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Comparison of four enhancement strategies for aerobic granulation in sequencing batch reactors

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ABSTRACT

Aerobic granules were developed in four identical sequencing batch reactors (SBRs) with synthetic wastewater to compare different strategies for the enhancement of granulation. The SBRs were operated by (a) increasing organic loading rate in R1; (b) reducing settling time in R2; (c) extending starvation period in R3; and (d) increasing shear force in R4. The results showed that four operational strategies were able to enhance aerobic granulation successfully in SBR, but that also showed different effect on the granulation process and characteristics of mature aerobic granules. The rapidest granulation was observed by using short settling time (R2) and the granules had higher extracellular polymeric substance (EPS) than other reactors. Extended starvation period (R3) and high shear force (R4) resulted in longer granulation period and the granules with higher integrity and smaller size. Higher organic loading rate (R1) resulted in the granules with larger size and higher *K* value. The maximum specific COD removal rates (q_{max}) of the granules in all SBRs were at a similar level (0.13–0.16 g COD/h-g VSS) but the granules in R1 and R2 had higher apparent half rate constant (*K*) of 18 and 16 mg/L, than those in R3 and R4 (2.8 and 3.3 mg/L).

1. Introduction

Aerobic granulation technology is proposed as a new and promising alternative approach of activated sludge process [1,2]. Compared to flocculent sludge, aerobic granules have good settling ability, high biomass retention, ability to withstand high-strength wastewater and shock load, and simultaneous nitrification–denitrification [3–5]. These advantages indicate that aerobic granular technology has great potential for the treatment of various municipal and industrial wastewaters.

Aerobic granulation has been mainly achieved in SBR and strongly related to operating conditions, which should be favorable for microorganisms to form aggregates and/or granular particles [6]. To date, published results indicate that four operational factors have significant influence on granulation process in SBR, including (a) control of organic loading rate, (b) settling time, (c) starvation period, and (d) shear force by aeration [7–10]. Control of organic loading rate to certain levels could be favorable for granulation, and the rate could be adjusted between famine and feast status in substrate to provide a driving force for aggregation or granulation [5,11]. In SBR the settling time is likely to exert a selection pressure to control the biomass remained in reactor based on settling ability. Granulation can be enhanced by reducing settling time to select sludge with good settling ability and wash-out of light flocculent biomass [12,13]. It was reported that aerobic starvation selected microorganisms which secrete more extracellular polymeric substances (EPS), and a longer starvation period had a significant impact on hydrophobicity and zeta potential of biomass [14,15]. Increasing shear force caused by aeration has been tested to select heavy sludge particles, and high shear force has a positive effect on the production of polysaccharides which impact on aerobic granulation and stability of granules formed [16]. Other factors (e.g. divalent metal ions, dissolved oxygen (DO), substrate composition) than above four could also influence the granulation but may not be essential in general because some factors are wastewater-specific except for DO concentration [6,17]. Research results have proved that supplementation of Ca^{2+} and Mg^{2+} enhanced granulation and improved the settling property but aerobic granules can be formed in reactors without metal ions addition [18–20]. The impact of DO on aerobic granulation has been controversial. One research group reported that a high DO concentration enhanced granulation because even when a high shear force was supplied, aerobic granules were not formed at DO below 5 mg/L [21]. But Peng et al. reported that aerobic granules were formed at DO concentration as low as 0.7–1.0 mg/L in a SBR [22]. In addition, the granules

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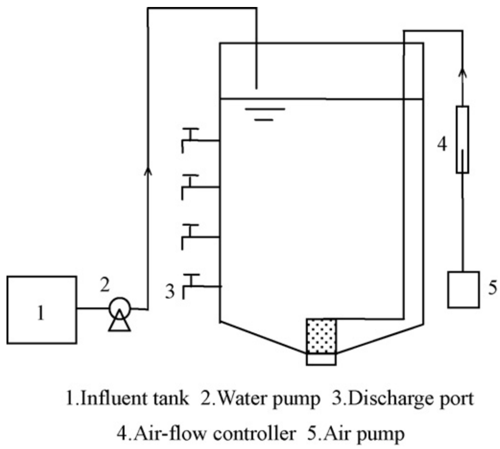

Fig. 1. The schematic diagram of SBR. (The operation procedure of four reactors was shown in Table 1.)

also could be cultivated with a wide variety of substrates including glucose, acetate, ethanol, phenol, and municipal wastewater [6].

