CrossMark

Understanding of aerobic granulation enhanced by starvation in the perspective of quorum sensing

Xiang Liu[1] · Supu Sun[1] · Buyun Ma[1] · Chen Zhang[2] · Chunli Wan[1] · Duu-Jong Lee[3]

Abstract Three sequencing batch reactors (M1, M2, and M3) were set up to investigate the influence of different lengths of starvation time (3, 5, and 7 h) on aerobic granulation in the perspective of quorum sensing (QS). Autoinducer-2 (AI-2) level was quantified to evaluate the QS ability of aerobic granules. The results indicated that AI-2 level increased steadily during a cycle of sequencing batch reactors, suggesting that starvation was closely related to AI-2 secretion. In the long-term operation, aerobic granules cultivated using a prolonged starvation period had a better integrity and a higher level of cell adhesiveness despite a slower formation speed. With the extension of the starvation period, the total amount of extracellular polymeric substances (EPS) displayed an increasing tendency. EPS with large molecular weight (MW) also reached a higher level using a prolonged starvation period. However, a higher level of AI-2 and cell adhesiveness was observed in M2, which might be related to more stable granules. The results implied that the starvation period could trigger AI-2 secretion and promoted the production of large MW EPS, leading to cell adhesiveness enhancement and granule formation. Therefore, a combination of different starvation periods was proposed in this study in order to improve aerobic granulation.

Keywords Aerobic granulation · Autoinducer-2 (AI-2) · Starvation · Cell adhesiveness · Extracellular polymeric substances (EPS) · Microbial community

Introduction

As one of the most promising wastewater treatment technologies, aerobic granular sludge is understood as a special form of biofilm which has more advantages in settling property, biomass retention, and ability to withstand organic loading rate (OLR) (Adav et al. 2008). However, one major obstacle against its wide application is the loss of granule stability (Lee et al. 2010). Some researches have been conducted to optimize manipulative conditions to improve granule stability (Mosquera-Corral et al. 2005; Wang et al. 2005). Starvation period has been investigated as a vital influential factor of aerobic granulation. Some researchers (Gao et al. 2011; Liu and Tay 2008) found that granules are more likely to form under short starvation period, while granules cultivated under long starvation period are reported more stable (Liu and Tay 2007). Starvation would also change the morphology and extracellular polymeric substances (EPS) characteristics of mature aerobic granules (Pijuan et al. 2009).

Bacteria communicate with each other by secreting autoinducers (AIs) to detect colonial density (Galloway et al. 2010). When accumulated AIs reach a threshold which means the increase of colonial density, certain genes will be expressed like bioluminescence, pathogenicity, and motility

✉ Chunli Wan
hitwan@163.com

✉ Duu-Jong Lee
djlee@ntu.edu.tw

[1] Department of Environmental Science and Engineering, Fudan University, No. 220 Handan Road, Yangpu District, Shanghai 200433, China

[2] Shanghai Municipal Engineering Design General Institute, Shanghai 200092, China

[3] Department of Chemical Engineering, National Taiwan University, Taipei 106, Taiwan

(Eberl 1999; Lesprit et al. 2003). This process was called quorum sensing (QS). Autoinducer-2 (AI-2) is an intra- and interspecies quorum sensing molecule that is responsible for communication between different microbial species (Galloway et al. 2010). QS-controlled behaviors have been demonstrated to be involved in the development of bacteria biofilms, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus mutans* (Zhang et al. 2011). In the research of aerobic granulation, Xiong and Liu (2012) discovered that higher level of AI-2 content was detected more in mature granules than in immature granules. With the decrease of AI-2 concentration, granule breakage was observed (Jiang and Liu 2013). Therefore, the secretion of AI-2 may be possibly related to the formation and stability of aerobic granules, and the relationship between QS ability and aerobic granulation induced by different manipulative conditions needs deep research.

In the present study, the influence of starvation period on QS system was investigated in aerobic granulation. Discrepancies of aerobic granules cultivated using three starvation periods were analyzed in terms of cell adhesiveness, EPS amount, and microbial communities. This study is expected to provide a unique insight into aerobic granulation mechanism, which reveals the influence of starvation length on QS ability of aerobic granules. The possible mechanism of aerobic granulation brought by starvation is also discussed in this paper. This work is aimed to promote the practical application of aerobic granules in terms of accelerated cultivation and stable operation.

Materials and methods

Reactor setup and sludge cultivation

Three sequencing batch reactors (SBRs), M1, M2, and M3, were set up to cultivate activated sludge using different cycle times, 4, 6, and 8 h, respectively. The influent chemical oxygen demand (COD) content fluctuated between 1250 and 7500 mg/L, while the change of influent COD content was synchronous in three reactors (Table 1). The reactors were cylindrical in shape, and fine air bubbles were introduced from the bottom of each reactor. The effective volume of reactors was 7 L, with an exchange ratio of 50 %. Reactor dimensions, operational parameters, and influent composition are shown in Table S1 and Table S2. The inoculated activated sludge was obtained from a municipal wastewater treatment plant in Shanghai. The sludge samples in three reactors were collected at a fixed time every day (the end of one cycle) for subsequent analysis.

A batch experiment was conducted to measure the COD consumption and AI-2 variation in one 8-h SBR cycle. The sludge samples were collected every hour for further analysis.

