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Research article

High-strength anaerobic digestion wastewater treatment by aerobic granular sludge in a step-by-step strategy

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ABSTRACT

To reduce the instability of aerobic granular sludge (AGS) caused by high-strength anaerobic digestion wastewater, a strategy of increasing proportion of anaerobic digestion wastewater step-by-step was adopted in this study. High-performance stable AGSs were successfully cultivated with sequencing batch reactors by this strategy, which could efficiently treat high-strength anaerobic digestion wastewater with an influent chemical oxygen demand (COD) up to 5090 $mg \cdot L^{-1}$. After six phases of stepwise increasing COD loads, the sludge sizes increased from 0.5 mm to 1.5 mm, with the final mixed liquor suspended solids increased to 13,814 $mg \cdot L^{-1}$, and the final sludge volume index decreased to 15 $mL \cdot g^{-1}$. The extracellular polymeric substance (EPS), which is crucial to keep the stability of AGS, increased continuously from 85.1 $mg \cdot g^{-1}$ SS to 307.8 $mg \cdot g^{-1}$ SS with the increase of COD loads. Moreover, the removal efficiency of COD and TN could reach 92% and 87% for real high-strength anaerobic digestion wastewater treatment. The bacterial community analysis revealed that the family *Rhodocyclaceae*, *Flavobacteriaceae*, and *Xanthomonadaceae* were the major microbes of AGS, and were responsible for COD and TN removal, as well as EPS secretion. These findings may provide novel information and enrich AGS treatment of high-strength real wastewater.

1. Introduction

With the advent of industrialization and population growth, the production of kitchen waste is increasing. According to statistics, about 97 million ton of kitchen waste was produced in China every year (De Clercq et al., 2017). Due to its high moisture concentration and organic loads, kitchen waste cannot be easily degraded in a short period of time, resulting in serious environmental pollution (Girotto et al., 2015).

Anaerobic digestion technology has been widely used to treat kitchen wastes for biogas production, which usually contains four procedures, i.e. high-temperature treatment, solid-liquid separation, oil-water separation, and anaerobic fermentation to produce clean energy as biogas (Tomei and Carozza, 2015). Anaerobic digestion of kitchen waste will produce a large amount of anaerobic digestion wastewater and residue, which still contains high concentration of chemical oxygen demand (COD) (5000–10,000 $mg \cdot L^{-1}$) (Whiting and Azapagic, 2014). In addition, many researches manifested that the anaerobic digestion wastewater is not only rich in nutriments (Guo et al., 2013), but it also contains high concentration of inorganic salts and ammonium (Kuo et al., 2012), which makes the anaerobic digestion wastewater even harder to be dealt with.

One wildly used approach for treating anaerobic digestion wastewater is ecological process, including oxidation pond, artificial wetland, and soil infiltration (Zhao et al., 2014). Another is advanced biochemical process, such as oxidation ditch (Poach et al., 2007), membrane reactor (Bertin et al., 2011), and upflow anaerobic sludge blanket (Mirzoyan and Gross, 2013). However, previous studies showed that, because of the poor biodegradability in wastewater, the efficiency of both approaches was low (Wen et al., 2016).

Aerobic granular sludge (AGS) has been developed widely in the past decades (Bandara et al., 2012). At present, AGS technology dominates in the field of biological sewage treatment, owning to its good settling ability, high biomass retention, dense bacterial structure, and high tolerance to organic loading and toxicity (Adav et al., 2008). Although,

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many of researches has been carried out with simulated wastewater to evaluate performances of AGS in sequencing batch reactor (SBR) (Nancharaiah and Kiran Kumar Reddy, 2018), only a few studies have been reported on the successful cultivation of AGS for treating real industry wastewater, especially for high-strength wastewater.

The industry high-strength wastewater, which usually contains COD concentration more than 4000 mg·L^{-1}(Chan et al., 2009), has become an urgent pollutant that is required to be treated. Wei et al. (2012) established SBRs for AGS cultivation to treat high-strength landfill leachate, which contained 4560 mg·L^{-1} COD and 945 mg·L^{-1} NH_4^+-N. However, the COD and NH_4^+-N removal was only 70% and 59% respectively, with an AGS diameter of 0.75 mm. Likewise, Kocaturk and Erguder (2015) achieved 87% COD removal, 57 ± 17% TN removal on the treatment of sugar beet processing wastewater at 4280 mg·L^{-1} COD and 49 mg·L^{-1} NH_4^+-N. Because the high-strength wastewater had an unstable shock to AGS, it still maintains an issue to treat the high-strength wastewater by AGS directly.

