Rapid aerobic granulation in an SBR treating piggery wastewater by seeding sludge from a municipal WWTP

Jun Liu¹, Jun Li²⁎, Xiaodong Wang², Qi Zhang³, Helen Littleton⁴

¹. College of Environment, Zhejiang University of Technology, Hangzhou 310014, China. E-mail: liujun521282@sina.com
². College of Civil Engineering and Architecture, Zhejiang University of Technology, Hangzhou 310014, China
³. Institute of Quality and Standard for Agro-products, Zhejiang Academy of Agricultural Science, Hangzhou 310021, China
⁴. WEF Municipal WWT Design Committee, Richboro, PA 18954, USA

Article Info

Article history:
Received 5 April 2016
Revised 27 May 2016
Accepted 8 June 2016
Available online 14 July 2016

Keywords:
Rapid aerobic granulation
Piggery wastewater
Microbial community
Granular structure

Abstract

Aerobic sludge granulation was rapidly obtained in the erlenmeyer bottle and sequencing batch reactor (SBR) using piggery wastewater. Aerobic granulation occurred on day 3 and granules with mean diameter of 0.2 mm and SVI₃₀ of 20.3 mL/g formed in SBR on day 18. High concentrations of Ca and Fe in the raw piggery wastewater and operating mode accelerated aerobic granulation, even though the seed sludge was from a municipal wastewater treatment plant (WWTP). Alpha diversity analysis revealed Operational Taxonomic Units, Shannon, ACE and Chao 1 indexes in aerobic granules were 2013, 5.51, 4665.5 and 3734.5, which were obviously lower compared to seed sludge. The percentages of major microbial communities, such as Proteobacteria, Bacteroidetes and Firmicutes were obviously higher in aerobic granules than seed sludge. Chloroflexi, Planctomycetes, Actinobacteria, TM7 and Acidobacteria showed much higher abundances in the inoculum. The main reasons might be the characteristics of raw piggery wastewater and granule structure.

© 2016 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

Keywords:
Rapid aerobic granulation
Piggery wastewater
Microbial community
Granular structure

Introduction

In the last decades, aerobic granular sludge in lab scale reactors has been extensively used for treating different types of wastewater such as soybean-processing wastewater (Su and Yu, 2005), brewery wastewater (Wang et al., 2007), abattoir wastewater (Cassidy and Belia, 2005), dairy wastewater (Schwarzenbeck et al., 2005), saline wastewater (Figueroa et al., 2008; Wang et al., 2015), palm oil mill effluent (POME) (Abdullah et al., 2011), domestic wastewater (Li et al., 2013) and livestock wastewater (Othman et al., 2013) due to its excellent characteristics of compact structure, diverse microbial species, good settling property, able to withstand shock loading and tolerance to unfavorable environmental conditions (Liu and Tay, 2004; Adav et al., 2008; Khan et al., 2013).

Typical of wastewater from livestock and poultry breeding, piggery wastewater is considered to have drastic effects on the environment and human health, because it contains high strength nitrogen and organic matter (Han et al., 2011). Previously, many researchers had sought solutions for treating piggery wastewater with various methods (Han et al., 2011; Xu and Shen, 2011; Zhang et al., 2011a, 2011b). Zhang et al. (2011a, 2011b) studied anaerobic codigestation of piggery wastewater and food waste and identified the key factors governing codigestion performance. Han et al. (2011) investigated the effect of feeding strategy on the treatment of swine wastewater and found that the feeding ratio had a more significant effect on the removal of phosphorus and nitrogen than on the removal of chemical oxygen demand (COD) in SBR system. Huang et al. (2011) found that high

http://dx.doi.org/10.1016/j.jes.2016.06.012
1001-0742/© 2016 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.
concentrations of K^{2+} and Ca^{2+} would be helpful for removing nutrients in piggery wastewater. These results have shown great improvements in treating piggery wastewater, but there are still some problems that hinder treatment. Also these methods belonged to conventional activated sludge and had some drawbacks. For example, conventional activated sludge process could not withstand higher organic loading, high strength nutrient compounds made active microorganism retention in the reactor difficult (Latif et al., 2011), and anaerobic digestion would increase the capital investment. Because of the good results obtained from considerable research, aerobic granules with excellent settling ability and withstand shock loadings may be applied on treating high-strength piggery wastewater.