Table 1 Operational conditions of three sequencing batch reactors (SBRs)

Operational conditions	M1	M2	M3
Cycle time (h)	4	6	8
Settling time (min)	5	5	5
Aeration time (min)	225	345	465
Discharge time (min)	4	4	4
Influent COD (mg/L)	1250–7500	1250–7500	1250–7500

Cell adhesiveness measurement by the packed-bed method

The test of cell adhesiveness was carried out according to Tsuneda et al. (2003) with some modifications. Glass beads were packed in a column with an inner diameter of 15 mm to the height of 200 mm. About 50 mL bacterial suspension was fed into the tube at a flow rate of 2 mL/min for 20 min. The effluent optical density at the wavelength of 600 nm (OD_{600}) was measured every 1 min in case it reached the saturation point. The cell adhesiveness X was calculated as follows:

$$X = \frac{\int_0^t \left(\frac{C_{in} - C_{out}}{C_{in}} \right) dt}{t}$$

where C_{in} and C_{out} represent the OD_{600} values of influent and effluent bacterial suspension, respectively.

EPS extraction and molecular weight distribution measurement

EPS extraction was performed according to the heating method described by Lv et al. (2014). The polysaccharides (PS) content was measured using phenol-sulfuric acid method (Herbert et al. 1971). The content of proteins (PN) was conducted by the Lowry method with some modifications (Lowry et al. 1951).

The molecular weight (MW) distribution measurement of EPS was conducted by the method of Yan et al. (2012). An Agilent high-performance liquid chromatography-size exclusion chromatography (HPLC-SEC, PL1149-6801) was adopted. Runs were performed at 0.5 mL/min in solvent which contained 0.001 M NaH_2PO_4, 0.001 M Na_2HPO_4, and 0.03 M NaCl. A 1260 diode array detector (DAD) was used to detect elution profiles at 254 nm, 25 °C.

AI-2 extraction and analysis

The AI-2 extraction was performed according to Xiong and Liu (2012). In brief, the sludge samples were homogenized for 1 min and centrifuged at 5000 rpm for 10 min. The supernatant was discarded, and fresh autoinducer bioassay (AB)

medium was added to 10 mL. Then, the mixture was homogenized for 5 min in ice bath and centrifuged at 8000 rpm for 15 min. Finally, the supernatant was filtered through 0.22-μm cellulose-acetate membranes to get cell-free AI-2 extraction. AB medium consisting of 17.55 g/L NaCl, 3.17 g/L MgSO$_4$, 2.00 g/L casamino acids, 0.174 g/L L-arginine, 2.12 g/L potassium phosphate, 9.21 g/L glycerol, 10 μg/L riboflavin, and 0.001 g/L thiamine was prepared in advance.

AI-2 was indicated by *Vibrio harveyi* BB170 (ATCC BAA-1117) according to the method described by Xiong and Liu (2012) with some modifications. *V. harveyi* BB170 is a bioluminescent reporter strain which can produce light in response to AI-2, while *V. harveyi* BB120 was used as a positive control (Han and Lu 2009). AI-2 concentration is determined according to the luminescence induction ability of *V. harveyi* BB170. These two report strains were first cultured in AB medium with a shaker (Blue Pard HZQ-X300C, China) at 150 rpm, 30 °C until the OD$_{600}$ of bacterial suspension reached 0.7–1.2. Then, BB170 and BB120 were diluted into fresh AB medium at 1:100 ratio. A mixture of 180 μL diluted bacterial suspension and 20 μL AI-2 extraction samples was added into a 96-well plate, shaken with a lid at 150 rpm, 30 °C for 6 h. A microplate reader (BioTek Synergy H1, USA) was adopted to measure the bioluminescence intensity from each well at a wavelength of 490 nm. The obtained optical density at the wavelength of 490 nm (OD$_{490}$) divided by volatile suspended solid (VSS) was calculated to characterize the relative AI-2 content of different samples. Each sample was analyzed in triplicate.

Microbial community analysis

The PCR method for bacteria and the primers used in this experiment were consistent with the previous works (Wan et al. 2013; Sun et al. 2016). After the purification of PCR products, pMD-18T and *Escherichia coli* DH5α were used as the vector and competent cells for cloning, respectively. Positive clones were randomly chosen for sequencing by Sangon Co., Ltd. (Shanghai, China). The representative sequences were compared on GenBank to identify their closest genes.

The raw sequences reported in this paper have been deposited in the GenBank (accession numbers: KT367732-KT367734; KT367736-KT367737; KT367739; KT367741-47; KT367749; KT367751-52; KT367754; KT367756).

Other analytical methods

Mixed liquor suspended solids (MLSS), VSS, and COD concentration were quantified according to APHA (1998).

Results

Organic degradation ability and AI-2 variations in one SBR cycle

To determine the starvation periods of three reactors, COD consumption was measured each hour in the batch experiment. As shown in Fig. 1, the COD concentration of one 8-h SBR cycle fell below 100 mg/L in the first hour and slowly dropped to around 50 mg/L at the end of the cycle, which indicated a 7-h starvation period in a cycle. Similarly, during the long-term operation of three reactors, the COD concentration could drop below 100 mg/L in the first hour and did not decrease much after that. Therefore, the starvation periods were regarded as 3 and 5 h in M1 and M2, respectively. The COD removal rate in three reactors remained over 90 % throughout the whole operation, which indicated strong organic degradation ability of sludge in this study.

