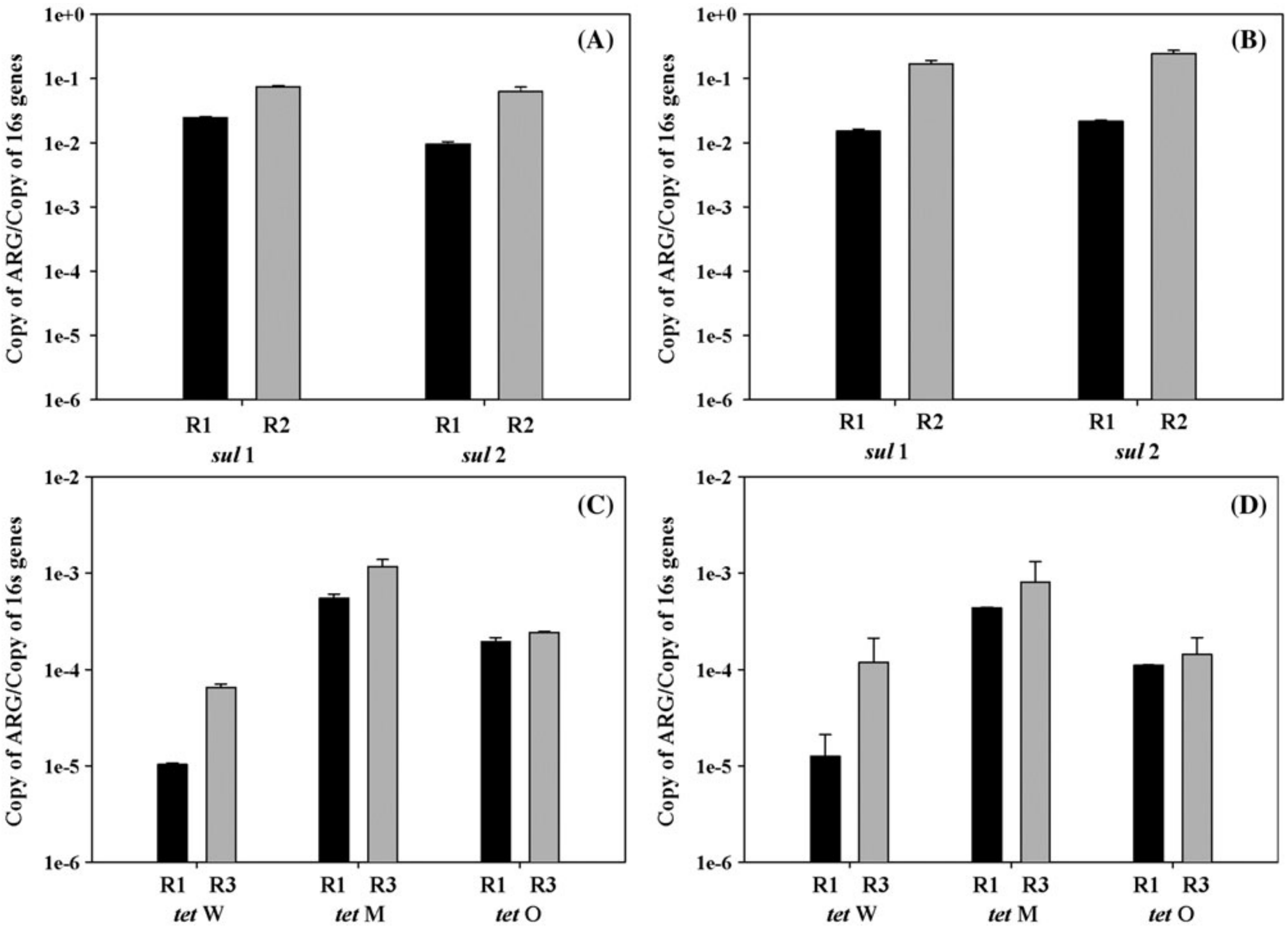

Fig. 4. Relative levels of *sul* genes and *tet* genes in the granules samples (R1, R2, and R3) on two sampling dates ((A) and (C): 50th day, (B) and (D): 120th day). Value is the mean ± SD of triplicate.

the control and test systems. However, the systems in the presence of different antibiotics showed a similar inhibition rate. The possible reason is that although action mode of three target antibiotics pertain differences, all of SM2, OTC, and CIP are designed to be broad spectrum drugs, and affect a broad range of micro-organism species [25].

