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RESEARCH ARTICLE

Treatment of swine wastewater in aerobic granular reactors: comparison of different seed granules as factors

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Abstract The granulation process, physic-chemical properties, pollution removal ability and bacterial communities of aerobic granules with different feed-wastewater (synthetic wastewater, R1; swine wastewater, R2), and the change trend of some parameters of two types of granules in long-term operated reactors treating swine wastewater were investigated in this experiment. The result indicated that aerobic granulation with the synthetic wastewater had a faster rate compared with swine wastewater and that full granulation in R1 and R2 was reached on the 30th day and 39th day, respectively. However, although the feed wastewater also had an obvious effect on the biomass fraction and extracellular polymeric substances of the aerobic granules during the granulation process, these properties remained at a similar level after long-term operation. Moreover, a similar increasing trend could also be observed in terms of the nitrogen removal efficiencies of the aerobic granules in both reactors, and the average specific removal rates of the organics and ammonia nitrogen at the steady-state stage were 35.33 $mg \cdot g^{-1}$ VSS and 51.46 $mg \cdot g^{-1}$ VSS for R1, and 35.47 $mg \cdot g^{-1}$ VSS and 51.72 $mg \cdot g^{-1}$ VSS for R2, respectively. In addition, a shift in the bacterial diversity occurred in the granulation process, whereas bacterial communities in the aerobic granular reactor were not affected by the seed granules after long-term operation.

Keywords aerobic granules, livestock wastewater, sequencing batch reactor, biological wastewater treatment, bacterial community

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1 Introduction

Along with the fast development of the pig breeding industry, swine wastewater containing high concentrations of chemical oxygen demand (COD), nitrogen and phosphorus has become an important source of environmental pollution worldwide. Some references indicated that the estimated daily swine wastewater production in China was up to 6.8 million tons, and approximately 25–48 million tons per year of swine slurry was produced in Spain [1–3]. Although anaerobic digestion is widely used to treat the carbonaceous compound in swine wastewater, the biogas slurry still contains a high concentration of nitrogen [2–4]. Thus, the nitrogen removal system is indispensable in the process of swine wastewater treatment.

Aerobic granules are spherical and compact aggregates of microorganisms and could be used for the aerobic degradation of organics and nutrient removal [5]. Compared with flocculent sludge, the main advantages of aerobic granules are their low facility area requirements, high biomass retention in the bioreactor and good settling property [6,7]. Thus, aerobic granulation technology is a good prospect for application for the treatment of swine wastewater or other livestock wastewater [2,3,8,9]. Figueroa et al., indicated that the nitrogen removal rate of the aerobic granular system was approximately 70% during the treatment of swine slurry with a nitrogen loading rate of 0.83 $kg \cdot m^{-3} \cdot d^{-1}$ at the laboratory scale [3]. A pilot-scale aerobic granular reactor was developed to treat swine slurry in Spain, and the removal rate of the organic and ammonia were 61%–73% and 56%–77%, respectively; the hybridized bacteria and nitrifying microbial population in the granules mainly belonged to the β-*Proteobacteria* subclass and *Nitrosomonas*, respectively [2,9]. However, information about the performance and distinct characteristics of aerobic granules treating swine wastewater is still sparse. Although the bacterial populations of aerobic granules in a swine wastewater treatment plant were detected by the fluorescence in situ hybridization (FISH) technique, this method could not obtain comprehensive information on the diversity of the bacterial community [2]. At present, a biodiversity analysis method based on high-throughput sequencing has been widely used to study the composition of microbial communities in activated sludge [10–12]. In addition, the granulation process and long-term stable operation of the aerobic granular reactor could be influenced by many factors and operational conditions, while seed sludge is widely thought to be an important factor and primary consideration [5]. Unfortunately, to date, most studies on the effect of seed sludge on aerobic granular reactor were performed in a sequencing batch reactor (SBR) with synthetic wastewater, and few studies used real wastewater with high nitrogen loading rates and variety of complex components [13–15]. Thus, in terms of the swine wastewater treatment, correlational research also needs to be further evaluated.

In the present study, the start-up and operation of aerobic granular reactors for the treatment of swine wastewater were investigated using two different strategies, and the operational strategies for each individual reactor are described as follows: R1) we first cultivated aerobic granules with synthetic wastewater, and the mature granules were then used as seed granules to treat swine wastewater; R2) flocculent sludge was directly used as inoculum to start the aerobic granular system with the swine wastewater. Thus, the objectives of this study were 1) to compare the granulation process and property differences of the aerobic granules using synthetic and swine wastewater, respectively, and 2) to determine a better operating strategy of the aerobic granular reactor to treat swine wastewater from a seed sludge point of view.