Moreover, it was found that AGS could easily become floccular in the treatment of high-strength wastewater, leading AGS systems less stable (Leal et al., 2020). Adav et al. (2010) reported that the critical COD values for AGS disintegration was 3000–4000 mg L^{-1} and the tested sludges did not grow in the medium at COD > 3000 mg L^{-1}. Intracellular protein hydrolysis, degradation at the anaerobic granule core (Zheng et al., 2006), and overgrowth of filamentous microorganisms (Liu and Liu, 2006) were the main reasons for AGS instability at high-strength wastewater. Corsino et al. (2018) found that proteins were hydrolyzed and sludge was unstable when AGS was used to treat citrus wastewater with 3525 ± 1615 mg·L^{-1}COD. Hamza et al. (2018) cultivated aerobic granular sludge with high influent COD concentration of 4500 mg·L^{-1}, and found that excessive growth of biomass at high COD concentrations negatively affected the stability of AGS, causing disintegration due to the presence of methanogens in the core of sludge particles. Zhang et al. (2011) fed pure petrochemical wastewater to SBR, and found that both granules properties and performances were deteriorated.

Therefore, it is urgent to develop an efficient strategy for direct treatment of such high-strength anaerobic digestion wastewater. In this study, stable high-performance AGSs were successfully obtained by a stepwise strategy. The impacts of different organic loads on the structural stability, the biodegradation efficiency, the change of extracellular polymeric substance, and the diversity of bacterial community were investigated. This study may provide the basic for AGS treatment of high-strength real wastewater.

2. Materials and methods

2.1. Seed sludge and wastewater composition

Aerobic granular sludge (AGS) with mixed liquor suspended solids (MLSS) of 2333 mg·L^{-1} and sludge volume index (SVI_{30}) of 110 mL·g^{-1} were used as seeding sludge, while sodium acetate was used as carbon source and ammonium chloride was used as nitrogen source in the synthetic wastewater, and the composition was showed by Dai et al. (2015).

The anaerobic digestion wastewater was collected from a 100 m^3 pilot-scale anaerobic digestion device in Changping Industrial Park (Beijing, China), with the composition of chemical oxygen demand (COD) was 5090 ± 50.0 mg·L^{-1}, total nitrogen (TN) was 1019 ± 15.0 mg·L^{-1}, ammonia nitrogen (NH_4^+-N) was 979.0 ± 10.0 mg·L^{-1} and total phosphorus (TP) was 28 ± 1.0 mg·L^{-1}.

2.2. Reactor set-up and operation

Two sets of cylindrical sequence batch reactors (SBR) were used for parallel experiments in this study. Each SBR has an internal diameter of 7 cm, an external diameter of 16 cm, a height of 63 cm, and a working volume of 1.5 L, and was used for ASG cultivation. The water inflows at the top of SBRs, and the volume exchange rate was set at 50%. With the intermittent operation, each cycle can be split into 4 subsequent process of influent (5 min), aeration (200 min), sedimentation (20 min), and effluent (5 min). The aeration was constant at 200 L h^{-1}. According to the pre-experiments with the different ratio of C/N on the performance of AGS, the ratio of C/N was maintained at 5:1. The schematic diagram of cylindrical SBR can be seen in Fig. 1.The SBR operating parameters of each phase are given in Table 1.

2.3. Analytical methods

Parameters such as COD, TN, MLSS and SVI were determined by reference methods (APHA, 1998). The COD was determined by the potassium dichromate method using a spectrophotometer (DR1010, HACH, USA). The TN content was determined by the potassium persulfate method using a digestion reactor (DRB200, HACH, USA). MLSS was measured by oven drying of samples at 105 °C for 1 h and SVI was determined in a 100 mL graduated cylinder after 30 min. The morphology and structure of sludge were observed using an optical microscope (SMZ-DV320, Chong Qing Aote, China) and acning electron micrographs (SU1510, Hitachi, Japan). In addition, liquid samples were collected from the reactor every three days after sedimentation when the stable performance was obtained at an operation cycle.

The extraction of extracellular polymeric substance (EPS) was conducted by the salt-heating method (Deng et al., 2016). Polysaccharide (PS) was measured by phenol/sulfuric acid method and protein (PN) was measured by the Coomassie brilliant blue method (Liu and Tay, 2002).