As a macromolecule polymers secreted by micro-organisms, EPS have been considered to be related to aerobic granulation and stability of microbial aggregates, and the composition of EPS can be affected by certain environmental conditions [3]. Some researchers have indicated that aerobic granules were sensitive to the VAs and produced more PN to protect them against VAs [5,6]. A similar result was observed in this study, namely PN rather than PS were the major active contents involved in the interaction. In addition, compared to the PN content in the four systems, the PS content in aerobic granules showed significant difference with antibiotics addition, and this result might be attributed to the fact that more organics absorption would stimulate the production of PS in the granules [28]. It is also interesting to notice that a negative correlation was found between PN/PS ratio and the zeta potential value of aerobic granules by previous studies [6,26]. However, in this study, a high PN/PS ratio was found to increase the negative charge of microbial aggregates, which can be explained by the fact that the surface charge was closely correlated to micro-organism community, while the selective antibiotic effects would alter the relative abundance of microbial species in systems as previously discussed [3,27]. In addition, compared to SM2 and OTC, CIP showed a more obvious effect on the gram-negative bacteria [25]. The previous studies and the analysis result of bacterial compositions of seed aerobic granules based on high-throughput sequencing in this study (detailed data not shown) indicated that *Proteobacteria* showed an important fraction in the sludge during wastewater treatment, while the *Proteobacteria*

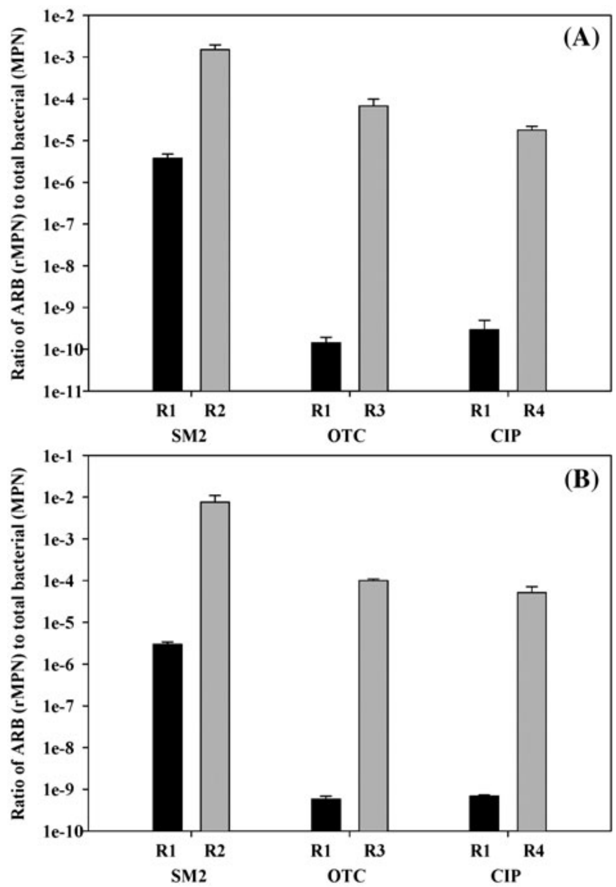

Fig. 5. Relative level of antibiotics-resistant culturable bacteria in aerobic granules (R1, R2, R3, and R4) on two sampling dates ((A): 50th day, (B): 120th day). Value is the mean ± SD of triplicate.

belong to gram-negative bacteria [2,5,29]. Thereby, these reasons maybe lead to more negative effect in the aerobic granules exposed to CIP.