2 Material and methods

2.1 Experimental setup and systems operation

Two SBR reactors (R1 and R2) were used in this experiment with an available capacity of 2L. The real-time pH control systems were applied to regulate and control pH level (7.2±0.1). The superficial gas velocity of the two reactors was kept at $1.0\ cm\cdot s^{-1}$ by air pump and gas rotameter. Both reactors operated at room temperature (approximately 25°C–28°C). According to the operating strategy, this experiment was composed of two phases, namely phase I (granulation process) and phase II (mature granules stage). In phase I, the synthetic wastewater and swine wastewater were added from the top of R1 and R2, respectively. The volumetric exchange ratio of the two reactors was set to 60%. The settling time of the two reactors was reduced gradually from 10 min to 1 min during aerobic granulation. The operation cycles of both reactors were three times per day, and each cycle contained influent feeding (1 min), aeration (470 min), settling (1–10 min) and effluent withdrawal (1 min). In phase II, swine wastewater was treated in two types of aerobic granular reactors, and the volumetric exchange ratio of R1 and R2 was increased to 80%. The total cycle time in both reactors was set to approximately 6 h (1 min of feeding, 350 min of aeration, 1 min of settling, and 1 min of discharging).

The swine wastewater was obtained from the anaerobic tank of a pig farm in Xiamen, and consisted of: COD 314–391 $mg\cdot L^{-1}$, ammonia nitrogen 301–371 $mg\cdot L^{-1}$, phosphate 18–24 $mg\cdot L^{-1}$, and nitrite and nitrate of less than 5 $mg\cdot L^{-1}$. As this study was conducted in the summer, the wash water yield was increased in the pig farm which led to the swine wastewater containing a low organic and nitrogen concentration compared to previous studies [2–4]. Synthetic wastewater was used with the following composition: the chemical oxygen demand (glucose) was 500 $mg\cdot L^{-1}$, ammonia nitrogen (NH_4Cl) was 80 $mg\cdot L^{-1}$, PO_4^{3-} -P (KH_2PO_4) was 10 $mg\cdot L^{-1}$, Ca^{2+} ($CaCl_2$) was 10 $mg\cdot L^{-1}$, and Mg^{2+} ($MgSO_4$) was 10 $mg\cdot L^{-1}$. In phase I, flocculent sludge taken from a local swine wastewater treatment plant was considered to be the seed sludge for R1 and R2 at a respective initial sludge concentration of 5100 $mg\cdot L^{-1}$ in mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) of 4300 $mg\cdot L^{-1}$. The ratio of MLVSS/MLSS was approximately 85%. The color of the seed flocculent sludge was grayish brown, and had a sludge volume index with 5 min measurements (SVI_5) of 156 $mL\cdot g^{-1}$. In phase II, the MLVSS in both granular reactors was kept at approximately 4600 $mg\cdot L^{-1}$ throughout this period.

2.2 Analytical methods

COD, ammonia nitrogen, nitrite and nitrate were measured following the standard methods [16]. The morphology and physic-chemical characteristics of the aerobic granules were immediately detected at the end of phase I and phase II. All of the experimental values reported in this study are the means±standard deviation (S.D.) of three measurements for each sample. The extracellular polymeric substances (EPS) in the aerobic granules were extracted and detected using the method described by Adav and Lee [17]. The analysis method of the physical strength and specific gravity of the aerobic granules were used as described by Liu et al. and Zhang et al., respectively [6,18]. A laser particle size analysis system was used to measure the size distribution of the aerobic granules (Malvern Mastersizer 2000, Malvern Instruments, England). The morphology and microstructure were observed by using digital camera (Canon 7D, Japan) and a scanning electronic microscope (SEM) (S-4800 Hitachi, Japan), respectively.

2.3 Microbial community analysis

Aerobic granules for the bacterial communities analysis were sampled at the end of phase I (32nd day for R1 and 40th day for R2) and phaseII(75th day for both reactors). The microbial communities of the five samples were analyzed in this study. An E.Z.N.A® DNA kit (OMEGA Bio-Tek USA) was utilized for extracted Genomic DNA from sludge simples of 0.5g in accordance with the manufacturer's instructions. The individual DNA extracts were visualized using 1.0% gel electrophoresis, and the DNA concentrations and purities were determined by spectrophotometric analysis (NanoDrop ND-1000, USA). PCR primer sets of 338F and 533R, which had been incorporated with barcodes for each primer pair, were used to amplify the bacterial-specific V3 hypervariable region of the 16S rRNA gene [19]. Each reaction was performed in a 25 µL volume containing 12.5 µL of buffer, 1.5 µL of each primer, 0.75 µL of MightyAmp® DNA Polymerase (TaKaRa, China), and 20–50 ng of genomic DNA [20]. The PCR amplification was conducted as follows: initial denaturation at 98°C for 2 min, followed by 30 cycles at 94°C for 20 s, 55°C for 20 s, 68°C for 30 s and a final extension step at 72°C for 5 min. The amplification products were examined by agarose (1.0%) gel electrophoresis. The PCR products were purified using PCR axygen PCR Product Purification Kit, and quantified using spectrometry (NanoDrop ND-1000, USA). All of the PCR products of the various samples were pooled as one sample by ensuring equal mass concentrations in the final mixture, and sequenced using the Illumina® HiSeq 2000 system at the BGI of Shenzhen with paired-end sequencing (2×150 bp). After sequencing, a barcoded Illumina paired-end sequencing (BIPES) pipeline was employed to trim off the barcodes and primers and filter the low-quality sequences. Chimeric sequences were detected and removed using UCHIME. Qualified sequences were clustered into operational taxonomic units (OTUs) by setting a 3% distance level using the Quantitative Insights into Microbial Ecology (QIIME) program. The gene sequences obtained from the five samples in this experiment were deposited in the NCBI sequence read archive database under accession number PRJNA274432.