However, rapid aerobic granulation was paid more attention at present, and various strategies have been put forward in many works. The earlier work demonstrated that shortening settling time, increasing organic loading rate, extending starvation period and increasing shear force were beneficial to enhance aerobic granulation in SBR with artificial wastewater (Gao et al., 2011). Recently, metal ions or metal such as Ca, Mg, Fe and Mn, were believed to be able to shorten the time of aerobic granulation by forming nucleus for bacteria to aggregation (Kong et al., 2014). In addition, fast aerobic granulation could be achieved by seeding anaerobic granules (Hu et al., 2005), crushed granular sludge (Pijuan et al., 2011), long-term stored aerobic granules (Liu et al., 2005). Adding external carrier, such as granular activated carbon (Li et al., 2011), poly aluminum chloride (Liu et al., 2014) could also be helpful to provide nucleus for rapid sludge granulation in the initial stage. Above all, the above-mentioned strategies could accelerate aerobic granulation in a varied degree, but these works were done using synthetic wastewater. Especially, the strategies of seeding anaerobic granules, crushed granular sludge, long-term stored aerobic granules were not easily carried out in practice. Furthermore, the methods of adding metal ions (Ca, Mg and Fe), granular activated carbon or poly aluminum chloride would increase the operational cost. The studies about piggery wastewater using aerobic granule technology were still a little reported (Zhang et al., 2013; Morales et al., 2013). Zhang et al. (2013) mentioned strategies could accelerate aerobic granulation in SBR using real wastewater. The volumetric exchange ratio was a great challenge in SBR using real wastewater. Therefore, it is necessary to explore the knowledge about rapid aerobic granulation processes directly fed with raw piggery wastewater in China.

Hence, the main purpose of this work is to investigate the feasibility of rapidly achieving aerobic granulation in erlenmeyer bottles and then in an SBR fed with piggery wastewater. The aerobic granular sludge formation process, physicochemical parameters and microbial variation were also investigated.

1. Materials and methods

1.1. Seed sludge and wastewater characteristics

The seed sludge was taken from a secondary sedimentation tank of Qige wastewater treatment in Hangzhou City, China. Mixed liquor suspended solid (MLSS), mixed liquor volatile suspended solid (MLVSS), sludge volume index with 30 min setting time (SVI30) of seed sludge were about 5000 mg/L, 3550 mg/L and 56 mL/g, respectively. Raw piggery wastewater was collected from an aeration flotation tank of a wastewater plant for treating piggery wastewater in Hangzhou City. This plant using conventional activated sludge processing, consisted of anaerobic tank, air flotation (adding Fe2(SO4)3), anaerobic/aerobic (A/O) tank, primary sedimentation tank, followed by A/O tank and secondary sedimentation tank. In the plant, adding 0.5% Fe2(SO4)3 is to solid–liquid separation in the air flotation. The dosage of Fe2(SO4)3 is based on the influent, suggested adding 5 g/ton piggery wastewater. The main characteristics of collected piggery wastewater are as following, total COD = 418–1600 mg/L, soluble COD = 210–832 mg/L, ammonia nitrogen (NH4-N) = 486–839 mg/L, Ca^{2+} = 25.4 mg/L, Fe^{3+} = 1.99 mg/L, total suspended solid (TSS) = 200–431 mg/L, and volatile suspended solid (VSS) = 152–364 mg/L. It is obviously found that influent NH4-N and COD exhibited a high fluctuation because of increasing washing water in hot weather (in summer). In addition, the concentration of Ca^{2+} is kept at 25.4 mg/L, and Fe^{3+} is 1.99 mg/L. The wastewater pH value stabled at 7–8.