During the wastewater treatment process, sludge has been documented as a reservoir of ARG and ARB, probably due to direct input of ARG, while VAs residue or metal in wastewater may potentially induce antibiotic resistance as well [15,16]. In addition, based on the previous studies, ARG transfer rate and ARB grown in aerobic granular reactor treating swine wastewater may be increased since bacterial cells in a biofilm stay in close contact, and higher gene transfer frequencies have also been measured in nutrient-rich environments [17,30]. In this study, the four systems remained at a similar granular range, thereby the increase in relative abundances of ARB and ARG in R2–R4 was mainly caused by antibiotic-induced response. This result confirmed that microbial resistance of aerobic granules has a strong correlation with VAs at low concentration. Some research also found that antibiotics help gene transfer at low concentrations (100 ppb), and pB10 plasmid transfer rate can significantly statistically increase with 10–100 ppb of VAs exposure [17,31]. However, given the differences of analytical methods to assess antibiotic resistance, the data of ARG and ARB showed a different resistant amplification [30]. Meanwhile, the results also indicated that the microbial resistance did not increase as time progressed, and the relative level of ARB and ARG was similar on the 50th day and 120th day. These results are in accordance with previous observations stating that a stabilization trend was obvious in the cases of ARG proportions in bacterial community of wetland media biofilm [32].

For the relative abundances of the target *tet* genes in R1, the trend was as follows: *tet*M > *tet*W and *tet*O, with a similar trend in R2–R4 also found after VAs treatment. One reason for this may be, in gram-positive species, *tet*M gene could be detected both in the chromosome and on conjugative elements, but *tet*O gene is mobile only when found on conjugative plasmids; further, due to the important regulation of upstream regions and low G + C contents in the structural genes, *tet*M could be easily spread into a significant number of other organisms including gram-negative and gram-positive bacteria and species lacking cell walls [33]. Meanwhile, Gao et al. also indicated that among the *tet* genes, *tet*M and *tet*W, had the widest and the second widest bacteria host range, respectively [34]. Compared to *tet* genes, all *sul* genes showed a higher level, while this may be explained as the *sul* genes is typically associated with integrons located in mobilizable broad-host-range plasmids that have an important positive effect on the genes spread in a wide range of bacterial species [34,35]. However, although *sul*II was usually slightly higher than that of *sul*I in municipal and rural domestic sewage treatment plants because *sul*II is generally associated with small non-conjugative or large transmissible multi-resistant plasmids as indicators of horizontal gene transfer [36], the absolute and relative abundances of two *sul* genes showed a similar level in this study. Thus, this result implies that the abundance of different types of *sul* genes in the sludge would be mainly affected by the content level of those in the wastewater.

5. Conclusion

The common antibiotics (SM2, OTC, and CIP) in swine wastewater at ppb level have no significant effect on the physical characteristics of aerobic granules, and the systems could retain sludge granulation and better settling properties. However, the presence of target antibiotics showed effect on the surface potentials, the EPS composition, and pollution removal rate of aerobic granules in SBR. In terms of microbial resistance, antibiotics at ppb level without effect of other induction factors can actually reach an effective content capable of spreading antibiotic resistance in aerobic granular reactor. Thus, the results in this study indicated that the role of residual antibiotics to aerobic granules in SBR during swine wastewater treatment should not be ignored.

Acknowledgements

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References

[1] Q. Xian, L. Hu, H. Chen, Z. Chang, H. Zou, Removal of nutrients and veterinary antibiotics from swine wastewater by a constructed macrophyte floating bed system, J. Environ. Manage. 91 (2010) 2657–2661.

[2] N. Morales, M. Figueroa, A. Fra-Vázquez, A. Val del Río, J.L. Campos, A. Mosquera-Corral, R. Méndez, Operation of an aerobic granular pilot scale SBR plant to treat swine slurry, Process. Biochem. 48 (2013) 1216–1221.