2.4 Statistical analysis

Statistical analysis was calculated using SPSS Version 16.0 (SPSS Inc., USA) to compare the characteristics and pollution removal capacity of the aerobic granules in both reactors ($P < 0.05$). The data of the bacterial communities were analyzed by principal components analysis (PCA) using SPSS Version 16.0 (SPSS Inc., USA), and the correlation between the bacterial communities in both R1 and R2 at different phases was further analyzed by the component score coefficient matrix (F1 and F2) which were calculated using a synthesizing evaluable function.

3 Results

3.1 Granulation progress and granular morphology

The granulation progress of the activated sludge and the morphology of the mature granules during the two phases are shown in Table 1, Fig. 1 and Fig. 2, respectively. The result indicated that granular sludge was first observed in R1 with synthetic wastewater after 5 days of cultivation, whereas the granules were not found in R2 until the 8th day. From that time on, the magnitude of aerobic granules increased, and their size gradually increased concurrently. Because granulation is a gradual process from flocculent sludge to granules, we defined complete granulation as sludge with a size of less than 0.3 mm, which accounted for less than 1% in the reactor. According to this definition, full granulation in R1 and R2 was reached on the 30th day and 39th day, respectively. Thereby, aerobic granulation with the synthetic wastewater had an obviously faster rate compared with the swine wastewater. In terms of the granular morphology, the color of the aerobic granules from R1 and R2 at the end of phase I were yellow and brown, respectively (Fig. 1).

However, the surface structure and bacterial shapes of the aerobic granules that developed with different wastewater were similar at the end of phase I (Fig. 2). In phase II, two types of aerobic granules were used to treat the swine wastewater, and morphology analysis showed that both reactors could maintain sludge granulation and a similar granule size distribution. The color of the sludge in both reactors changed to black. Moreover, microscopic observation revealed an abundance of bacteria and cocci in all of the samples, and no filamentous fungi were observed in this study. The aerobic granules from both reactors at different phases had a compact structure with uniform pores and channels. In addition, except for bacteria, ciliate protozoa were found to be very abundant on the surface of almost all swine wastewater-feed aerobic granules (Fig. 2 (a)), and a detailed representation of the ciliate protozoa is shown in Fig. 2(b). This finding agrees with results from a previous study [21].

3.2 Physical and chemical characteristics of the aerobic granules

Samples of the aerobic granules were taken from both reactors at the end of each phase, and the single average value of the several test data of the physic-chemical properties were calculated for each reactor. The analysis results of the physical and chemical characteristics were summarized in Table 1.

In terms of the physical strength, specific gravity and SVI_5, the aerobic granules that developed with different wastewater remained at a similar level ($P > 0.05$), and the values of these parameters did not show an obvious change

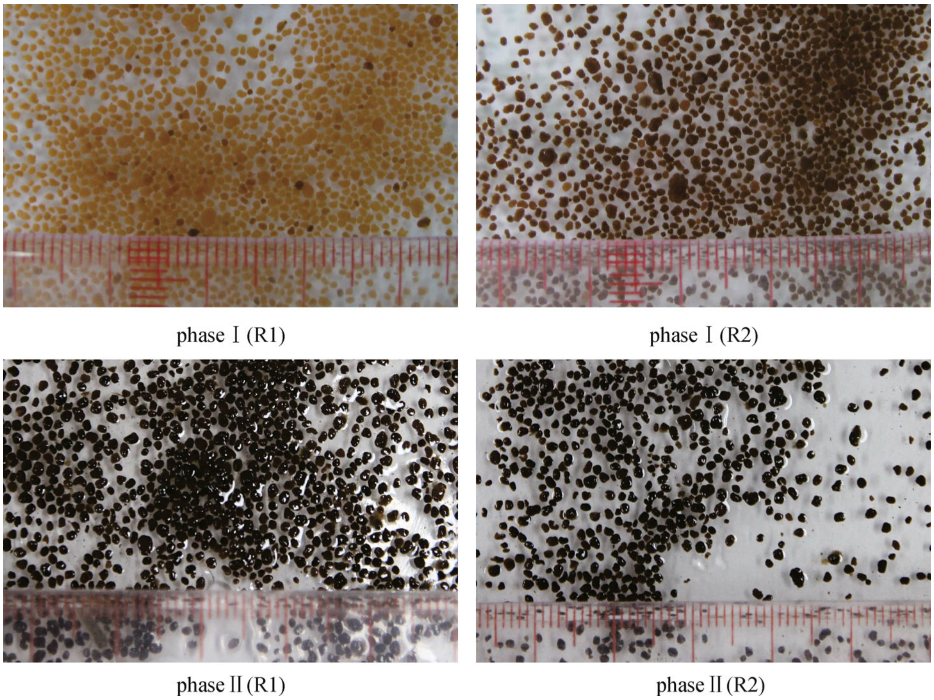

Fig. 1 Images of aerobic granules in R1 and R2 during phase I (32nd day for R1, 40th day for R2) and phase II (75th day for both reactors)