2.4. Bacterial community analysis

Six samples in each phase of sludge were selected for community analysis. Genomic DNA of the sludge samples was extracted with the E. Z.N.A. Soil DNA Kit (OMEGA), and were sent to Novogene (Beijing, China) for shotgun 16S rDNA library construction with an Illumina Hiseq2500 platform. The raw sequences were analyzed according to Wang et al. (2017), using Quantitative Insights Into Bacterial Ecology (QIIME, version 1.7.0). The resulting high-quality sequences were clustered into operational taxonomic units (OTUs) at the 97% sequence similarity threshold by Uclust clustering.

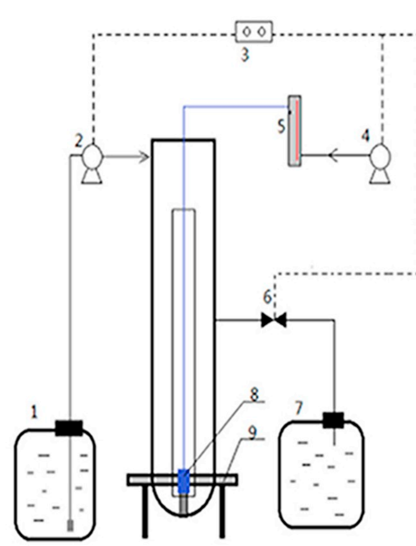


Fig. 1. Schematic diagram of cylinder SBR (1-influent tank; 2,4-peristaltic pump; 3-electromagnetic relay; 5-flow counter; 6-solenoid valve; 7-effluent tank; 8-aerator; and 9-flange).

Table 1
The operation parameters of SBRs.

Phase	Time (d)	C/N	Proportion of anaerobic digestion wastewater (%)	COD ($mg{\cdot}L^{-1}$)
I	1–26	5 ± 0.1	29.4	1500 ± 20.0
II	27–59	5 ± 0.1	39.0	2000 ± 20.0
III	60–89	5 ± 0.1	49.0	2500 ± 30.0
IV	90–119	5 ± 0.1	68.7	3500 ± 30.0
V	120–149	5 ± 0.1	88.3	4500 ± 40.0
VI	150–180	5 ± 0.1	100.0	5095 ± 50.0

3. Results and discussion

3.1. Reactor performance and characteristic of aerobic granular sludge

3.1.1. Settling property and biomass concentration

The biomass concentration of aerobic granular sludge (AGS) is indicated by the mixed liquor suspended solids (MLSS). The greater value of MLSS, the higher biomass concentration of sludges (Beun et al., 2002). The sludge volume index (SVI) is used to reflect the settleability of granular sludges. In general, denser and faster settling biomass reduces SVI value, thus enhancing the settling ability of sludge and resulting in effluent low in suspended solids (Toh et al., 2003).

As shown in Fig. 2, MLSS increased gradually while SVI decreased with the increase of organic loads, implying that sludge granulation increased gradually. In the initial stage of the experiment (phase I and phase II, 1–60 d), the sludge particles were unstable because of the increase of influent COD concentration, leading to the MLSS and SVI fluctuated greatly. Especially in phase II, the granular sludge inflated when the COD concentration increased to 2000 $mg{\cdot}L^{-1}$. At the same time, the SVI increased to 95 $mL{\cdot}g^{-1}$ rapidly, and the MLSS decreased to 2738 $mg{\cdot}L^{-1}$. The main reason was that the increase of COD concentration could promote the overgrowth of the filamentous bacteria in the sludge, and the granular sludge swelled in a short time (27–35 d), which decreased the densification of the sludge, deteriorated the settling performance, and decreased the sludge concentration. After a period of adaptation, SVI fluctuated slightly with the impact of COD loads from phase III to phase VI. This phenomenon implied that the anaerobic digestion wastewater was advantageous to enhance the sludge granulation and to resist the COD loads shock. It can be observed in Fig. 2 that the SVI showed a downward trend after 60 days. During the phase VI where the complete anaerobic digestion wastewater was used, the SVI decreased to 15 $mL{\cdot}g^{-1}$ and the MLSS increased to 13,814 $mg{\cdot}L^{-1}$.

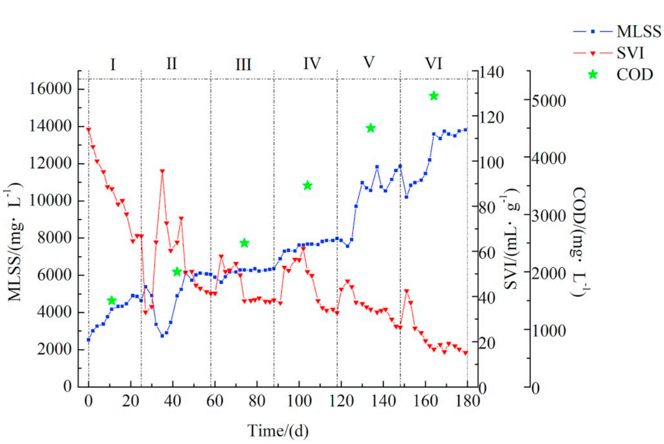


Fig. 2. Variation of sludge parameters in different phases.