A brief review of aerobic granulation showed that different operational strategies would lead to different characteristics of sludge, thereby affecting the performance of reactor and the cost of sludge treatment. However, to date, no research has been done to compare above four factors together to verify the extent of their influence on aerobic granulation. Thus, the purpose of this study was to find the better granulation-enhancement strategies leading to good granulation, and investigate the physical and chemical characteristics of the granules with different operational strategies. It is expected that the results derived from this study would be useful for the cultivation of aerobic granules in SBR.

2. Materials and methods

2.1. SBR system

Four identical SBRs with a working volume of 12 L were operated for aerobic granulation (Fig. 1). The temperature of the reactors was maintained at 24 ± 1 °C using a temperature controller. The reactor pH was maintained at 7.3–7.8 during operation. Air was introduced through a diffuser at reactor bottom using an air pump. The upflow shear force was adjusted by changing air flow rate.

Synthetic wastewater prepared with tapwater was used in this study with glucose as a carbon source. The tapwater contained low concentrations of temporary hardness (5.7 mg/L as $CaCO_3$) and ammonium-N (0.52 mg/L). The chemicals added in the synthetic wastewater (per liter) were: NH_4Cl, 125 mg; $NaHCO_3$, 250 mg; and KH_2PO_4, 25 mg; trace element solution, 0.1 mL. The trace element solution contained (mg/L): $MgSO_4 \cdot 7H_2O$, 20; $FeCl_3$, 15; $CuSO_4$, 30; $MnSO_4 \cdot H_2O$, 50; $CoCl_2 \cdot 6H_2O$, 50; KCl, 18; and $AlCl_3$, 15.

Activated sludge from an aeration tank of Wenchang Municipal Wastewater Treatment Plant, Harbin, China was used as inoculum. The activated sludge was aerated with air for 3 days and then inoculated to each SBR to achieve an initial concentration of approximately 3500 mg SS/L.

2.2. Operational strategy for granulation

The granulation process was investigated using four different strategies in each SBR. The reactors were operated for two sequential cycles and then maintained under resting condition till next day. The filling time (1 min) and discharging time (5 min) were the same for all reactors. The detailed operational strategies for individual SBR are described as below (Table 1).

Table 1
Operational conditions of four SBRs.

	Reactor			
	R1	R2	R3	R4
Influent COD (mg/L)	300–1000	500	500	500
COD load (g/(L-d))	0.45–1.5	0.75	0.75	0.75
Added Ca^{2+} (mg/L)	50	0	0	0
Settling time (min)	15	15 and then 1	15	15
Aeration time (min)	280	280	445	280
Total cycle time (min)	300	300	465	300
SRT (days)	15	Various	15	15
Air flow (m^3/h)	0.3	0.3	0.3	0.6

2.2.1. COD loading rate and Ca addition (R1)

The influent COD concentration of R1 was increased from 300 to 1000 mg/L over a 90-day period. In R1, Ca^{2+} (50 mg/L) was supplemented in order to enhance granulation [18,19].

2.2.2. Settling time (R2)

The settling time of R2 was 15 min at the beginning and then reduced gradually from 15 to 5 min from day 1 to day 11. Afterwards, the settling time was further shortened to 4 min on day 20, 3 min on day 28, and 2 min on day 37. The settling time was reduced to 1 min on day 45 and then maintained throughout remaining period.