1.2. Pre-experiment in two erlenmeyer bottles in a shaker

The pre-experiment was firstly performed in a shaker (HY-5A, Baitaxinbao Electric Co., Ltd., Jintan City, China) for aeration and shear force at a speed of 260–280 r/min, and each erlenmeyer bottle had an effective volume of 100 mL as a reactor (250 mL). Each erlenmeyer bottle was inoculated with 5 mL of activated sludge, fed with domestic sewage (COD = 530–830 mg/L, NH4-N = 400–430 mg/L) and piggery wastewater (COD = 1500–1600 mg/L, NH4-N = 640–830 mg/L). This was done to verify the feasibility of rapid aerobic granulation with piggery wastewater compared to feed with sewage wastewater. A 12 hr operation cycle was implemented, including 11 hr of aeration and 55 min of filling, effluent and idling time, while the settling time was decreased from 5 to 1 min according to actual situation. The volumetric exchange ratio was 90%. The mentioned operation was done manually.

1.3. SBR setup and operation

A lab scale SBR with a working volume of 4 L, an internal diameter of 9 cm and a total height of 100 cm was setup for aerobic granulation with raw piggery wastewater. The reactor was operated with 1 cycle of 24 hr with 5 min of feeding, 23 hr of aeration, 5 min of settling time, 7 min of effluent discharge and 43 min of idling time. The volume of effluent decanting was 2 L each cycle (exchange ratio = 50%). A gas velocity of about 0.87–1.31 cm/sec was supplied during the aeration. The reactor was operated at room temperature (25 ± 5°C).
1.4. Analytical methods

COD, NH$_4^+$-N, MLSS, MLVSS, SVI$_{30}$ TSS and VSS were measured according to standard methods (APHA, 2006). The concentrations of Fe$^{3+}$ and Ca$^{2+}$ were determined using Atomic Absorption Spectrometer (AA6300, Shimadzu, Japan). In the initial stage (0–20 days), the metal ions were analyzed every 4 days. After complete granulation (21–80 days), they were detected every 5 days. The morphology of aerobic granular sludge was observed under an optical microscope (CX31, Olympus, Japan).

1.5. Sample collection, DNA extraction, and metagenomic analysis

The seed sludge (day 0) and aerobic granular sludge (day 60) from the SBR were taken into two tubes (5 mL) and stored at –20°C for DNA extraction. DNA extraction was performed using E.Z.N.A. Mag-Bind Soil DNA Kit (OMEGA, USA) according to the manufacturer’s instruction. Metagenomic analysis was conducted at the Sangon Biotech (Shanghai Co., Ltd., China) using Illumina Miseq 2 × 300. About 10 ng DNA of the seed sludge and granular sludge was used for library construction and 16s rDNA sequencing.

Metagenomic data were processed by the Quantitative Insights into Microbial Ecology (Qime) pipeline (http://qiime.org/) and Mothur (http://www.mothur.org/). Briefly, any sequence with a length < 50 bp, or mean quality < 20 bp, and ambiguous bases were removed from the dataset. After quality control, the mean reads long was about 440 bp and the sequences were clustered and assigned to Operational Taxonomic Unit (OTU) using Ribosomal Database Project (RDP) with sequence identity more than 97%. The abundance curve, abundance-based coverage estimator (ACE), Chao1, Simpson, Shannon and coverage were calculated using Mothur software.

2. Results and discussion

2.1. Aerobic granulation in the erlenmeyer bottles using piggery wastewater and sewage wastewater

As can be seen in Fig. 1, the initial seeding sludge had a fluffy and irregular structure (Fig. 1a). After one week, aerobic granulation was occurred using domestic wastewater, but flocs sludge was still predominant (Fig. 1b). Then settling time was shortened from 5 to 4 min, compact granules with clear boundary appeared on day 20, but there were still few flocs (Fig. 1c). However, small dark granules fed with piggery wastewater were clearly presented and flocs gradually disappeared on day 7 (Fig. 1d). Two weeks after inoculums, aerobic granulation was completely finished, and the flocs sludge were completely replaced by aerobic granules with a clear outer shape and round structure, the average diameter being about 600 μm (Fig. 1e). On day 60, aerobic granules become compact and denser with spherical and smooth outer morphology, the size increased to 1–2 cm (Fig. 1f). Hence, rapid granulation is feasible using the raw piggery wastewater than the domestic wastewater at the same conditions. Simultaneously, longtime of aeration (11 hr) and shorten settling time (5–1 min) were beneficial to enhance aerobic granulation. These results provided the basic information of fast granulation with the raw piggery wastewater in SBR.