The relative AI-2 concentration in one 8-h SBR cycle was also detected in the batch experiment. As shown in Fig. 1, the ascending trend of AI-2 level was rather obvious during the whole cycle, especially since the third hour. The AI-2 rose steadily since the first hour, climbing from 1.35 OD$_{490}$/g VSS to 1.38 OD$_{490}$/g VSS till the second hour. Then, the AI-2 level upsurged to 1.59 OD$_{490}$/g VSS in the next hour and increased evenly to 1.69 OD$_{490}$/g VSS at the end of the cycle. Therefore, it is reasonable to infer that starvation might have key influence on quorum sensing ability of sludge.

The aerobic granulation performance of sludge using different starvation periods

The morphological divergences between activated sludge cultivated using three starvation strategies were quite evident. The seed sludge of M1, M2, and M3 was from the same origin, while the formation of aerobic granules took different periods of time. Judging from Fig. S1, M1 was the first to
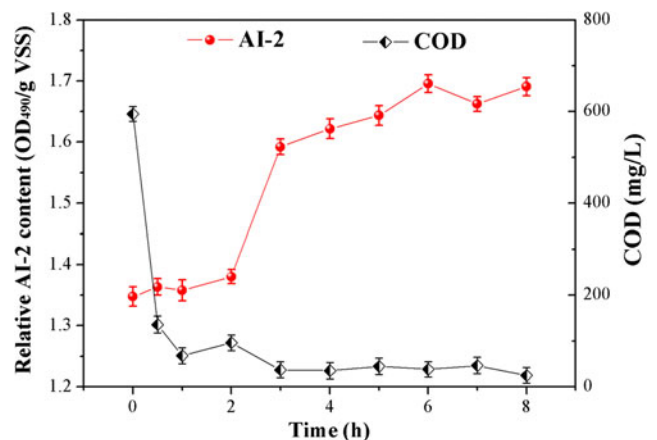

Fig. 1 The variation of AI-2 level and COD consumption in one SBR cycle of the batch experiment

form granules, which tiny aggregates were observed on day 15. The mean size of aerobic granules was between 2.5 and 3 mm, but the breakage of granules happened immediately (Fig. S1 (a)). On day 59, aerobic granules with a mean diameter of 3–4 mm were observed again in M1. In comparison, loose microbial aggregates appeared in M2 on day 19, and they grew larger on day 24 (Fig. S1 (b)). The granules were irregular with a mean diameter of 3–4 mm. Tiny aggregates did not appear until day 30 in M3 (Fig. S1 (c)), and granules of clear shape were observed on day 53.

Cell adhesiveness

Figure 2 reveals the relationship between the influent COD concentration and cell adhesiveness in M1, M2, and M3. From day 1 to day 19, the cell adhesiveness of M1, M2, and M3 increased from about 90 to around 95 % despite some fluctuations on day 4, due to the instability during reactors' startup. The cell adhesiveness of all reactors experienced a drastic rise on day 19 along with the increase of influent COD content to 5000 mg/L. M2's cell adhesiveness kept rising since day 19 and reached 97.57 % on day 50, and the value undulated in the last 10 days around 96 to 97 %. The peak of M3 appeared on day 42, hitting 97.15 %. Nevertheless, the cell adhesiveness of M3 dropped to 93.02 % on the last day of operation though the value bounced to 96 % on day 59 due to the sudden rise of influent COD. In comparison, the cell adhesiveness of M1 did not show a significant rising tendency since day 19. It decreased to 93.31 % on day 34 and then fluctuated around 93 to 94 %, which was the lowest among the reactors.

Differences of starvation period seemed to produce distinctions in cell adhesiveness as well as granulation speed and quality. Although cell adhesiveness in three reactors all experienced rising tendencies, the rising extent of M2 was 6.56 % which was the highest. The cell adhesiveness of M1 rose from 90.33 to 93.81 %, which only increased 3.48 %. During the
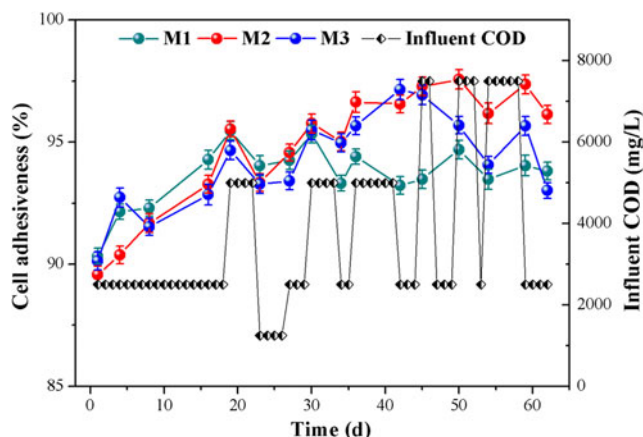

Fig. 2 Bacterial attachment ability of sludge in M1, M2, and M3

late period of operation, aerobic granules in M2 possessed the highest cell adhesiveness of over 95 %, along with good granule stability. Conversely, the cell adhesiveness of M1 was the lowest and its granules were relatively weak.