[3] D.W. Gao, L. Liu, H. Liang, W.M. Wu, Aerobic granular sludge: Characterization, mechanism of granulation and application to wastewater treatment, Crit. Rev. Biotechnol. 31 (2011) 137–152.

[4] M. Figueroa, A. Val del Rio, J.L. Campos, A. Mosquera-Corral, R. Mendez, Treatment of high loaded swine slurry in an aerobic granular reactor, Water Sci. Technol. 63 (2011) 1808–1814.

[5] C.L. Amorim, A.S. Maia, R.B. Mesquita, A.O. Rangel, M. van Loosdrecht, M.E. Tiritan, P.M. Castro, Performance of aerobic granular sludge in a sequencing batch bioreactor exposed to ofloxacin, norfloxacin and ciprofloxacin, Water Res. 50 (2014) 101–113.

[6] Y.J. Shi, S.F. Xing, X.H. Wang, S.G. Wang, Changes of the reactor performance and the properties of granular sludge under tetracycline (TC) stress, Bioresour. Technol. 139 (2013) 170–175.

[7] R.H. Song, X.H. Wang, G.H. Zhang, L. Zhao, X.F. Sun, S.G. Wang, B.Y. Gao, Simulation of equilibrium system and release behaviors of both oxytetracycline and copper on aerobic granules in a sequencing batch reactor, Biochem. Eng. J. 56 (2011) 198–204.

[8] Y.G. Zhu, T.A. Johnson, J.Q. Su, M. Qiao, G.X. Guo, R.D. Stedtfeld, S.A. Hashsham, J.M. Tiedje, Diverse and abundant antibiotic resistance genes in Chinese swine farms, Proc. Nat. Acad. Sci. USA 110 (2013) 3435–3440.

[9] L. Liu, C.X. Liu, J.Y. Zheng, X. Huang, Z. Wang, Y.H. Liu, G.F. Zhu, Elimination of veterinary antibiotics and antibiotic resistance genes from swine wastewater in the vertical flow constructed wetlands, Chemosphere 91 (2013) 1088–1093.

[10] H.N. Storteboom, M. Arabi, J.G. Davis, B. Crimi, A. Pruden, Identification of antibiotic-resistance-gene molecular signatures suitable as tracers of pristine river, urban, and agricultural sources, Environ. Sci. Technol. 44 (2010) 1947–1953.

[11] C.W. McKinney, K.A. Loftin, M.T. Meyer, J.G. Davis, A. Pruden, *tet* and *sul* Antibiotic resistance genes in livestock lagoons of various operation type, configuration, and antibiotic occurrence, Environ. Sci. Technol. 44 (2010) 6102–6109.

[12] N. Wu, M. Qiao, B. Zhang, W.D. Cheng, Y.G. Zhu, Abundance and diversity of tetracycline resistance genes in soils adjacent to representative swine feedlots in China, Environ. Sci. Technol. 44 (2010) 6933–6939.

[13] X. Ji, Q. Shen, F. Liu, J. Ma, G. Xu, Y. Wang, M. Wu, Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; China, J. Hazard. Mater. 235–236 (2012) 178–185.

[14] C. Baker-Austin, M.S. Wright, R. Stepanauskas, J.V. McArthur, Co-selection of antibiotic and metal resistance, Trends Microbiol. 14 (2006) 176–182.

[15] C. Bouki, D. Venieri, E. Diamadopoulos, Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: A review, Ecotoxicol. Environ. Saf. 91 (2013) 1–9.

[16] E. Peltier, J. Vincent, C. Finn, D.W. Graham, Zinc-induced antibiotic resistance in activated sludge bioreactors, Water Res. 44 (2010) 3829–3836.

[17] S. Kim, Z. Yun, U.H. Ha, S. Lee, H. Park, E.E. Kwon, Y. Cho, S. Choung, J. Oh, C.A. Medriano, K. Chandran, Transfer of antibiotic resistance plasmids in pure and activated sludge cultures in the presence of environmentally representative micro-contaminant concentrations, Sci. Total Environ. 468–469 (2014) 813–820.