2.2. Rapid aerobic granulation in a SBR using piggery wastewater

Based on the mentioned experiment, it is concluded that rapid aerobic granulation can be achieved in the shaker fed with piggery wastewater under suitable conditions. Hence, it is necessary to investigate the feasibility of fast aerobic granulation in a lab scale SBR with this wastewater. Fig. 2 shows the sludge morphology in the SBR, the seeding sludge with a fluffy and loose structure (Fig. 2a) gradually disappeared and was replaced by small dark granules after 3 days (Fig. 2b). At this stage, developed aerobic granules were unstable and could easily break up if the conditions changed. After 18 days, granules steadily increased and became clearly, the average size increasing to 200 μm, although the flocs sludge did not completely disappear (Fig. 2c). The sludge granulation was faster than the work (Zhang et al., 2013), which spent 42 days in sludge granulation with digested piggery wastewater in SBR. Moreover, the characteristics of granular sludge at the end of the experiment were clearer with a regular outer morphology, indicating that dense and compacted aerobic granules had been formed (Fig. 2d). This suggested that rapid aerobic granulation and compact granules were successfully obtained in the SBR using the raw piggery wastewater by seeding activated sludge from a WWTP.

Fig. 3 illustrates the profile of sludge concentration and SVI$_{30}$ throughout 80 days of the experimental process. During day 0–20, MLSS was increased from 5650 to 7450 mg/L on day 3, then decreased greatly, gradually tended stable and finally kept at 3500 mg/L after 48 days running. This was due to the washing out of a large amount of seed sludge with poor settling properties in a short settling time that took place in the beginning. Although small granules appeared, they were not stable and easily broke up. With the reactor running, more and more granules with average size of 0.2 mm were observed in the reactor and MLSS started tending stable after 18 days. Once stable granules formed, biomass activity could be maintained in the reactor. The settling properties can be described in term of SVI$_{30}$. After inoculation, SVI$_{30}$ values were sharply declined from the initial 56.1 to 20.3 mL/g. After the formation of aerobic granules on day 18, the SVI$_{30}$ was gradually balanced and maintained around 25 mL/g at the end, which was obviously lower than the works with higher SVI$_{30}$ of 27–60 mL/g (Zhang et al., 2013; Othman et al., 2013; Morales et al., 2013). One reason is that aerobic granules became the dominant form of biomass in the reactor and the settling properties gradually improved. Another explanation is relatively high Ca and Fe in the effluent were clearly decreased in the beginning, indicating Ca and Fe were necessary in the rapid formation of compact aerobic granules structure with excellent settling properties during sludge granulation (Yu et al., 2000; Ren et al., 2008).

Aerobic granules with excellent settling ability were rapidly obtained in the lab scale SBR with the raw piggery wastewater, and suggested an explanation of fast aerobic granulation. The
high contents of Ca and Fe in the raw piggery wastewater played the key roles on fast formation of aerobic granulation. In this study, the fact that Ca existed in piggery wastewater was the intrinsic character (Huang et al., 2011) and adding Fe³⁺ (0.5%) in the flotation tank for solid liquid separation during pretreatment. Moreover, it was reported that ferric can be chelated with protein or alginate like exopolysaccharides to form iron-porphyrin which is a co-enzyme. It not only carries oxygen efficiently but also can stimulate extracellular polymeric substrates (EPS) formation (Ren and Littleton, 1992). Hence, these inorganics, composed of Ca and Fe in the raw wastewater, were obviously beneficial to the acceleration of microbial aggregation. Similar results were also found in these works (Ren et al., 2008; Othman et al., 2013; Kong et al., 2014). They considered that the positive charge of the metals (Ca and Fe) neutralized the overall negative charge on the surface of microbial biomass and EPS and thus helped to shorten the time of aerobic granulation in SBR. In addition, the presence of high amounts of TSS in the wastewater feed could contribute to the mineral fraction in the aerobic granules.