2.2.3. Aerobic starvation (R3)

A long starvation time was considered to help aerobic granulation [14,15]. The total cycle time of 465 min was maintained in reactor R3 (1 min filling, 445 min aeration, 15 min settling, and 4 min discharging) and much longer than other SBR (about 300 min).

2.2.4. Shear force and DO (R4)

An air flow of 0.6 m^3/h (or an upflow velocity of 3.9 cm/s) was used in R4 and was 2 times of that in other SBRs. The DO concentration in R4 was above 5.0 mg/L and was higher than those (2.0–4.0 mg/L) in other reactors.

2.3. Analytical methods

Measurement of COD, ammonium-N, nitrate-N, nitrite-N, total phosphorus, suspended solids (SS), volatile solids (VS), and sludge volume index (SVI) were performed in accordance with Standard Methods for the Examination of Water and Wastewater [23]. The extraction of EPS from granules was performed using heating-centrifugation extraction method and the phenol-sulfuric acid method was used to quantify polysaccharides in EPS [24,25]. The concentration of protein in the extracted EPS was determined using the modified Lowry method [26]. The granule samples were taken for size distribution analysis which was conducted based on the dry weight of the granules passed through different sized wet sieves [20]. The settling velocity of sludge was measured as reported by Zheng et al. [27]. The integrity coefficient (%) was measured based on the ratio of the weight of residual granules after shaking at 300 rpm for 5 min on a platform shaker versus the weight of test sample [28].