[18] L. Liu, D.W. Gao, M. Zhang, Y. Fu, Comparison of Ca^{2+} and Mg^{2+} enhancing aerobic granulation in SBR, J. Hazard. Mater. 181 (2010) 382–387.

[19] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, 1998.

[20] S.S. Adav, D.J. Lee, Extraction of extracellular polymeric substances from aerobic granule with compact interior structure, J. Hazard. Mater. 154 (2008) 1120–1126.

[21] L. Liu, D.W. Gao, H. Liang, Effect of sludge discharge positions on steady-state aerobic granules in sequencing batch reactor (SBR), Water Sci. Technol. 66 (2012) 1722–1727.

[22] R. Pei, S.C. Kim, K.H. Carlson, A. Pruden, Effect of River Landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG), Water Res. 40 (2006) 2427–2435.

[23] R.I. Aminov, N. Garrigues-Jeanjean, R.I. Mackie, Molecular ecology of tetracycline resistance: Development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins, Appl. Environ. Microbiol. 67 (2001) 22–32.

[24] H. Heuer, K. Smalla, Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months, Environ. Microbiol. 9 (2007) 657–666.

[25] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement, M100-S20, Wayne, PA, 2010.

[26] Y. Liu, H.H.P. Fang, Influences of extracellular polymeric substances (EPS) on flocculation, settling, and dewatering of activated sludge, Crit. Rev. Environ. Sci. Technol. 33 (2003) 237–273.

[27] C. Ding, J.Z. He, Effect of antibiotics in the environment on microbial populations, Appl. Microbiol. Biotechnol. 87 (2010) 925–941.

[28] L. Wu, C. Peng, Y. Peng, L. Li, S. Wang, Y. Ma, Effect of wastewater COD/N ratio on aerobic nitrifying sludge granulation and microbial population shift, J. Environ. Sci. 24 (2012) 234–241.

[29] T.C. Yadav, A.A. Khardenavis, A. Kapley, Shifts in microbial community in response to dissolved oxygen levels in activated sludge, Bioresour. Technol. 165 (2014) 257–264.

[30] L. Rizzo, C. Manaia, C. Merlin, T. Schwartz, C. Dagot, M.C. Ploy, I. Michael, D. Fatta-Kassinos, Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review, Sci. Total Environ. 447 (2013) 345–360.

[31] D.E. Salcedo, J.H. Lee, U.H. Ha, S.P. Kim, The effects of antibiotics on the biofilm formation and antibiotic resistance gene transfer, Desalin. Water Treat. (in press), doi: http://dx.doi.org/10.1080/19443994.2014.923206.

[32] H. Nõlvak, M. Truu, K. Tiirik, K. Oopkaup, T. Sildvee, A. Kaasik, Ü. Mander, J. Truu, Dynamics of antibiotic resistance genes and their relationships with system treatment efficiency in a horizontal subsurface flow constructed wetland, Sci. Total Environ. 461–462 (2013) 636–644.

[33] I. Chopra, M. Roberts, Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance, Microbiol. Mol. Biol. Rev. 65 (2001) 232–260.

[34] P.P. Gao, D.Q. Mao, Y. Luo, L.M. Wang, B.J. Xu, L. Xu, Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment, Water Res. 46 (2012) 2355–2364.

[35] N. Collado, G. Buttiglieri, E. Marti, L. Ferrando-Climent, S. Rodriguez-Mozaz, D. Barceló, J. Comas, I. Rodriguez-Rod, Effects on activated sludge bacterial community exposed to sulfamethoxazole, Chemosphere 93 (2013) 99–106.

[36] H. Chen, M. Zhang, Occurrence and removal of antibiotic resistance genes in municipal wastewater and rural domestic sewage treatment systems in eastern China, Environ. Int. 55 (2013) 9–14.