3.1.2. Morphology and structure of aerobic granular sludge

In general, the average particle size of sludge particles increased simultaneous, with the continuous increase of sludge settleability. AGS was reported to maintain structural stability when the organic loads fluctuated within a certain range (Hamza et al., 2018; Li et al., 2008). From phase I to phase II, the average sizes of granular sludges were about 0.5 mm with clear flocculent sludges in the unstable state (Fig. 3a and b). From phase III to phase V, compact granules could be observed with average diameters of 0.8–1.3 mm (Fig. 3e, f and 3g). With the stepwise increase of organic loads, the final size of AGS could reach 1.5 mm (Fig. 3h).

Scanning electron micrographs images (Fig. 4) showed that the compact and complete AGSs were formed after high-strength wastewater treatment. The granular sludges were ellipsoidal in shape and were well-delineated (Fig. 4b), and their surfaces were mainly composed of rod-shaped microorganisms and spheroidal microorganisms (Fig. 4c). Filamentous microorganisms were used as the skeleton structure of aerobic granular sludge, mainly distributed inside the granular sludge (Ma et al., 2012; Wang et al., 2015). Meanwhile, a large number of gullies and wrinkles were distributed on the surface of the particles (Fig. 4d). These pleats greatly increased the specific surface area of the sludge particles, allowing the sludge to be in full contact with the pollutants, thereby increasing the pollutant removal rate.

3.1.3. Variation of extracellular polymeric substance

The extracellular polymeric substance (EPS) secreted by the microorganisms contained in the AGS also has influences on the stability of the granular sludge. The reason is that EPS can change surface properties of AGS such as surface charge and hydrophobicity (Rusanowska et al., 2019). In this experiment, the concentration of EPS increased continuously with the increase of COD loads. Microorganisms contained in sludge converted the excess carbon and nitrogen sources to polysaccharides (PS) and proteins (PN) after the adequate nutrients met the demand of their own growth and reproduction.

As showed in Fig. 5, at the beginning of the reaction (phase I, 1–25 d), the EPS concentration remained stable. However, when the influent COD concentration increased to 2000 $mg{\cdot}L^{-1}$ in phase II (26–59 d), the value of PN/PS greatly reduced. The reason was that sludge granulation has great impact on protein secretion. The higher the degree of granulation, the higher the concentration of secreted protein in the granule. Under large load shocks, most of the organic substances were used for the proliferation of filamentous bacteria, which resulted in sludge swelled. Therefore, the protein secretion decreased while the concentration of PS was stable during the process of granulation of the sludge. The concentration of EPS reached 307.8 $mg{\cdot}g^{-1}$ SS (average value) when the proportion of anaerobic digestion wastewater increased to 100% in the influent. The average concentration of PN and PS was 229.2 $mg{\cdot}g^{-1}$ SS, and 78.6 $mg{\cdot}g^{-1}$ SS and PN/PS increased to 2.91, respectively.

The curve of PN/PS performed an overall upward trend, associating with changes in SVI (Fig. 2). The average values of PN/PS were 2.34, 2.20, 2.37, 2.61, 2.79 and 2.91 from phase I to phase VI, respectively. Increasing granularity of AGS was reflected in the increase of PN/PS. This also indicated the important role of PS in sludge granulation.

3.2. Pollutants removal efficiencies

The impact of different organic loading on effluent COD concentration was given in Fig. 6A. The initial COD concentration was 1500 $mg{\cdot}L^{-1}$, and the average removal rate reached to 88.3% with the increase of sludge concentration in phase I. In phase II, the COD concentration was adjusted to 2000 $mg{\cdot}L^{-1}$. The flora structure has changed due to the fluctuation of the COD load, which caused the MLSS decreased. The COD removal efficiency decreased continuously to 82.8% during 29–33 d as the sludge SVI increased, and the average removal rate of COD was 84.6%. Afterwards, the COD removal