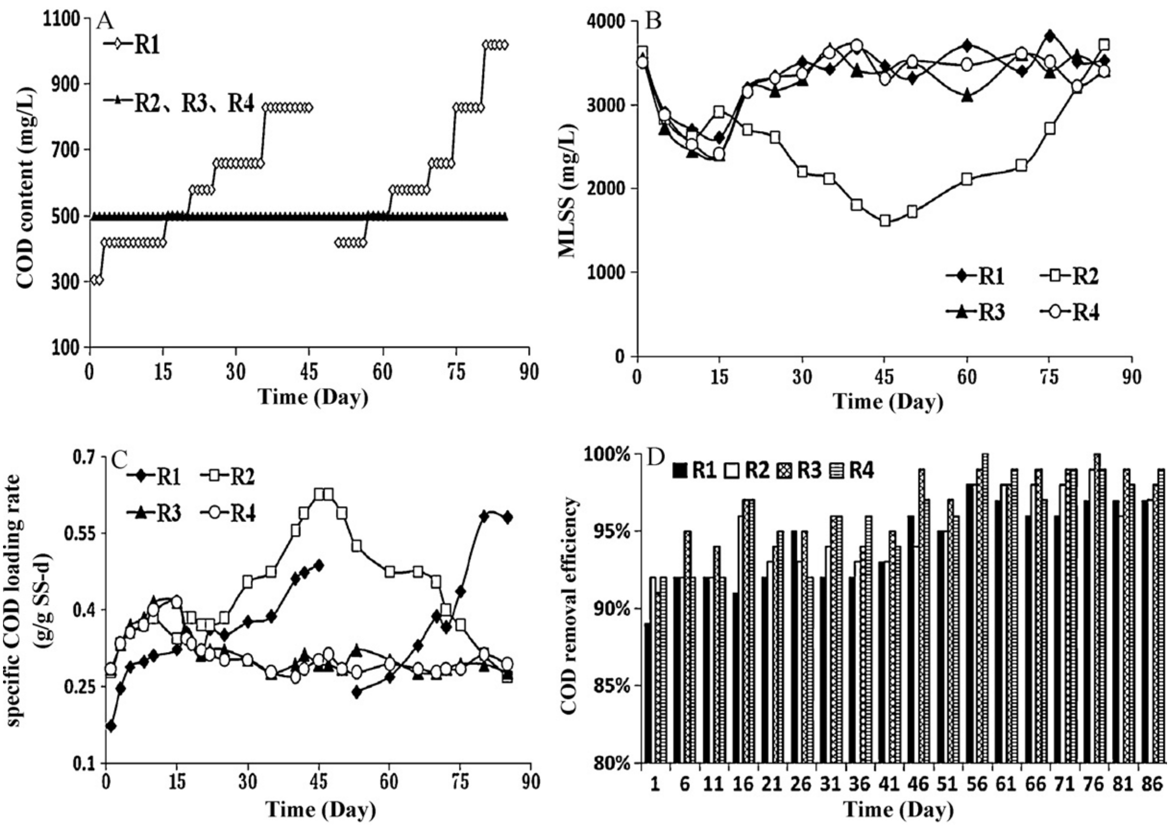

Fig. 2. (A) Changes in influent COD concentration. (B) Sludge concentration changes in R1–R4 reactors. (C) Changes in specific COD loading rates in R1, R2, R3 and R4 during granulation process. (D) COD removal efficiencies of SBRs during granulation.

3. Results and discussion

3.1. Granulation in SBR reactors

3.1.1. Granulation process with four start-up strategies

The operational results of four reactors are illustrated in Fig. 2. The granulations in the four SBRs were quite different, and the sequence of appearance of granules in SBRs was R2 (day 11), R3 (day 16), R4 (day 16) and R1 (day 21). COD loading rate in four reactors was calculated on the basis of daily COD refilled and sludge concentration. When the granules appeared in four reactors, specific COD loading rate was 0.36, 0.38, 0.41, 0.35 gCOD/gSS-d in R1, R2, R3 and R4, respectively (Fig. 2C). The rates had significantly increased from start-up date. It may be implied that a relative high specific loading rate near 0.4 gCOD/gSS-d could be favorable for the initial formation of aerobic granules. It is interesting that a similar level (greater than 0.3–0.5 g COD/g VSS-d) was observed during anaerobic granulation [29,30].

The initial COD concentration of R1 was 300 mg/L and then gradually increased (Fig. 2A). The specific COD loading rate of R1 had been lower than other reactors for more than two weeks (Fig. 2C). Granules appeared in R1 on day 21 as the rate increased to about 0.36 g COD/g VSS-d. The relatively lower specific rate in R1 than other SBRs could delay the appearance of granules in R1 as discussed above. After aerobic granules appeared, the specific rate of R1 increased continuously to about 0.48 g COD/g VSS-d but flocculent sludge remained predominant. On day 46, influent filling was stopped and R1 was aerated continuously without influent COD for 5 days. The sludge in R1 turned to granular form and the granules become predominant in R1 on day 48. It was likely that a famine status stimulated formation of granules by small granules and flocculent sludge during this period. The influent filling was restarted on day 50 with influent COD of 420 mg/L. Subsequently, the COD concentration was increased to 1000 mg/L on day 80 (Fig. 2A).

Aerobic granules appeared in R2 on day 11 when the settling time was 5 min. Afterwards, both the fraction of the granules in sludge and the size of granules increased gradually. On day 31, small-sized granules become dominated in R2. The settling time of R2 reactor was decreased further to 1 min on day 45 and then maintained the same throughout the rest period. Due to heavily washing out of flocculent sludge, the MLSS concentration in R2 reached the lowest level on day 45 (Fig. 2B) while specific COD loading rate reached 0.68 g COD/g VSS-d. Afterwards, the MLSS concentration started increasing due to the growth of granules as the specific loading rate decreased.

Aerobic granules were observed in R3 and R4 after 16 day operation. The granules with relatively small size dominated in R3 and R4 after 43 day operation. During this period, the MLSS remained unchanged as the same as specific COD loading rate (Fig. 2B and C). No obvious differences in size distribution of granules were observed in R3 and R4.