between phase I and phase II ($P > 0.05$). However, the ratio of the MLVSS to the MLSS of the aerobic granules with synthetic wastewater was approximately 93.4%, noticeably higher than that with the swine wastewater (82.6%–84.5%), as the sludge contains a greater biomass fraction in R1 during phase I. Proteins (PN) and polysaccharides (PS) are major constituents of the EPS in aerobic granules and influence the biomass structure and stability [5,22]. At the end of phase I, the PS/PN ratio and the content of the PN and PS in R1 were higher than those in R2 ($P < 0.05$). However, compared to the seed granules, the content and composition proportion of EPS in the two types of granules showed a similar decreasing trend and maintained similar values at the end of phase II ($P > 0.05$). The PS/PN ratio, PS content and PN content in both granular reactors were 26.62%–27.05%, 25.9–26.71 $mg \cdot g^{-1}$ VSS, and 97.3–98.7 $mg \cdot g^{-1}$ VSS, respectively. These results indicated that although feed-wastewater had an obvious effect on the MLVSS/MLSS ratio and EPS in sludge during the granulation process, the difference in these characteristics decreased after long-term operation.

3.3 Comparison of the operational performance

The operational performance of the aerobic granular reactors with different seed granules that were separately cultivated from synthetic wastewater and swine wastewater was investigated during phase II; the removal efficiencies of the organics and nitrogen in the 75 days of operation are shown in Fig. 3.

In this experiment, the effluent of both reactors contained the highly concentrated nitrite, and the existence of nitrite could lead to an increase of the COD detected content (1 g nitrite equals 1.14 g COD) [3]. Thus, the data of the COD content in the influent and effluent after correction were analyzed. The result indicated that the seed granules would not obviously affect the organics removal efficiency of the aerobic granular reactor and the COD removal rate ranges of R1 and R2 were 43%–76% and 41%–74%, respectively ($P > 0.05$). Compared with the aerobic granules in R2, the nitrogen removal ability of the aerobic granules cultivated with the synthetic wastewater showed a lower level at the beginning of the inoculation. The ammonia nitrogen removal rate in R2 (69%–82%) was higher than that in R1 (65%–78%) during the first 16 days, whereas a similar removal rate was observed in both reactors at the end of phase II ($P < 0.05$). Moreover, a similar removal trend was reflected by the specific removal rates (per cycle) of the COD and ammonia nitrogen, which were detected to analyze the differences of biological function in both systems. After 20 days of operation, the average specific removal rates of the COD and ammonia nitrogen were 35.33 $mg \cdot g^{-1}$ VSS and 51.46 $mg \cdot g^{-1}$ VSS for R1 and 35.47 $mg \cdot g^{-1}$ VSS and 51.72 $mg \cdot g^{-1}$ VSS for R2, respectively. Besides, over the course of this study, nitrite was always the main product of nitrification, while the concentration of nitrates was very low (average value