It was reported that most of aerobic granules had been successfully cultivated in SBR, indicating that aerobic granules were easily formed by the SBR operation. In SBR mode, shortened settling time and the volume exchange ratio served as selection pressures to wash out flocs sludge resulted in rapid granulation and they were easily controlled in the SBR operation (Li et al., 2014). Meanwhile, the periodic feast-famine condition facilitated microbial aggregation. Hence, these factors can enhance the fast formation of aerobic granules in the SBR. In our experiment, the advantages of 5 min setting time, 50% exchange ratio and 23 hr aeration were applied for enhancing the fast granulation in this study. In brief, we took full advantages of the raw piggery wastewater and SBR mode for rapid aerobic granulation in this study.

2.3. Removal efficiencies in SBR

Although aerobic granular sludge was successfully developed in this work, the removal efficiencies of COD and NH₄⁺-N stabilized at 51% and 50% after complete granulation (Fig. 4). It
can be observed that COD and NH₄⁺-N in the influent were fluctuated sharply from day 20 to day 26. COD in the effluent sharply decreased from 700 to 200 mg/L in the end. This met the Discharged Standard of Pollutants for Livestock and Poultry Breeding of China (2001). Meanwhile, the COD removal ratio had a similar trend throughout the operation period. Effluent NH₄⁺-N decreased from 401 to 249.8 mg/L, suggesting that granular sludge could not remove all of the ammonium. Effluent NH₄⁺-N did not meet the discharged standard and needed improvement in the next process. The removal efficiencies in this work were significantly lower than in the previous works (Arrojo et al., 2004; Othman et al., 2013; Zhang et al., 2013) whose efficiencies were found in treating the industrial wastewater, livestock wastewater and digested piggery wastewater using aerobic granule technology. Although effluent NH₄⁺-N was not completely removed by granular SBR in this study, the residual-ammonia could be improved through the following processing of the next A/O in the treatment plant in practice. Moreover, aerobic granular sludge with excellent settling ability of 20.3 mL/g could resolve the problem of sludge retention difficult in the reactor.

It is worth noting that the ratio of MLVSS/MLSS was significant reduced from 0.71 to 0.48 throughout the granulation process. The ratio of MLVSS/MLSS was roughly evaluated the bioactivity, and the results indicated biological activated decreased greatly in this study and further resulted in the poor removal performances. It might due to raw wastewater always contain a large amount of Ca²⁺. In the experiment, the concentration of Ca²⁺ in influent was always kept at 25.4 mg/L, but Ca²⁺ concentration in effluent was decreased to 10.4 mg/L in the first 15 days and then gradually increased to 22.4 mg/L after sludge granulation. It was suggested that a large amount of Ca accumulated in granular sludge in initial stage of granulation process and beneficial to enhance sludge granulation. But excessive Ca enriched in aerobic granules led to a reduced granular sludge MLVSS, which was similar to earlier work (Ren et al., 2008). Hence, although Ca²⁺ was widely recognized to play an important role in the self-immobilization of microbial aggregates (Yu et al., 2001; Wan et al., 2015), excessive Ca²⁺ enriched in the aerobic granules might have a negative effect on
the microbial growth (Ren et al., 2008). However, previous study was considered high concentrations of K\(^{2+}\) and Ca\(^{2+}\) of piggery wastewater were beneficial to N remove (Huang et al., 2011), which was opposite to this work. In addition, the poor COD removal can be attributed to a high concentration of COD non-biodegradable, which was similar to the earlier study (Morales et al., 2013).