The full granulation was defined as when the difference between SVI_5 and SVI_{30} of the same sludge was less than 10%, and clear granular particles were observed [9]. The sequence of full granulation was R2 (day 54), R1 (day 70), R4 (day 72) and R3 (day 75). This demonstrated that the settling time is the most influential factor for granulation process.

3.1.2. Changes in EPS content during granulation

EPS are organic macromolecule polymer secreted by bacterial cells, and the major substances are the protein and polysaccharide [31]. The role of EPS in granulation was suggested to facilitate cells to aggregation by forming polymeric matrix [32,33]. The changes in

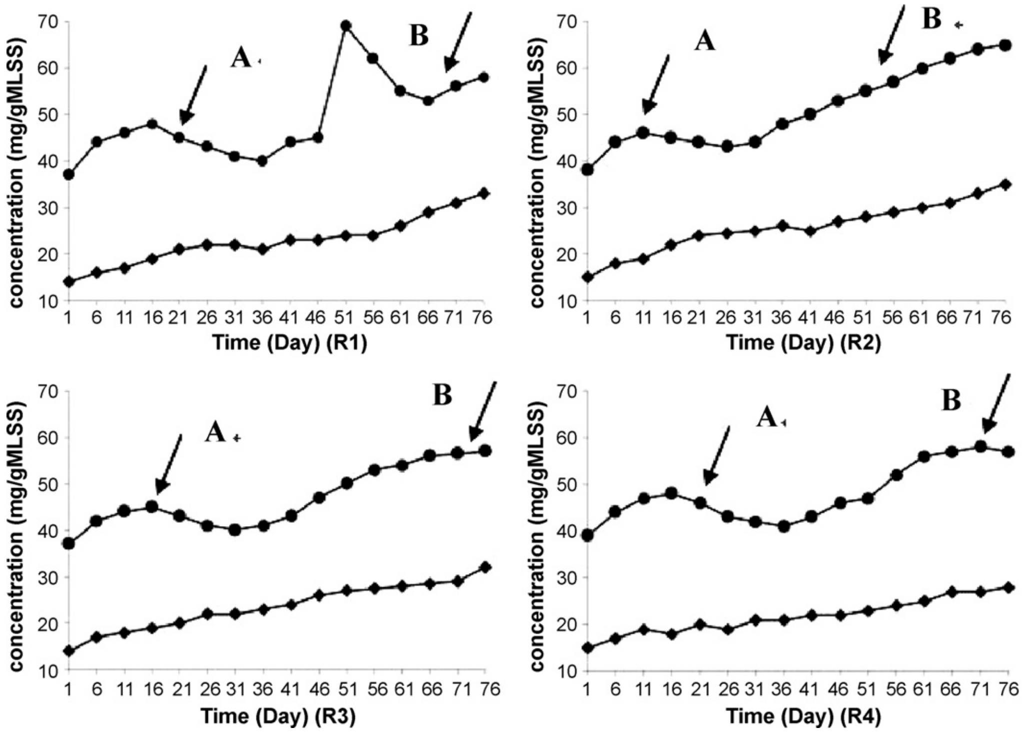

Fig. 3. Changes of EPS concentration during aerobic granulation by different control strategies. (●) Proteins in EPS; (♦) polysaccharides in EPS. (A) Aerobic granules appeared in reactor; (B) full granulation in reactor.

polysaccharide and protein contents of extracted EPS from sludge are presented in Fig. 3.

After granules appeared (A arrows in Fig. 3), the protein content declined for several weeks and then resumed increasing again. In R1, the protein content decreased from 48.2 to 40.1 mg/g SS on day 36 with a declining period 22 days but in R2 it decreased from 46.7 to 43.4 mg/g SS with a 15-day period. It is unknown why the protein content declined for several days after granules appeared. However, the increase in the protein content was likely related to the progress of granulation, especially in R2, R3 and R4. The increase in protein content in R2 was faster than R1, R3 and R4. In all reactors, high protein content was accompanied as full granulation was achieved (B arrows in Fig. 3). Liu and Tay suggested that the increase in protein content of EPS enhanced neighboring microbial cells to form a cross-linked network by attraction of organic and inorganic materials and helped granulation [6].