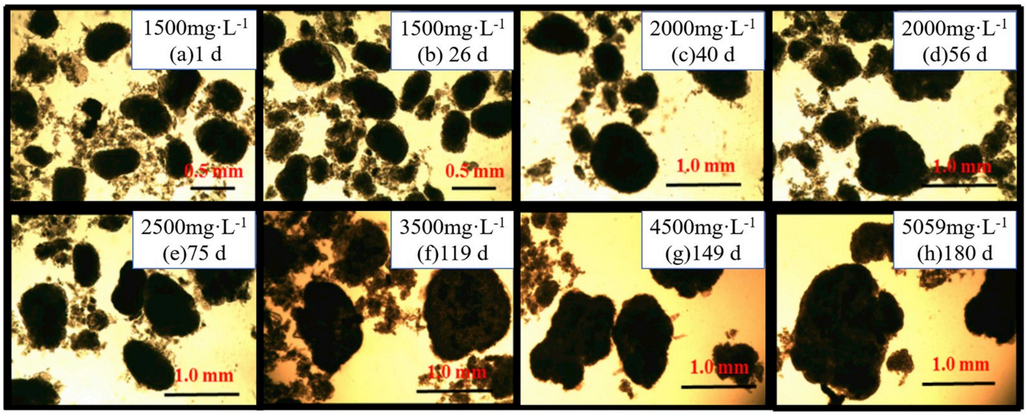


Fig. 3. Variation of sludge morphology in different phases.

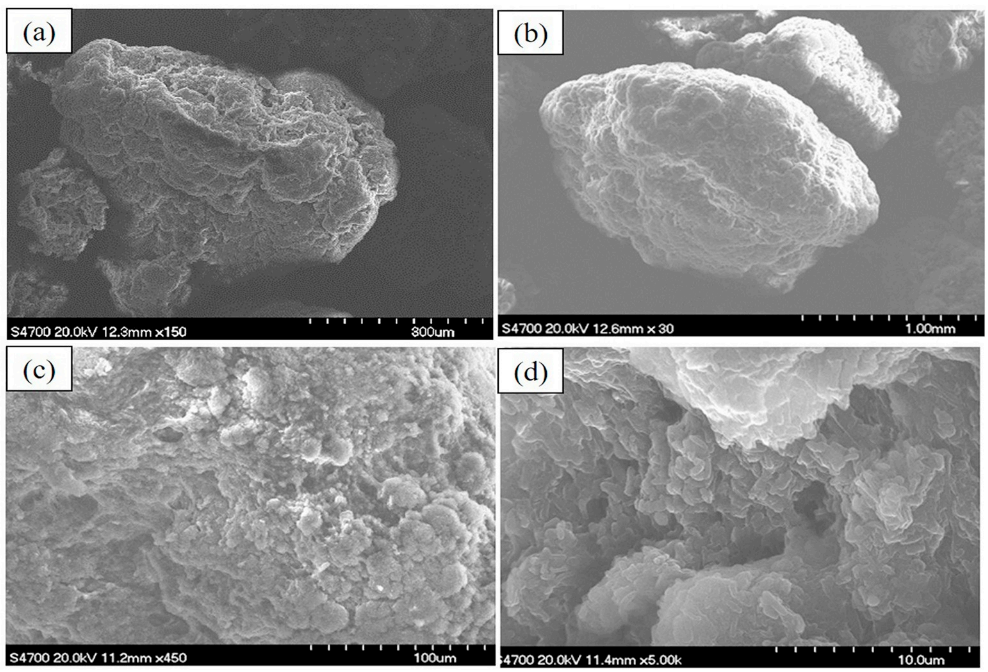


Fig. 4. Scanning electron micrographs of aerobic granular sludge: (a) before the treatment, (b, c, d) after the treatment.

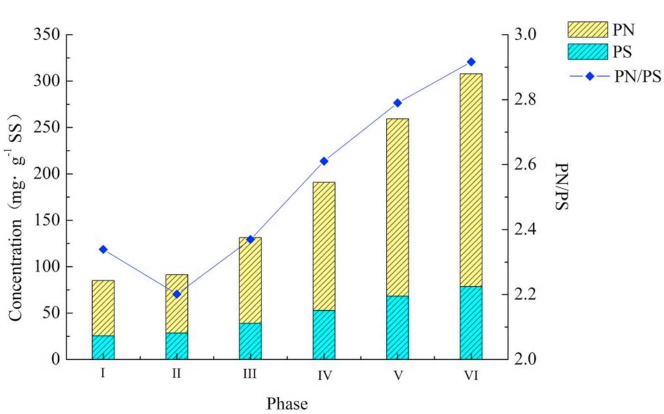


Fig. 5. Variation of EPS in different phases.

efficiency increased to 88.7%, 90.2%, 91.5%, and 92.2% when the influent COD concentration increased to 2500 mg·L^{-1} (phases III), 3500 mg·L^{-1} (phases IV), 4500 mg·L^{-1} (phases V), and 5059 mg·L^{-1}(phases VI), respectively. The average removal efficiency was 87.0%, 87.5%, 90.9%, and 90.1%, respectively. Especially in phase VI, where the influent was all anaerobic digestion wastewater, the COD concentration was degraded from 5059 mg·L^{-1} to 396 mg·L^{-1}. In these phases, the granularity of sludge was strengthened slightly, which provided a good aerobic-anaerobic environment for removing organic matter (Gao et al., 2011).