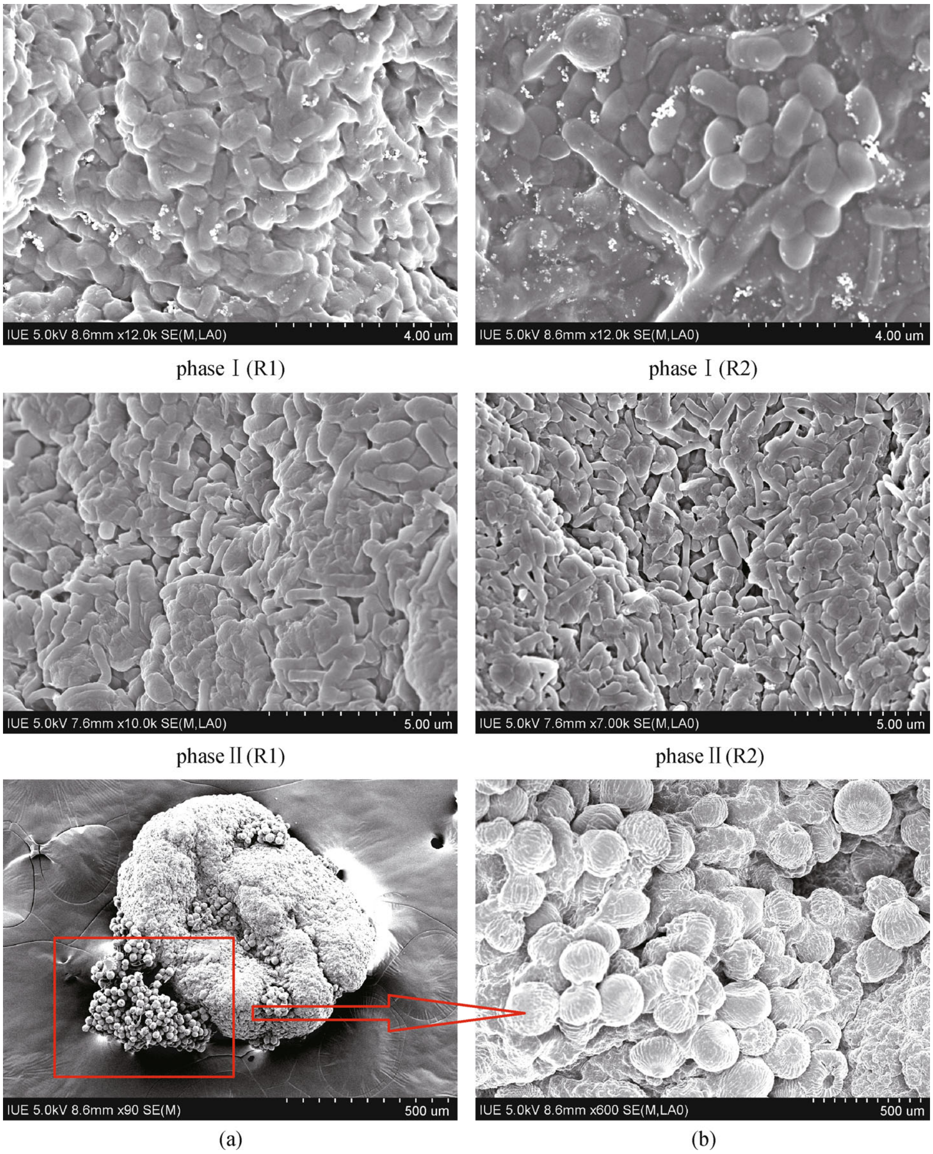

Fig. 2 Scanning electron microscope photographs of aerobic granules during phase I (34th day for R1, 42nd day for R2) and phase II (75th day for both reactors)

Table 1 Physic-chemical characteristics of aerobic granules in both reactors at the end of each phase. All values include standard error of the measurements, with 3 samples

characteristics	units	phase I		phase II	
		R1	R2	R1	R2
granulation progress	granule appearance /d	5	8	–	–
	full granulation /d	30	39	–	–
physical properties	physical strength /%	98.2±0.4	97.8±1.1	97.2±0.3	97.4±0.5
	specific gravity /($g \cdot cm^{-3}$)	1.13±0.04	1.14±0.05	1.09±0.04	1.14±0.03
	SVI_5 /($mL \cdot g^{-1}$)	15±1	14±1	13±1	14±1
	MLVSS/MLSS /%	93.4±1.3	84.5±2.1	82.6±1.8	84.1±2.4
chemical properties	proteins /($mg \cdot g^{-1}$ VSS)	132.2±4.82	123.71±3.28	98.74±3.1	97.3±4.89
	Polysaccharides /($mg \cdot g^{-1}$ VSS)	79.43±2.53	50.1±1.34	26.71±2.4	25.9±1.72
	PS/PN ratio /%	60.08	40.5	27.05	26.62

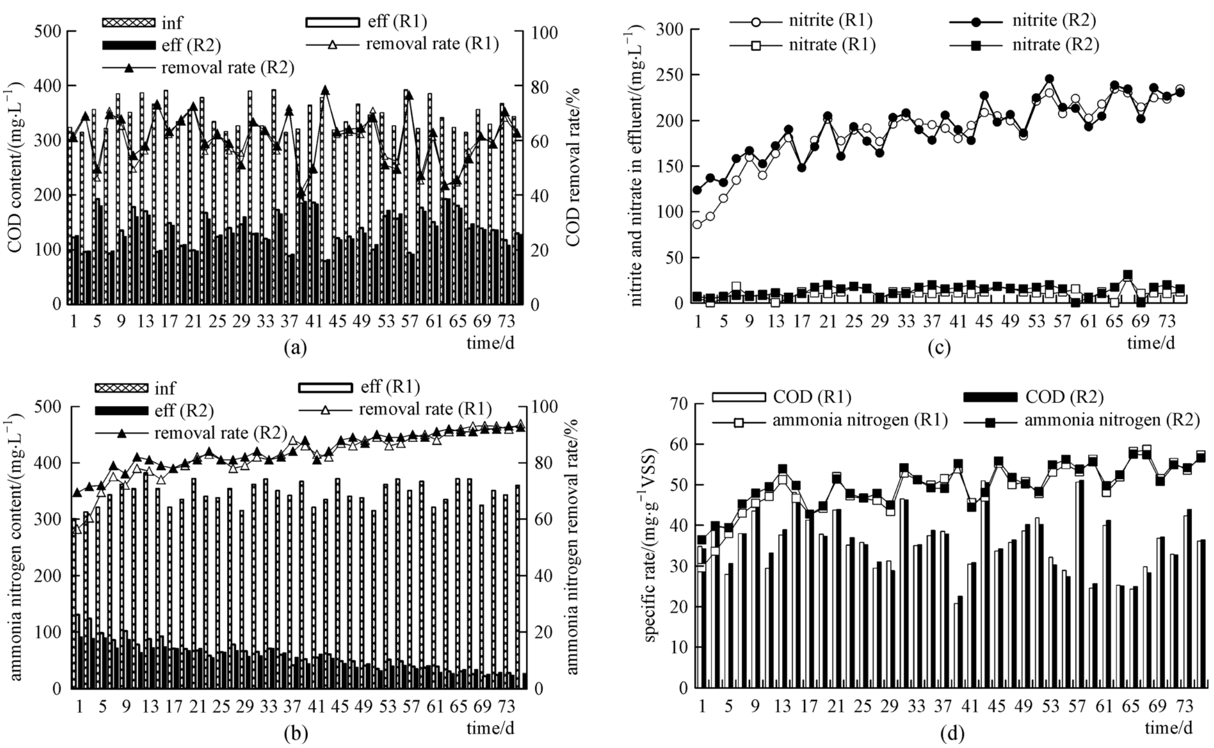

Fig. 3 Operational performance of R1and R2 during phaseII(75 days). (a) COD removal efficiency; (b) ammonia nitrogen removal efficiency; (c) changes in concentration of nitrite and nitrate in effluent; (d) changes in specific rate per cycle of COD and ammonia nitrogen