The change in polysaccharide content in EPS was different from that of protein content. It appeared that no distinct difference in the change in polysaccharide content during the granulation using different strategies (Fig. 3). Tay et al. proposed that the increase of polysaccharides in EPS could help microbial cells to form aggregates by bridging the cells together specially during initial microbial aggregation [34]. Research on anaerobic granules indicated that polysaccharide content was low (<4%) and unlikely plays an important role in anaerobic granulation [35]. The role of polysaccharides in aerobic granulation has remained unclear so far. In this study, it is noted that between day 46 and 50, no influent was filled in R1 as aeration continued, and the protein content in EPS had significantly increased as granulation accelerated. While there was declining of proteins in EPS after day 50, as the influent filling was restarted with influent COD of 420 mg/L. The results of EPS analysis implied that the change in protein content in EPS was closely related to the formation of aerobic granules, whereas the granulation process was less sensitive to increase in polysaccharide content and the contribution of polysaccharides to granulation was likely less significant than proteins did.

Table 2
Reactor performance after full granulation (day 95).

	Reactor			
	R1	R2	R3	R4
Influent COD (mg/L)	689	681	689	681
Effluent COD (mg/L)	29	29	7	7
Cycle time (min)	300	300	300	300
COD loading rate (g/(L-d))	1	1	1	1
COD removal (%)	95.7	97.3	98.9	99.5
Influent NH_4^+ as N (mg/L)	35.7	34.8	35.7	34.8
NH_4^+-N removal (%)	98.3	96.2	97.5	95.1
Effluent NO_3^- as N (mg/L)	0	0	0.73	0.94
Effluent NO_2^- as N (mg/L)	0.37	0.86	0.86	1.22
Total N removal (%)	97.3	94.2	93.2	90.5
Influent PO_4^{3-} as P (mg/L)	6.44	6.85	7.05	6.85
P removal (%)	99.5	97.5	99.5	96.1
MLSS (mg/L)	3464	3921	3341	3595
MLVSS (mg/L)	3048	3734	3240	3451
VSS/SS (%)	88	95	97	96
Specific rate (g COD/g VSS-d)	0.34	0.28	0.32	0.3
Sludge yield (g VSS/g COD)	0.502	0.455	0.411	0.428

Note: The COD loading rate was calculated based on two operational cycles per day.

3.2. Characterization of aerobic granules developed

3.2.1. Comparison of operational performance

After full granulation, operational data were collected to compare reactor performance (Table 2). Similar removal efficiencies for COD, NH_4^+-N and P were achieved in all reactors. The removal of NH_4^+-N was mainly due to cell synthesis and nitrification but the presence of simultaneous nitrification–denitrification (SND) reaction could not be ruled out as research on microbial community analysis of the granular sludge has indicated the presence of both nitrifying and denitrifying bacteria [36,37]. The removal of P was mainly due to cell synthesis and biomass adsorption.