### 2.4. Variations of microbial communities between seed sludge and granular sludge

Microbial communities of the inoculum (day 0) and aerobic granular sludge (day 65) were detected by metagenomic analysis. The 24,685 and 20,604 microbial reads in the inoculum and granular sludge were assigned to different taxa levels from phylum to genus using the RDP Classifier at 97% identity. Table 1 shows the results of Alpha diversity analysis in two sludge samples. The results count 4003 OUT in the inoculum, which is significantly higher than that in aerobic granular sludge (2013 OUT). The ACE and Chao 1 indices of inoculated sludge were significantly higher than that in granular sludge, suggesting that the inoculum community richness was superior to granular sludge. Compared to inoculated sludge, the Shannon index of granular sludge was slightly lower but the Simpson valve was obviously higher. It was indicated community diversity of aerobic granular sludge was inferior to that of the inoculum. The coverage of two sludge samples was over 0.9, but granular sludge coverage exceeded the inoculated sludge. This means that the unclassified taxa of granular sludge are lower than that of the inoculated sludge. As well, the microbial composition of seed sludge is more complex. It was also found that the unclassified sequences in community decreased form 55 to 32 from phylum level to species level in the seed sludge and granular sludge. The seed sludge was taken from the domestic sewage treatment plant with low COD and N loading rates, but the raw piggery wastewater used in this study had relatively high COD and N loading rate. This suggested that high-loading rate could reduce sludge microbial diversity, confirming the earlier works (Li et al., 2008; Adav et al., 2009; Zhao et al., 2013).

The variation in pollutant removal and physical and biochemical properties between the inoculum and granular sludge is probably attributable to the difference in microbial diversity. Microbial compositions of the seed sludge and granular sludge were also analyzed. As shown in Fig. 5, Proteobacteria was the most abundant phylum in the seed sludge and granular sludge, accounting for 36.7% and 46.0%, respectively. The other dominant phyla were Chloroflexi (19.72%, 2.81%), Bacteroidetes (18.52%, 34.58%), Firmicutes (5.95%, 11.95%), Planctomycetes (5.38%, 0.16%), Actinobacteria (3.54%, 2.01%), TM7 (2.18%, 0.51%) and Acidobacteria (2.59%, 0.08%). Significant differences in the proportion of dominant microorganisms have been found in two sludge samples. The percentages of Proteobacteria, Bacteroidetes and Firmicutes in granular sludge were higher than that in the inoculum, suggesting that they adopted to live in granular sludge fed with high N loading piggery wastewater. The abundances of Chloroflexi, Planctomycetes, Actinobacteria, TM7 and Acidobacteria in seed sludge were significantly more than that in the inoculum, suggesting that they adopted to live in granular sludge fed with high N loading piggery wastewater. The abundances of Chloroflexi, Planctomycetes, Actinobacteria, TM7 and Acidobacteria in seed sludge were significantly more than that in aerobic granular sludge, indicating that the piggery wastewater had a negative effect on the bacterial growth. Similar results were also found in the minor phyla (abundance <1%). Particularly, the phylum of Nitrospirae accounted for 0.17% in the seed

### Table 1 - Results of Alpha diversity analysis.

<table>
<thead>
<tr>
<th>Samples</th>
<th>OTU</th>
<th>Shannon</th>
<th>Simpson</th>
<th>ACE</th>
<th>Chao1</th>
<th>Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>4003</td>
<td>6.85</td>
<td>0.003</td>
<td>13,835.7</td>
<td>9257.2</td>
<td>0.907</td>
</tr>
<tr>
<td>Granular sludge</td>
<td>2013</td>
<td>5.51</td>
<td>0.024</td>
<td>4665.5</td>
<td>3734.5</td>
<td>0.953</td>
</tr>
</tbody>
</table>

Fig. 4 – COD (a) and NH\(_3\)-N (b) removal efficiency of the SBR during the operation.
sludge, but the ratio was decreased to 0 after aerobic granular sludge formation. This means the removal efficiency of NH$_4$-$N$ was greatly influenced by Nitrospirae having played an important role in N removal. Therefore, we concluded that microbial diversity could affect the performance of the aerobic granular sludge process.