below 13 mg·L^{-1}) in the effluent of the two reactors. During phase II, the average nitrite nitrogen concentrations of the effluent in R1 and R2 were 189 mg·L^{-1} and 191 mg ·L^{-1}, respectively.

3.4 Microbial and genetic characteristics

The composition and structure of the bacterial communities of the seed flocculent sludge and aerobic granules in both reactors at the end of phase I and phase II were characterized using an Illumina sequencing system. The taxonomic affiliation of the dominant bacteria (relative abundance > 1%) in the five samples at different classification levels is shown in Fig. 4, and the detailed datum is summarized in the Appendices (Table A1, Figs. A1 and A2). The bacterial compositions of the five samples at the phylum level, which accounted for 95.71%, 94.06%, 91.74%, 92.76%, and 95.55% of the reads, were similar and mainly consisted of *Proteobacteria* and *Bacteroidetes*. There were 6 dominating classes, 11 orders and 16 families detected in the total bacterial population, and the result could obviously describe the differentiation in the bacterial diversity of the five samples. Component score coefficient matrix of all of the samples at the family level based on the PCA analysis further indicated that the seed flocculent sludge and aerobic granules in R1 and R2 at phase II (Seed, R1(II) and R2(II) in Fig. 4) were closely related with each other. The dominating bacteria at the family level were *Saprospiraceae* (8.93%, 5.36%, and 5.84%), *Comamonadaceae* (17.84%, 18.55%, and 18.21%), *Rhodocyclaceae* (11.64%, 10.01%, and 11.92%) and *Xanthomonadaceae* (40.96%, 40.02%, and 42.44%). Although aerobic granules were cultivated using different types of wastewater, the relative abundance of common bacteria in both reactors were similar. The bacterial communities in R1 and R2 at the initial stage of sludge granulation (namely R1 (I) and R2 (I) in Fig. 4) were also dominated by *Comamonadaceae* and *Xanthomonadaceae*, whereas *Rhodocyclaceae* and *Xanthomonadaceae,* respectively, showed a slight decrease (approximately 10 percentage points) and a slight increase (ranging from 5 to 10 percentage points) compared to the seed flocculent. In addition, the relative abundances of *Flexibacteraceae*, *Chitinophagaceae*, *Nitrosomonadaceae* and *Rhodocylaceae* in the activated sludge showed an obvious effect from the sludge granulation process, whereas their proportions in the bacterial compositions at phase II returned to the primary levels of the seed sludge. These results indicated that during the swine wastewater treatment by aerobic granular reactor, the bacterial diversity shifted in the sludge granulation process, but the composition structure of the bacterial communities of aerobic granules would recover