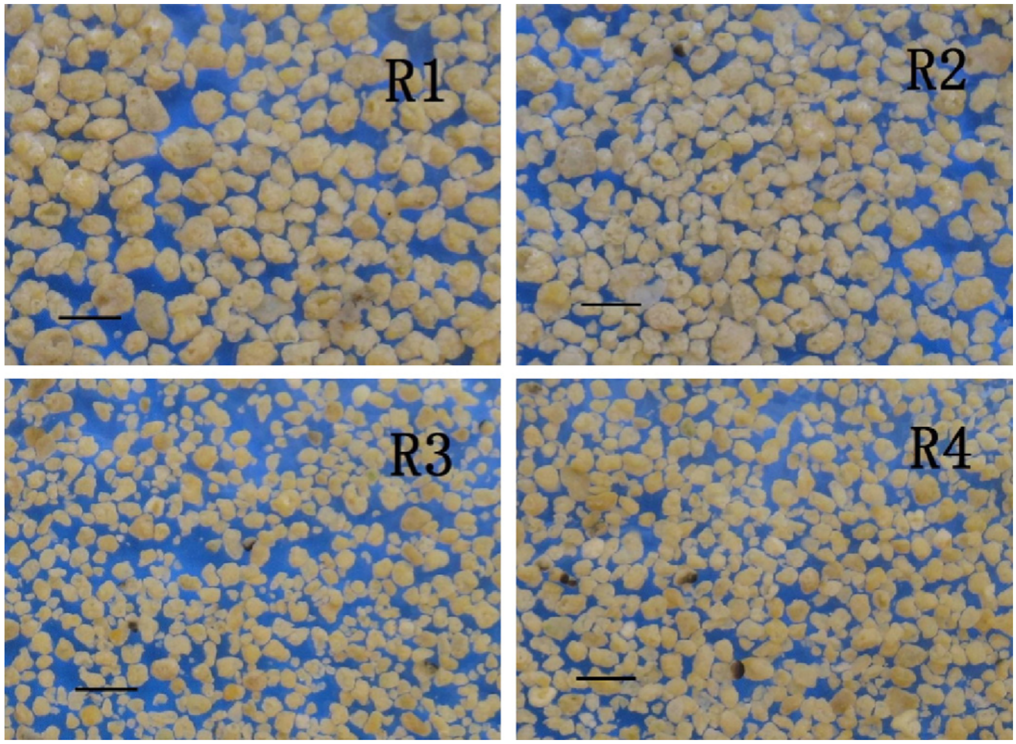

Fig. 4. Granules developed in R1, R2, R3 and R4 after 100 day operation (bar = 2 mm). The sequence of the average diameter of granules is R1 > R2 > R3 = R4.

3.2.2. Microscopic observation of granules

Aerobic granules from all reactors had a clear and regular outer shape indicated by an aspect ratio higher than 0.7 (Fig. 4). The color of aerobic granules was yellow. Microscopic indicated no significant amount of filamentous cells in the granules. This observation was similar as that observed during aerobic granulation using glucose-containing synthetic wastewater by other researchers [38]. In addition, we cultured aerobic granules with the synthetic wastewater with relatively high N/C ratio (N/C ranging from 12 to 3.5:100 mg/mg for R1 and N/C of 7:100 for R2, R3 and R4) in this study. This result suggested that the different N/C ratio in SBR may have an influence to select non-filamentous cells in the granules.

3.2.3. Size distribution of aerobic granules

Fig. 5 shows the granules size distributions. The highest volume percentage of the granules was in the range of 1.0–1.2 mm for R1, 0.8–1.0 mm for R2, R3 and R4. The granules with a size smaller than 0.2 mm contributed to less than 2% of total volume of the granules in all reactors. The size of the granules was influenced by the operational strategies used. The result indicated that a higher organic loading rate applied could stimulate formation of larger granules than a lower rate. The development of larger granules at a high loading rate was likely due to deep penetration of substrates inside granules without substrate limitation. However, smaller sized granules can be developed under a high shear conditions (R4) or with a long starvation period (R3). The high shear force causes more frequent collision among granules and/or stronger friction between granules and liquid. As results, the cells loosely grown on the surface are removed and the formation of big granules is prevented. With a longer starvation period, aerobic granules experienced longer endogenous respiration phase at slower microbial growth rate. This also limited the size of the granules as observed in the case of R3.

In addition, the pattern of size distributions of the granules in R1, R3 and R4 was similar i.e. more than 50% of the granules measured was within a narrow size range (0.8–1.0 mm in R1 and 0.5–0.8 mm in R3 and R4). However, the relative even size distribution with only 35% in 0.5–0.8 mm was observed in R2. This suggests that short settling time applied could result in even sized granules.