Influent COD loads have a great impact on the removal efficiency of nitrogen (N) source (Adav et al., 2008; Toh et al., 2003). With the increase of influent COD concentration, the removal efficiency of total nitrogen (TN) increased gradually. Due to the microorganisms were unstable in swelled sludge, the TN removal efficiency dropped to 51.5% during the phase II (Fig. 6B). As the density of the sludge structure was improved, the AGS remained a relative high efficiency despite the fact the influent TN concentration increase continuously. The removal efficiency of TN was 72.9%, 75%, 84.3% and 87.3%, when the influent TN concentration was 500 mg·L^{-1}, 700 mg·L^{-1}, 900 mg·L^{-1} and 1019 mg·L^{-1}, respectively. The results implied that AGS, which can provide a strict aerobic-anaerobic environment (Bassin et al., 2012), had high biodegradation efficiency to COD and TN in anaerobic digestion

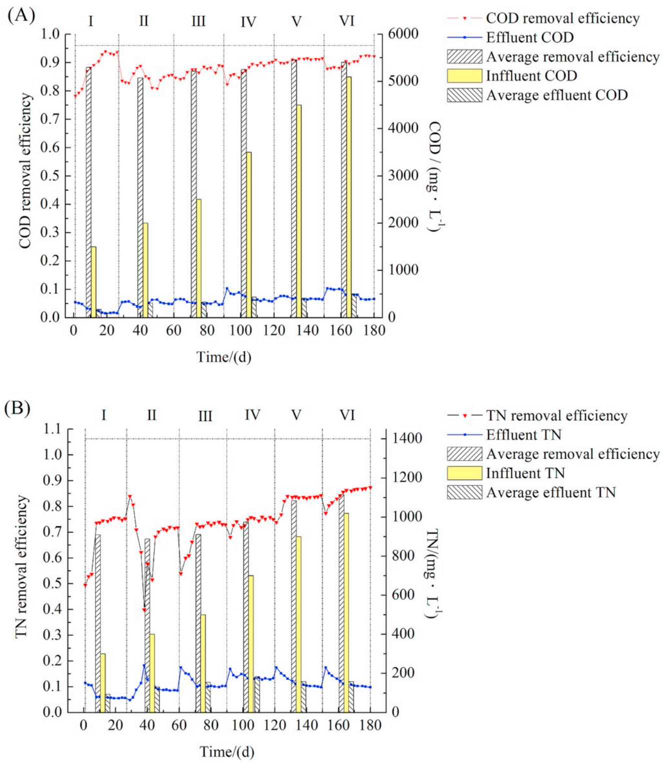


Fig. 6. Variation of (A) COD removal efficiency and (B) TN removal efficiency under different phases.

wastewater. After the strategy operation, AGS was able to effectively removed pollutants in all high-strength anaerobic digestion wastewater during the last phase for 30 days.

3.3. Bacterial community analysis

The diversity of bacterial communities reflects the species richness of a particular ecosystem. In biological wastewater treatment systems, bacterial diversity not only determines its water treatment performance (Wang et al., 2013a, 2013b), but also affects the stability of the systems (Mielczarek et al., 2013). The species richness of microbes also plays an important role in granulation (Ma et al., 2013), while the bacterial community varies from place to place (Sun et al., 2014). For example, Wang et al. (2010) surveyed eight sewage treatment plants and showed that ammonia-oxidizing bacteria in municipal wastewater were higher than those of industrial wastewater or mixed wastewater, indicating that the impact of wastewater composition had effect on the bacterial community structure.

3.3.1. Analysis of OTU clustering for aerobic granular sludge

Organic loads have direct effects on the growth and bacterial structure of AGS. Li et al. (2008) studied the effects of different organic loads on the growth of microbes in the process of AGS granulation. The results showed that organic loads directly affected the morphology and flora structure of sludge particles.

In general, as indicated in Table 2, the bacteria in the AGS have experienced dynamic changes in terms of species richness and dominant bacteria throughout the granulation process. The bacteria with the highest substrate loading rate had the lowest bacterial diversity index, while the lowest substrate loading rate had the highest diversity index. The operational taxonomic units (OTU) showed a downward trend from phase I to phase VI, indicating that the species richness decreased and dominant species were enriched with the increase of COD concentration. Interestedly, when the COD concentration was increased to 2000 mg·L^{-1}

Table 2
Variation of aerobic granular sludge bacterial diversity index.