It was reported that Proteobacteria were universal in aerobic granular sludge and could produce EPS for the cohesion of floc sludge and formation of aerobic granules (Sirous et al., 2010; Jiang, 2011). The abundance of Proteobacteria was obviously increased by 9.3% from seed sludge to aerobic granular sludge, suggesting that Proteobacteria played a key role in sludge granulation and aerobic granular sludge structure further facilitated its growth. Proteobacteria belonged to Gram-negative bacteria and classified to the heterotrophic community, and played important roles in removing COD and N. The other dominant phyla were Chloroflexi, Bacteroidetes, Firmicutes, Planctomycetes, Actinobacteria, TM7 and Acidobacteria. Chloroflexi, Bacteroidetes, Acidobacteria and Planctomycetes were classified to autotrophs. Firmicutes and Actinobacteria were classified to heterotrophs. The microorganisms not only could consume carbon and nitrite resources, but also produced glue-like extracellular polymers for microbial aggregation (Sirous et al., 2010; Li et al., 2008). It was observed that Bacteroidetes was easily washed out at short settling time in the development of aerobic granulation in the previous study (Zhang et al., 2011a, 2011b). However, the proportion of Bacteroidetes increased significantly after sludge granulation due to excellent settling ability of aerobic granular sludge (SVI$_{30}$ = 20.3 mL/g) caused most of them enriched and blooming in granular sludge. Many Firmicutes strains as denitrifiers took part in dinitrification in an anaerobic environment (Liu et al., 2008), and granular sludge could provide this micro-environment. Hence, the phylum of Firmicutes proliferated in aerobic granular sludge with raw piggery wastewater.

It is well known that high organic loading rate has a negative effect on filamentous bacterial growth, and some of Chloroflexi and Planctomycetes were identified as the filaments in activated sludge (Kragelund et al., 2007) and aerobic granules (Figueroa et al., 2015). The piggery wastewater with a relatively high N and COD loading rate limited the filamentous bacteria growth, so the abundance greatly decreased in granular sludge in this study. The other microorganisms had a similar phenomenon due to a high loading impact to microbial abundance in granular sludge. This indicated that high loading rate might reduce the microbial diversity of aerobic granular sludge. As well know, activated sludge has been used as seed in most aerobic granulation studies. Microbial communities in seed sludge exhibited different behaviors with various types of wastewater or substrate, especially the microbial diversity and dominance. Therefore, it can be concluded that microbial diversity was not only related to the structure of the granules, but also to the wastewater in which they were developed.

The taxonomic profile analyses from two sludge types were mapped onto NCBI taxonomy by MEGAN soft. Fig. 6 shows all dataset profiles from the inoculum and granular sludge metagenome at class level. The subsequent classification and abundance catalog clearly demonstrated that bacteria comprised the most abundant domain; the remaining sequences were distributed in Euryarchaeota. Particularly, results revealed that the microorganisms in the seed sludge and aerobic granular sludge had significant evolutions from phylum to class level. Some species including the phyla of Proteobacteria, Bacteroidetes and Firmicutes, were adopted in granular sludge and the abundances increased. Meanwhile, other species, such as Chloroflexi, Planctomycetes, Actinobacteria and Acidobacteria, did not survive and decreased or varnished during the changes of wastewater and sludge structure in this study. These differences consisted with the results shown in Fig. 5.

Fig. 6 also demonstrates the differences in the case of the unassembled and assembled reads. In Bacteroidetes, the abundance of unassembled reads was higher, while in Proteobacteria and Firmicutes, the number of assembled reads was higher. Overall, combining data from assembled and
unassembled reads, a good diversity profile was obtained. In addition, \( \alpha \), \( \beta \), and \( \gamma \)-Proteobacteria contain the maximum number of reads and became the predominated classes in two types sludge (Fig. 6). It was also found that some reads could not be identified into any taxonomy, since they were unidentified bacteria.
3. Conclusions

Rapid aerobic granulation was archived in the SBR with raw piggery wastewater by seeding activated sludge after 3 days operation. Granules with excellent settling ability (SVI₃₀ = 20.3 mL/g) were obtained in SBR after 18 days. Although this work used the activated sludge as the inoculum, fast formation of aerobic granules was successfully obtained in this work. This was due to high contents of Ca and Fe in raw piggery wastewater and SBR operating mode. Microbial analysis demonstrated that the communities of aerobic granules had less richness and abundance than seed sludge in terms of Alpha diversity and microbial composition. The characteristic of raw piggery wastewater and granule structure may play crucial roles in microbial variations.

Acknowledgments

This work was supported by the National Nature Science Foundation of China (No. 51478433).

References