EPS characterization

Figure 3 shows the variation of EPS in M1, M2, and M3. It can be seen that all reactors were predominated by PN, whereas differences in total EPS amount and composition in M1, M2, and M3 were observed. The initial total EPS amount was about 12 mg/g MLSS in three reactors, but both the rising rate and extent in M2 and M3 were much higher than that in M1 (Fig. 3). In M2, an obvious increasing trend was observed since day 25 and the total EPS amount reached 31.93 mg/g MLSS on day 31, almost 2.6 times of the initial amount. After that, it slowly climbed to about 35 mg/g MLSS until day 58. Similarly, the total EPS amount of M3 experienced some fluctuations between 15 and 20 mg/g MLSS before day 20. Since day 25, the amount of total EPS amount increased steadily and hit 44.29 mg/g MLSS. In comparison, the highest amount of EPS in M1 was only 22.27 mg/g MLSS, which was only half of the highest amount in M3.

In addition, the level of PN concentration in M2 and M3 was much higher than in M1. On day 31, the PN content of M2 upsurged to 28.08 mg/g MLSS and steadily climbed to 32.49 mg/g MLSS on day 58. The PN concentration of M3 also ascended to 38.42 mg/g MLSS on day 58 with some fluctuations. In comparison, the rising extent of PN in M1 was relatively mild, with an increase from 9.67 mg/g MLSS to 20.44 mg/g MLSS. The PS result showed obvious differences between three reactors. In M1, the PS concentration fluctuated at a low level between 1.3 mg/g MLSS and 2.5 mg/g MLSS. M2 possessed a PS content which was a little higher than that of M1. In comparison, PS level of M3 was relatively higher which kept rising to 5.32 mg/g MLSS till day 62.

MW distribution of EPS

Figure 4 displays MW distribution of EPS in three reactors. EPS of large MW (10,000–1,000,000 Da) appeared since day 15 in M1 and M3, while in M2, this section of EPS was not detected until day 20. The proportion of EPS with large MW in M2 reached 37.46 % on day 31. After a gentle drop, this section of EPS climbed to 22.99 % in the end, which occupied the second largest proportion of EPS in the reactor. Similarly, this section of EPS in M3 rose from 5.53 % on day 43 to 39.21 % on day 62. At the same time, EPS with extremely large MW (>1,000,000 Da) appeared on day 15 and reached 43.24 % on the last day of M3 operation with a slight descend during day 31 to day 48.
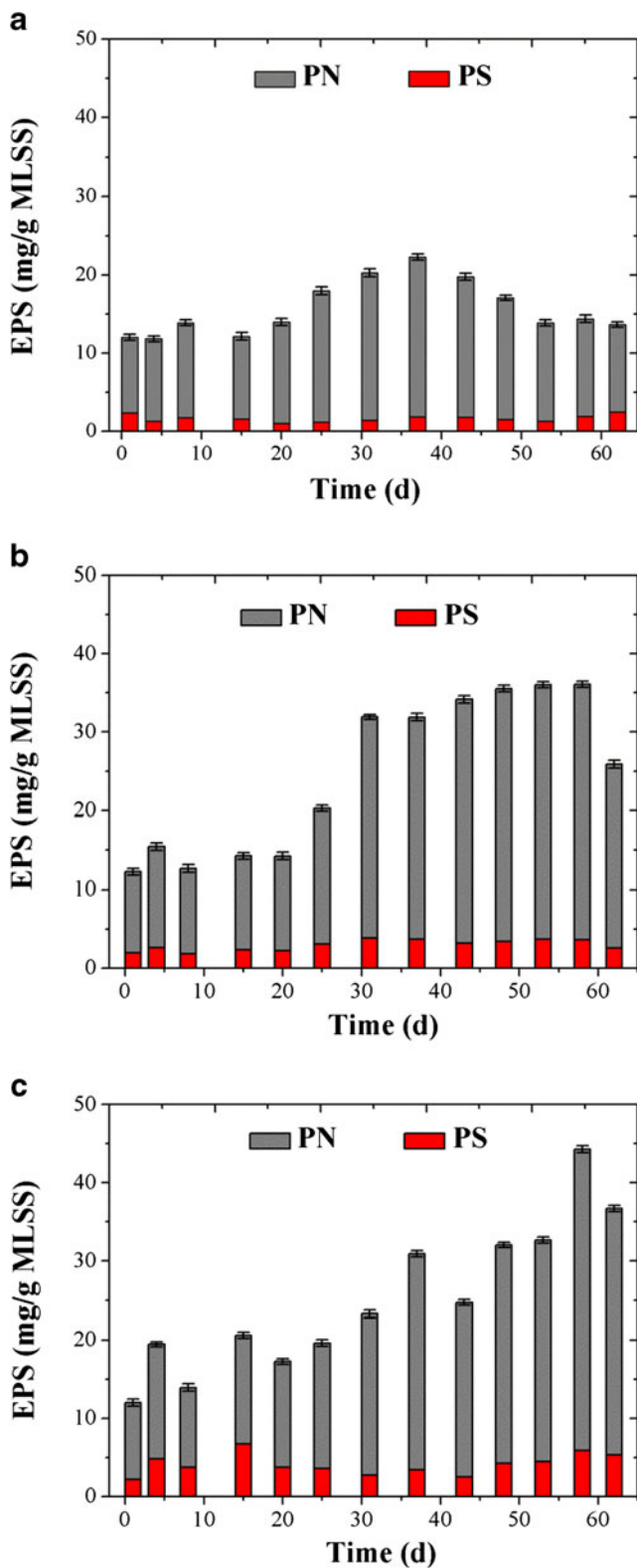
Fig. 3 Extracellular protein (*PN*) and polysaccharide (*PS*) concentrations during the whole operation of **a** M1, **b** M2, and **c** M3