Sample	COD (mg·L^{-1})	OTU	Observed species	Shannon[a]
Phase I	1500 ± 20	268	226	2.29
Phase II	2000 ± 20	220	179	3.13
Phase III	2500 ± 30	163	140	1.20
Phase IV	3500 ± 30	179	151	2.32
Phase V	4500 ± 40	154	141	2.21
Phase VI	5095 ± 50	120	120	2.75

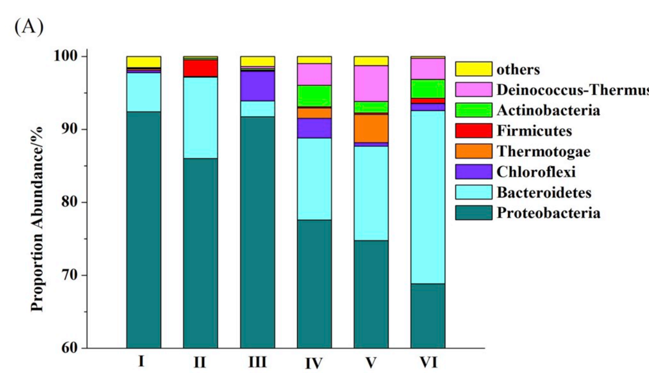
[a] a higher value represents a higher species richness.

(phase II), the AGS was swollen and bacterial structure was adversely changed, which may be attributed to the high influent fluctuation. However, when the COD concentration was continually increased to 2500 mg·L^{-1} (phase III), some bacteria became dominant while a large number of other bacteria diapered. Therefore, the species richness in phase III decreased. From phase IV to phase VI, the species richness decreased with the increase of COD concentration.

3.3.2. Analysis of bacterial dynamic changes of aerobic granular sludge

The bacterial composition and structure of six different phases at the phylum and family level were analyzed in detail (Fig. 7). Obviously, the community of different phases were quite different. At phylum level (Fig. 7A), seven identified phyla were observed in six samples, *Proteobacteria* and *Bacteroidetes* were the main phyla, followed by *Chloroflexi*, *Deinococcus−Thermus*, *Actinobacteria*, *Thermotogae*, and *Firmicutes*. *Proteobacteria* and *Bacteroidetes* maintained the foremost phyla during the whole wastewater treatment. With increasing COD concentration up to 5000 mg·L^{-1}, an opposite trend was shown between the phyla *Proteobacteria* and *Bacteroidetes.* The *Proteobacteria* decreased slightly from 92.43% (phase I) to 68.83% (phase VI), while *Bacteroidetes* gradually increased to 23.72% (phase VI) from 5.32% (phase I).

Meanwhile, *Chloroflexi*, which is a kind of photosynthetic bacteria with biological nutrient removal (Gupta, 2013), decreased from 2.69%



(B)

Phylum	Family	I	II	III	IV	V	VI
Proteobacteria	*Comamonadaceae*	0.84	50.91	0.12	0.45	0.06	0.18
	Rhodocyclaceae	4.59	0.21	86.53	68.02	67.86	49.24
	Xanthomonadaceae	1.1	2.07	3.21	1.79	1.29	13.77
	Pseudomonadaceae	0.45	26.96	0.05	0.65	0.02	0.05
	Rhodobacteraceae	75.85	2.53	0.53	3.63	3.00	1.75
Deinococcus−Thermus	*Trueperaceae*	0	0.01	0.28	2.97	4.91	2.88
Chloroflexi	*Anaerolineaceae*	0.23	0.04	4.04	2.66	0.46	0.92
Bacteroidetes	*Flavobacteriaceae*	0.16	2.98	0.64	10.21	10.87	20.80
Thermotogae	*Thermotogaceae*	0.22	0.05	0.11	1.42	3.86	0.06
unidentified	unidentified	4.17	0.33	0.97	0.79	0.68	0.55

Fig. 7. (A) The changes of microbial community structure in the phylum level in different phases; (B) Percentages of the major families in each treatment phase.

to 0.99% in the final phase. It seems that autotrophic bacteria were suppressed while heterotrophic bacteria clearly dominated at high-strength anaerobic digestion wastewater. It worth noting that during the phase III-VI, *Deinococcus−Thermus* (2.97%, 4.91%, and 2.88%) and *Actinobacteria* (2.94%, 1.60%, and 2.59%) increased significantly comparing with the former stages.