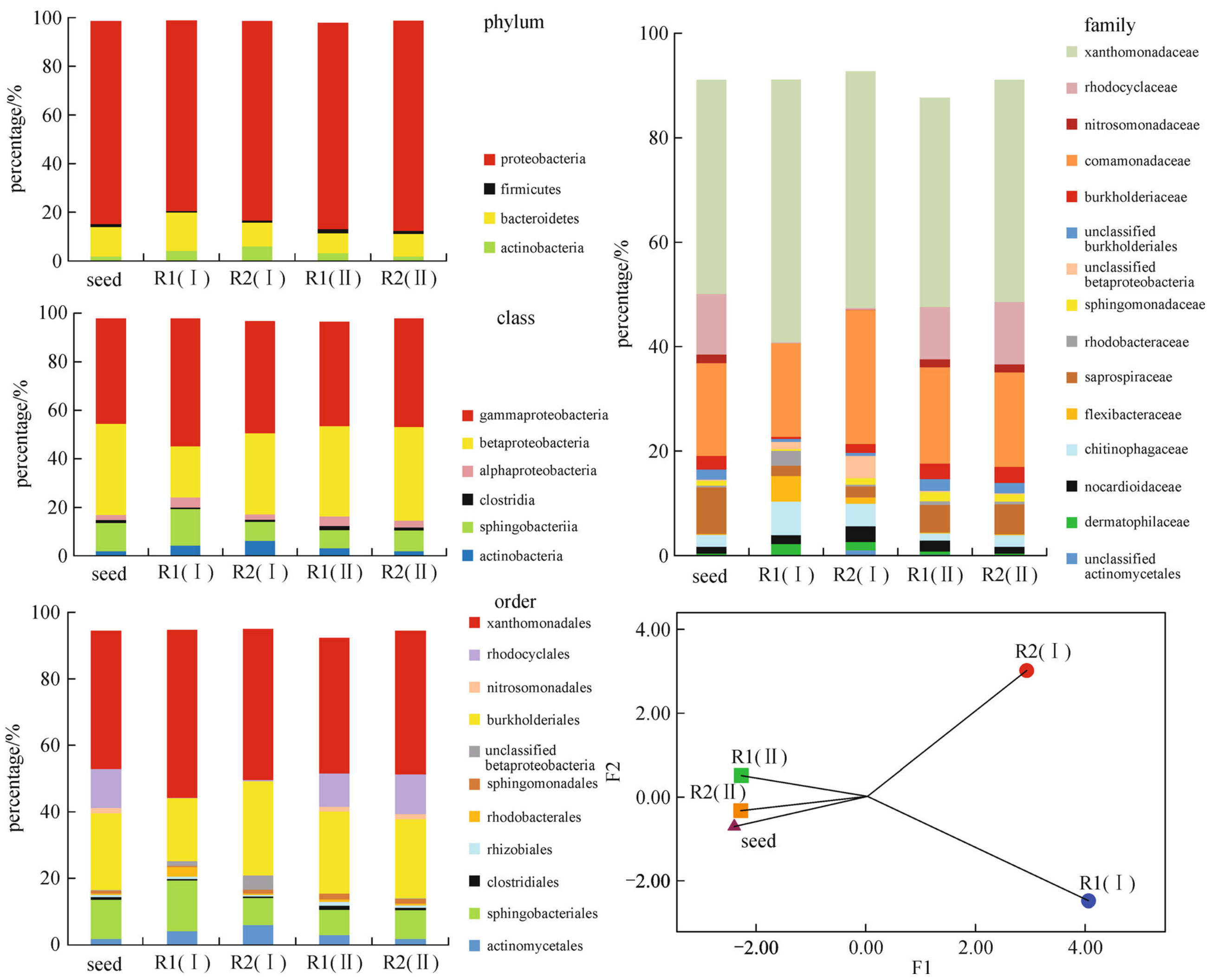

Fig. 4 Classification of dominant bacterial diversity (relative abundance > 1%) at phylum, class, order and family level of seed flocculent sludge and aerobic granules in both reactors at the end of phase I and phase II. Component Score Coefficient Matrix of all samples at family level based on the PCA analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article)

to the level of initial seed flocculent sludge, and was not affected by the seed granules after long-term operation.

4 Discussion

The granulation process and some physic-chemical properties of the aerobic granules in the SBR could be affected by many operating conditions and influential factors, which accounted for different wastewater types [5]. Compared to the synthetic wastewater, the swine wastewater showed a lower COD/N ratio and a higher content of hazardous materials (such as antibiotics), and these factors were proven to have a negative effect on the course length of aerobic granulation [23,24]. Furthermore, *Rhodobacteraceae* was the main bacterial difference in the two seed granules because of the different types of wastewaters used, and Elifantz et al. indicated that *Rhodobacteraceae* are key members of the microbial community at the initial stage of the biofilm [25].

In this experiment, the PS/PN ratio and PS content of the granules with synthetic wastewater were noticeably higher than those with swine wastewater ($P < 0.05$) at phase I. In general, aerobic granules would produce more EPS to protect them against the toxic substances (e.g. heavy metals) in the wastewater, and the increase of the protein content in the granules far exceeded that of other components in the EPS [22]. According to a recent study by our team, the PS/PN ratio of aerobic granules also showed a positive correlation with the antibiotics in swine wastewater [26]. Moreover, this phenomenon might also be ascribed to the fact that the ratio of COD to nitrogen of the wastewater in this experiment, and Wu et al., indicated that the production of PS could be simulated by a higher substrate COD/N ratio in the granules [23]. However, previous research indicated that PN and PS were the hydrophobic and hydrophilic components of EPS, respectively, and an excessive PS secretion may lead to a lower cell surface hydrophobicity of aerobic granules [23,27]. Thus, although the aerobic sludge with the synthetic wastewater had a faster granulation process, the mature granules with swine wastewater may show the better structure stability. At phase II, the swine wastewater was treated in an aerobic granular reactor with two types of the granules as seeds. The morphology and physic-chemical properties of both granules showed a similar trend of change, and there were no obvious differences among these parameters in the two systems, especially for the EPS. The EPS change trend agreed with research by Sheng et al., who indicated that the EPS content in sludge would decrease with the completion of granulation because of a change in the food-to-microorganism (F/M) ratio in the granular system [22]. Thus, these results implied that seed granules had little potential effect on the sludge properties in the aerobic granular reactor after long-term treatment of the real wastewater. A similar result was also found in a study about mature aerobic granules during synthetic wastewater treatment [28].