Fig. 4 The molecular weight (*MW*) distribution of extracellular polymeric substances (EPS): **a** M1, **b** M2, and **c** M3

This part of EPS proportion grew fastest in M3 among three reactors, which implied that long starvation was favorable to large MW EPS secretion by microorganisms.
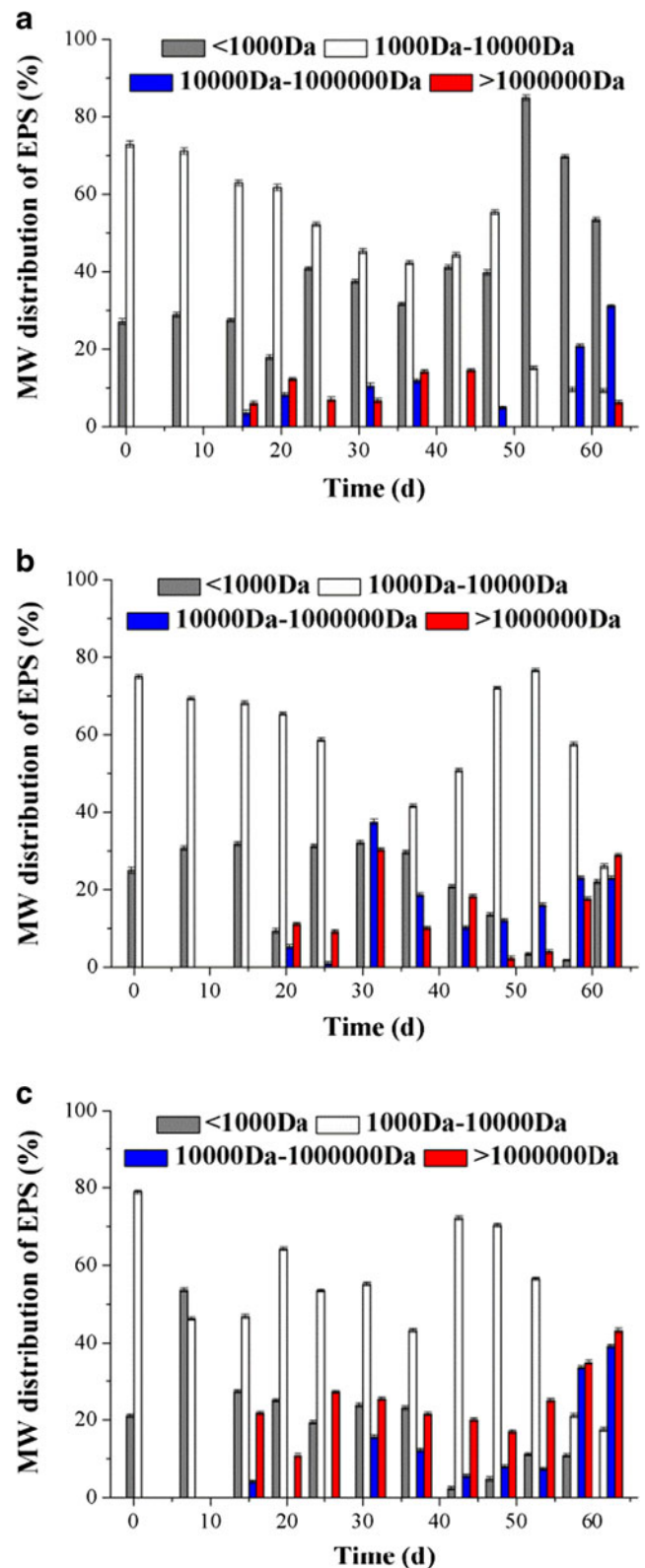
EPS with medium and small MW (<10,000 Da) dominated the three reactors, especially in M1. The EPS proportion

within this MW range dropped to 73.96 % on day 37 and then rose gradually to nearly 80 % on the last days of operation. However, the proportion of EPS with medium MW (1000–10, 000 Da) in M1 decreased from 72.92 % since day 1. On the other hand, the amount of small MW EPS (<1000 Da) was decreasing throughout the whole operation.

Variation of relative AI-2 content

According to Fig. 5, The AI-2 concentration of three reactors was different from one another. On day 16, an elevation of AI-2 concentration was detected in M1, M2, and M3 from the original value of 0.32 OD_{490}/g VSS. After that, the AI-2 content of M2 continued to ascend steadily and achieved 1.70 OD_{490}/g VSS at the end of the operation. Comparatively, M3 displayed the most intense upsurge of AI-2 content, hitting 1.50 OD_{490}/g VSS on day 16. After that, the value slightly decreased but rebounded to 1.53 OD_{490}/g VSS on the last day. It was worth noticing that although AI-2 level of M2 was lower than that of M3, it kept climbing during the whole operation and exceeded M3 since day 38. Different from the AI-2 variation observed in M2 and M3, the AI-2 content in M1 ascended to 0.99 OD_{490}/g VSS on day 16 and fell to 0.44 OD_{490}/g VSS on day 36. Then, the value increased to 1.10 OD_{490}/g VSS on day 54.