Further analysis at family level was shown in Fig. 7B. The communities at family level varied a lot from phase I to phase III. In phase I, the family of *Rhodobacteraceae* (75.85%) , with the function of denitrification (Huang et al., 2013), was the dominate bacteria. The Comamonadaceae (50.91%) and *Pseudomonadaceae* (26.96%) families were dominated in phase II. The Comamonadaceae was reported to play a major role in denitrification processes in the presence of acetate in AGS (Adav et al., 2009). The *Rhodobacteraceae* and Comamonadaceae, belong to the *Proteobacteria* phylum, encompassed enormous morphological, physiological and metabolic diversity, and were of great importance to global carbon, nitrogen and sulphur cycles (Kersters et al., 2006). The elimination of unacclimated bacteria was attributed to the toxicity of the high-strength wastewater and the ascending of organic load rate (Chen et al., 2019). The family *Pseudomonadaceae* was reported to biodegrade various organic micropollutants, including sulfamethoxazole, chlorinated compounds and several complex dyes (Herzog et al., 2017). However, significant bacterial community shift occurred that the *Rhodocyclaceae* became the foremost in phase III (86.53%). During these three phases, the bacterial structure changed visibly at the family level with increasing COD concentration. This means that the stepwise influent anaerobic digestion wastewater had an impact on microbial community structure and AGS showed its adaptation to different composition wastewater.

After a course three-month operation of feeding high-strength anaerobic digestion wastewater, the bacterial community and core families of phase IV, phase V, and phase VI were similar to some extent. From phase IV to phase VI, *Rhodocyclaceae* kept the highest percentages accounting for 68.02%, 67.86%, and 49.24%, respectively. *Flavobacteriaceae*, which was reported to resist in the breakdown of complex organic wastewater (Liu et al., 2005), was the second dominant family (10.21%, 10.87%, and 20.80%, respectively) during these periods. *Rhodocyclaceae* and *Flavobacteriaceae* were two functionally important and closely related species that often occurred together and responsible for denitrifying and resisting in fluctuation in AGS (Tian et al., 2015). *Rhodocyclaceae* was reported to be involved to denitrification and have the ability to reduce nitrate or nitrite (Lu et al., 2018). Therefore, the family *Rhodocyclaceae* might be well adapted to the specific nutrient conditions in high-strength anaerobic digestion wastewater. When the COD concentration was increased to more than 3500 $mg\cdot L^{-1}$, *Flavobacteriaceae* increased greatly after phase III. Analysis has reported that an increase in *Flavobacteriaceae* improved the capacity of fatty acid, lipid, and protein degradation (Jin et al., 2011). It proved the degradation potential of AGS in treatment of high-strength wastewater, with high COD removal (92%) and TN removal (87%) in phase VI. *Trueperaceae*, with the capability of ammoxidation, changed significantly from phase IV to phase VI (Qian et al., 2017). Additionally, because of the function of EPS secretion (Zhang et al., 2019), *Xanthomonadaceae* enriched gradually to 13.77%, with concentration of highest EPS reached to 307.8 $mg\cdot g^{-1}$ SS in phase VI.

4. Conclusions

In this study, AGS was successfully cultivated by the step-by-step strategy for the real high-strength anaerobic digestion wastewater treatment. During an 180-day-operation, AGS demonstrated its adaptability and good performance in the reactors. The formed granules achieved high COD and TN removal efficiency and had an average diameter of 1.5 mm eventually. At the same time, MLSS of sludge increased to 13,814 $mg\cdot L^{-1}$, SVI dropped to 15 $mL\cdot g^{-1}$ with EPS secretion growing consistently. The family *Rhodocyclaceae*, *Flavobacteriaceae* and *Xanthomonadaceae* maintained the foremost contents in high-strength stage. With the stepwise strategy, the stability and integrity of AGS enhanced and bacterial community changed stably. This strategy reduced the negative impact of real wastewater. The results warranted further investigation on the potential of AGS technology for the direct treatment of real high-strength wastewater.

Declaration of competing interest

The authors declare no competing interests.

CRediT authorship contribution statement

Wei Xiong: Conceptualization, Validation, Data curation, Writing - original draft, Writing - review & editing. **Luxi Wang:** Methodology, Investigation, Data curation. **Nan Zhou:** Methodology, Investigation, Data curation. **Aili Fan:** Resources, Software. **Shaojie Wang:** Resources, Supervision, Writing - review & editing. **Haijia Su:** Writing - review & editing, Supervision, Project administration, Funding acquisition.

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