The organics and nitrogen removal abilities in the two granular reactors with different seed granules under the same operating conditions were measured during phase II. The COD removal efficiency of R1 and R2 was not affected by the different seed granules, but both granular reactors showed a lower and unstable COD removal ability for the real swine wastewater compared with the synthetic wastewater. Andreadakis indicated that non-biodegradable materials had a higher proportion in the total organic matter of swine wastewater (approximately 40%), and the hydrolysis of these materials can be a limiting step in the process of COD removal [29]. A similar result was also observed in other research that studied the livestock treatment in the granular or flocs reactors with hydraulic retention times of as long as 4–6 h [4,8]. In terms of nitrogen removal, the effect of the seed granules on the performance of the aerobic granular reactors was only obvious at the initial stage (1–16 days), and a similar removal rate in both reactors increased as time progressed. The reason for this phenomenon could be that: 1) compared with the swine wastewater, the synthetic wastewater in this study showed a higher COD/N ratio, whereas the nitrification ability and nitrifying bacteria population were remarkably enriched at a low COD/N ratio and 2) previous research showed a significantly negative correlation between the nitrification efficiency and the amount of EPS in the aerobic granules, and that organic assimilation in EPS would hamper nitrogen removal [23]. In addition, the transformation path of nitrogen in this study is similar to previous research by Figueroa et al. and Morales et al., namely, ammonia was mainly oxidized to nitrite [2,3]. Yan et al. indicated that a short sludge retention time (<7 days), a high free ammonia (FA) concentration ($>0.1\ mg\cdot L^{-1}$) or a low DO level ($<1\ mg\cdot L^{-1}$) were the important effect factors for the inhibition of nitrite oxidizing bacteria [4]. However, the sludge retention time and DO level in this study were 14–16 days and 4–7 $mg\cdot L^{-1}$, respectively. Therefore, we thought the main reason for nitrite accumulation might be the high FA content, as the FA content in the influent was 2.77–3.52 $mg\cdot L^{-1}$ in this study. Anthonisen et al. also found that the FA inhibition threshold for nitrite oxidizing bacteria was 0.08–0.82 $mg\cdot L^{-1}$, whereas that for ammonium oxidizing bacteria was 8.2–41.2 $mg\cdot L^{-1}$ [30].

The bacterial population dynamic of aerobic sludge during granulation and the long-term operation process was widely studied by FISH and denaturing gradient gel electrophoresis (DGGE), and the bacterial communities usually showed an obvious response to the effect of the operational conditions [14,18,23,31]. In this study, the evolution of the bacterial communities mainly occurred in the process of aerobic granulation, while the feed-wastewater and seed granules types showed little influence on the bacterial communities. This result is similar to the research by Sheng et al. [13]. Settling time controlling, which could select the fast settling sludge particles and remove flocs from the SBR reactor, was used to enhance aerobic granulation at phase I. Therefore, the aerobic granulation process comes with an intense evolution of the bacterial communities in sludge, and our research further indicated that the evolution mainly occurred at the level of order and family. To date, a physical parameter of the sludge (such as SVI and granule size) usually is used to identify the granulation state, and the evaluation method of complete aerobic granulation dose not form a unified standard [5,18]. The results of this experiment implied that the evolution of bacterial communities in sludge can also be used as the evaluation index of the granulation process, especially for *Nitrosomonadaceae* and *Rhodocylaceae* which are affected by selective pressure.

Although the community diversity of the aerobic sludge at the family level showed an obvious difference during this experiment, the community composition in all of the samples demonstrated the dominancy of *Comamonadaceae* and *Xanthomonadaceae* which belong to the β-*Proteobacteria* and the γ-*Proteobacteria* subclasses, respectively. Adav et al. [32] indicated that *Comamonadaceae* play an important role in the denitrification processes in the presence of acetate, and *Xanthomonadaceae* may be affected by the strong binding strength of the EPS matrix of the aerobic granules. It is noteworthy that the relative abundance of *Xanthomonadaceae* also showed a positive correlation with the content of EPS in aerobic granules. In an aerobic granular pilot SBR plant, β-*Proteobacteria* also exhibited a crucial role in sludge during the treatment of swine slurry based on FISH analysis [2]. Comparative 16S rRNA sequence analyses of cultured ammonium oxidizing bacteria indicated that members of these physiologic groups could be confined to two monophyletic lineages, namely the β-*Proteobacteria* and γ- *Proteobacteria* subclasses [33]. In addition, *Nitrospirae* was identified as the dominant nitrite oxidizing bacteria in activated sludge [34], whereas in this study, this phylum only showed a small fraction in the bacterial communities of the aerobic granules (relative abundance < 0.2%). Therefore, this result could further explain the transformation path of nitrogen and nitrite accumulation in both granular reactors during the treatment of the swine wastewater. Different types of wastewater showed an obvious effect on the relative abundance of *Rhodobacteraceae* in the two seed granules (approximately 7.5 times), and we thought that the lower DO level in R2 which was coursed by the higher organics and nitrogen content in the swine wastewater may be the main reason for this observation. Lv et al., indicated that *Rhodobacteraceae* showed a higher relative abundance at the surface of the aerobic granules, which is where the aerobic heterotrophic zone was located [35].

5 Conclusions

Aerobic sludge with synthetic wastewater had a faster granulation process, yet the mature granules with swine wastewater maybe showed the better structure stability and pollution removal ability. Moreover, seed granules had little potential effect on the physic-chemical properties and bacterial communities of sludge in aerobic granular reactors after a long-term treatment of the real swine wastewater. Thus, we thought that the operating conditions of the SBR reactor were more important than the seed sludge over the course of practical application, and the real wastewater with manual adjustments may be a better choice for the commercialization of aerobic granules during the cultivation process.

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