Microbial communities

The microbial evolution of M1, M2, and M3 was detected during the whole operation. Judging from the DGGE patterns, microbes in three reactors all experienced diversity expansion (Fig. S2). The sequence analysis results are listed in Table 2 and detected bacteria can be classified into three groups: *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* at the genus level.
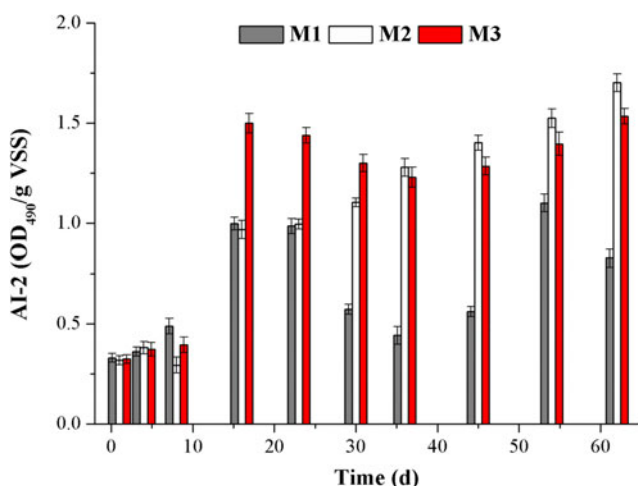

Fig. 5 The QS ability of sludge in M1, M2, and M3

Species like *Paracoccus* sp. (bands 7 and 8) and *Rhodobacter* sp. (band 9) were prevalent in three reactors. They existed throughout the whole operation of M1, M2, and M3, which suggested their dominant roles in aerobic granulation. They all belong to *Proteobacteria* which has been found essential in biodegradation of organic pollutants and carbon cycling, as well as various biogeochemical processes in aquatic ecosystems (Cheng et al. 2014). *Azoarcus* sp. (band 2) and *Alpha proteobacterium* (band 3) only existed in the beginning stages of M1, M2, and M3. It could be inferred that these two strains were dominant at first in the reactors, whereas they lost prevalence and were finally eliminated from the reactors.

Several species were only detected in the latter period of operation such as *Sphingobacterium* sp. (band 18), *Bacterium rJ7* (band 17) and *Ornithinibacter aureus* strain (band 12). *Sphingobacterium* sp. has been reported to play an important role in the flocculation of microorganisms, which is the prerequisite of aerobic granulation (Jin et al. 2003). *Ornithinibacter* sp. was reported able to secrete EPS (Xiao et al. 2011). Similarly, the reported isolates from phylum *Verrucomicrobia* were detected on day 45 in M1 and M3, which could utilize various carbon compounds (Zhang et al. 2014). On the other hand, *Rhizobium* sp. (band 15) and *Arthrobacter* sp. (band 16) were detected in M2 and M3 on day 60. The previous was reported able to produce large amounts of EPS, as well as to promote microalgae growth and lipid secretion (Zhou et al. 2015). The latter was discovered to have a strong capacity for phosphate removal (Yi et al. 2008). The existence of these strains contributed to the rise of cell adhesiveness and EPS secretion of sludge, which stimulated aerobic granulation in three reactors.

Discussion

This study gave a comprehensive view of correlation between starvation time and aerobic granulation in the perspective of interspecies quorum sensing. The AI-2 level variation in one SBR cycle (Fig. 1) indicated that starvation period was correlated with QS ability of sludge, which varied a lot in different nutrient status. In more than 60 days' operation, alternating OLR strategy was used to promote the cultivation of aerobic granules, which has been studied in the previous work (Sun et al. 2016). Although alternating OLR had impact on AI-2 level, the discrepancies between M1, M2, and M3 were mainly caused by different starvation periods since the change of influent was synchronous in three reactors. Judging from AI-2 results of different starvation periods, long starvation period may also promote AI-2 production persistently. During the process of aerobic granulation, microbes have to secrete more AI-2 to survive the starvation

Table 2 Sequence analysis of bands excised from the DGGE gel of M1, M2, and M3

Band ID	Accession no.	Closest relative	Taxonomic group	Similarity (%)
1	KT367732	*Leucobacter* sp.	*Actinobacteria*	93
2	KT367733	*Azoarcus* sp.	*Proteobacteria*	95
3	KT367734	*Alpha proteobacterium*	*Proteobacteria*	97
4	KT367736	*Paracoccus* sp.	*Proteobacteria*	99
5	KT367737	*Rhodobacter* sp.	*Proteobacteria*	96
6	KT367739	*Brevundimonas* sp.	*Proteobacteria*	96
7	KT367741	*Paracoccus aminovorans partial 16S rRNA* gene	*Proteobacteria*	99
8	KT367742	*Paracoccus marcusii* strain	*Proteobacteria*	96
9	KT367743	*Rhodobacter* sp.	*Proteobacteria*	99
10	KT367744	*Paracoccus kawasakiensis* gene	*Proteobacteria*	96
11	KT367745	*Roseococcus* sp.	*Proteobacteria*	94
12	KT367746	*Ornithinibacter aureus* strain	*Actinobacteria*	99
13	KT367747	*Luteolibacter* sp.	*Verrucomicrobia*	95
14	KT367749	*Luteolibacter* sp.	*Verrucomicrobia*	95
15	KT367751	*Rhizobium* sp.	*Proteobacteria*	96
16	KT367752	*Arthrobacter* sp.	*Actinobacteria*	99
17	KT367754	*Bacterium rJ7* gene	*Actinobacteria*	94
18	KT367756	*Sphingobacterium* sp.	*Proteobacteria*	99

pressure. The longer the starvation period lasts, the higher AI-2 level is needed to help bacteria resist external threats, which was previously indicated as an effective survival strategy (Galloway et al. 2010). Judging from the long-term effect of starvation period on aerobic granules, granulation tended to happen faster using shorter starvation time, while granules cultivated using 7-h starvation period took the longest time to form. A short starvation time provides a stronger hydraulic selection pressure and biomass are flushed away more frequently, both of which are beneficial to acceleration of aerobic granulation. Nevertheless, aerobic granules cultivated using 3-h starvation time lost stability in 5 days, which corresponded with previous conclusions that short starvation period had negative effect on granule stability (Liu and Tay 2008). On the other hand, aerobic granules cultivated under prolonged starvation time displayed better integrity during reactor operation.

EPS are sticky substances secreted by microbes to resist environmental pressure, which lay the basis for the spatial structural growth and maintenance of aerobic granules (Tay et al. 2001; Xiong and Liu 2012). In the study, the amount of EPS ascended in three reactors during the process when flocculent sludge was transforming into granules, which was also reported by Tan et al. (2014). Therefore, the EPS content was positively correlated with AI-2 level. Moreover, long starvation period was more favorable to the secretion of large MW EPS which was suggested as the prerequisite of granulation (Garnier et al. 2005). The results of MW distribution indicated that the ascending trend of AI-2 was also consistent with the increasing amount of large MW EPS.

On the other hand, although M3 had the longest starvation period and produced higher level of AI-2, it was observed that the total EPS amount in M2 was larger than that of M3 between days 20 to 50. Since EPS is both the carbon source and energy source of cells during starvation period, microbes have to consume the EPS they have produced after being starved for a long time. Wang et al. (2006) discovered that about 50 % EPS was consumed during starvation period which was almost equal to EPS produced during feast period. That is the reason why M3 had less EPS than M2 though it had longer starvation time. According to the results obtained in this study, the granules cultivated using 5-h starvation length were more stable than those cultivated in the other two reactors. Since nonbiodegradable EPS mainly existed on the outer shell of aerobic granules while degradable EPS were most detected in the inner layers of granules (Wang et al. 2007), the consumption of degradable EPS might lead to instability of M3 granules. Therefore, if the starvation period is too long, it may cause negative effect on granulation performance.

Cell adhesiveness was also found correlated with starvation period. Sludge cultivated using prolonged starvation period was more likely to develop higher cell adhesiveness. It is assumed that higher bacteria adhesiveness was more favorable to granulation due to stronger adhesion ability (Tsuneda et al. 2003). Besides, long starvation period might result in EPS consumption as stated above. This may account for the decrease of AI-2 level in M3, lower than that in M2. Therefore,

the AI-2 content of three reactors was positively related to cell adhesiveness and EPS characterization during the stage of steady operation. So it can be inferred that bacteria strengthen cell adhesiveness by secreting AI-2 and sensing its content variation, which protects bacteria against starvation pressure by triggering the production of large quantity of EPS with large MW. Therefore, starvation could promote aerobic granulation by enhancing bacterial adhesion and microbial aggregation.

In conclusion, starvation could promote QS ability of aerobic granules. Since short starvation period could accelerate granulation while long starvation period was favorable to granule stability. Therefore, a combination of different starvation periods can be adopted in order to achieve aerobic granulation effectively. More specifically, short starvation period could be adopted at the beginning of aerobic granulation to accelerate formation of granules, while starvation period can be prolonged to enhance granule stability.

Compliance with ethical standards This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no competing interests.

References

Adav SS, Lee DJ, Show KY, Tay JH (2008) Aerobic granular sludge: recent advances. Biotechnol Adv 26:411–423

APHA (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington DC

Cheng W, Zhang J, Wang Z, Wang M, Xie S (2014) Bacterial communities in sediments of a drinking water reservoir. Ann Microbiol 64:875–878

Eberl L (1999) N-acyl homoserinelactone-mediated gene regulation in gram-negative bacteria. Syst Appl Microbiol 22:493–506

Galloway WR, Hodgkinson JT, Bowden SD, Welch M, Spring DR (2010) Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and AI-2 quorum sensing pathways. Chem Rev 111:28–67

Gao D, Liu L, Wu WM (2011) Comparison of four enhancement strategies for aerobic granulation in sequencing batch reactors. J Hazard Mater 186:320–327

Garnier C, Görner T, Lartiges BS, Abdelouhab S, De Donato P (2005) Characterization of activated sludge exopolymers from various origins: a combined size-exclusion chromatography and infrared microscopy study. Water Res 39:3044–3054

Han X, Lu C (2009) Detection of autoinducer-2 and analysis of the profile of *luxS* and *pfs* transcription in *Streptococcus suis* serotype 2. Curr Microbiol 58:146–152

Herbert D, Phipps P, Strange R (1971) Chemical analysis of microbial cells. Meth Microbiol 5:209–344

Jiang B, Liu Y (2013) Dependence of structure stability and integrity of aerobic granules on ATP and cell communication. Appl Microbiol Biotechnol 97:5105–5112

Jin H, Lee NK, Shin MK, Kim SK, Kaplan DL, Lee JW (2003) Production of gellan gum by *Sphingomonas paucimobilis* NK2000 with soybean pomace. Biochem Eng J 16:357–360

Lee DJ, Chen YY, Show KY, Whiteley CG, Tay JH (2010) Advances in aerobic granule formation and granule stability in the course of storage and reactor operation. Biotechnol Adv 28:919–934

Lesprit P, Faurisson F, Join-Lambert O, Roudot-Thoraval F, Foglino M, Vissuzaine C, Carbon C (2003) Role of the quorum-sensing system in experimental pneumonia due to *Pseudomonas aeruginosa* in rats. Am J Respir Crit Care Med 167:1478–1482

Liu YQ, Tay JH (2007) Characteristics and stability of aerobic granules cultivated with different starvation time. Appl Microbiol Biotechnol 75:205–210

Liu YQ, Tay JH (2008) Influence of starvation time on formation and stability of aerobic granules in sequencing batch reactors. Bioresour Technol 99:980–985

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Lv J, Wang Y, Zhong C, Li Y, Hao W, Zhu J (2014) The effect of quorum sensing and extracellular proteins on the microbial attachment of aerobic granular activated sludge. Bioresour Technol 152:53–58

Mosquera-Corral A, De Kreuk M, Heijnen J, Van Loosdrecht M (2005) Effects of oxygen concentration on N-removal in an aerobic granular sludge reactor. Water Res 39:2676–2686

Pijuan M, Werner U, Yuan Z (2009) Effect of long term anaerobic and intermittent anaerobic/aerobic starvation on aerobic granules. Water Res 43:3622–3632

Sun S, Liu X, Ma B, Wan C, Lee DJ (2016) The role of autoinducer-2 in aerobic granulation using alternating feed loadings strategy. Bioresour Technol 201:58–64

Tan CH, Koh KS, Xie C, Tay M, Zhou Y, Williams R, Ng WJ, Rice SA, Kjelleberg S (2014) The role of quorum sensing signalling in EPS production and the assembly of a sludge community into aerobic granules. ISME J 8:1186–1197

Tay JH, Liu QS, Liu Y (2001) The effects of shear force on the formation, structure and metabolism of aerobic granules. Appl Microbiol Biotechnol 57:227–233

Tsuneda S, Aikawa H, Hayashi H, Yuasa A, Hirata A (2003) Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. FEMS Microbiol Lett 223:287–292

Wan C, Zhang P, Lee DJ, Yang X, Liu X, Sun S, Pan X (2013) Disintegration of aerobic granules: role of second messenger cyclic di-GMP. Bioresour Technol 146:330–335

Wang F, Yang FL, Zhang XW, Liu YH, Zhang HM, Zhou J (2005) Effects of cycle time on properties of aerobic granules in sequencing batch airlift reactors. World J Microbiol Biotechnol 21:1379–1384

Wang Z, Liu L, Yao J, Cai W (2006) Effects of extracellular polymeric substances on aerobic granulation in sequencing batch reactors. Chemosphere 63:1728–1735

Wang ZW, Liu Y, Tay JH (2007) Biodegradability of extracellular polymeric substances produced by aerobic granules. Appl Microbiol Biotechnol 74:462–466

Xiao C, Huang H, Ye J, Wu X, Zhu J, Zhan B, Bao S (2011) *Ornithinibacter aureus* gen. nov., sp. nov., a novel member of the family Intrasporangiaceae. Int J Syst Evol Microbiol 61:659–664

Xiong Y, Liu Y (2012) Essential roles of eDNA and AI-2 in aerobic granulation in sequencing batch reactors operated at different settling times. Appl Microbiol Biotechnol 93:2645–2651

Yan M, Korshin G, Wang D, Cai Z (2012) Characterization of dissolved organic matter using high-performance liquid chromatography (HPLC)-size exclusion chromatography (SEC) with a multiple wavelength absorbance detector. Chemosphere 87:879–885

Yi Y, Huang W, Ge Y (2008) Exopolysaccharide: a novel important factor in the microbial dissolution of tricalcium phosphate. World J Microbiol Biotechnol 24:1059–1065

Zhang SH, Yu X, Guo F, Wu ZY (2011) Effect of interspecies quorum sensing on the formation of aerobic granular sludge. Water Sci Technol 64:1284–1290

Zhang J, Zhang X, Liu Y, Xie S, Liu Y (2014) Bacterioplankton communities in a high-altitude freshwater wetland. Ann Microbiol 64: 1405–1411

Zhou D, Li Y, Yang Y, Wang Y, Zhang C, Wang D (2015) Granulation, control of bacterial contamination, and enhanced lipid accumulation by driving nutrient starvation in coupled wastewater treatment and *Chlorella regularis* cultivation. Appl Microbiol Biotechnol 99: 1531–1541