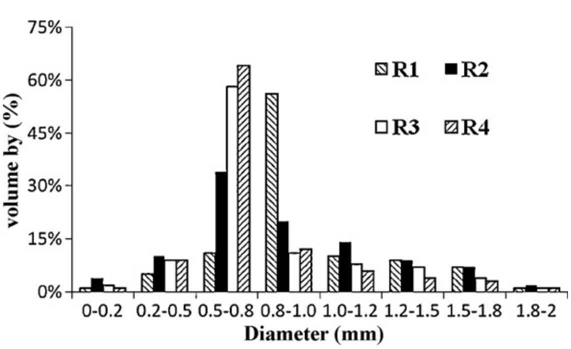

Fig. 5. Size distributions of the granules in all reactors on day 95.

3.2.4. EPS in aerobic granules developed

After full granulation, the contents of EPS, protein and polysaccharides of the granular sludge in all reactors were higher than the activated sludge inoculated (Fig. 6). The operational strategies used had influenced the EPS content and polysaccharides/protein (PS/PN) ratio. The granules in R2 had the highest EPS and protein content and followed by granules in R1. Granules in R1 had the highest polysaccharide content and PS/PN ratio. The highest EPS content of the granules may correspond to the size of granules. The influent C/N ratio (mg/mg) was 66, 55, 56, and 52 for R1, R2, R3 and R4, respectively. The granules with the highest PS/PN ratio are likely related to the influent C/N ratio. More available organic carbon source (glucose) would cause more polysaccharides formation and develop granules with larger size. The EPS content and PS/PN ratio was related to physical characteristics of the granules.

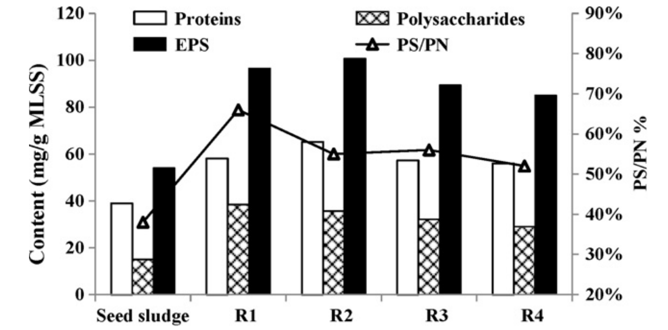

Fig. 6. EPS content and the ratio of polysaccharides/protein (PS/PN) of the granules in SBRs and inoculum activated sludge. Granule samples were taken on day 90.

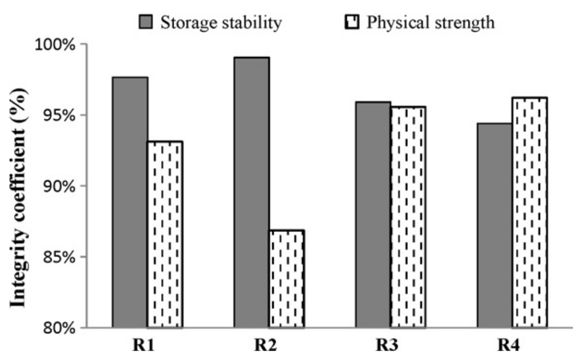

Fig. 7. Physical strength and storage stability of aerobic granules in R1–R4.

3.2.5. Physical strength and storage stability of aerobic granules

Physical strength and storage stability of aerobic granules was expressed as the integrity coefficient (%) in this study. The results of the granules from the four reactors are presented in Fig. 7. With longer starvation or higher shear force in R3 or R4, harsh hydrological conditions prevented development of loose, large-sized granules in these two reactors. Aerobic granulation occurred under selection pressure through changing microbial surface properties and metabolic behavior. Other previous studies indicated that bacterial cells increased their surface hydrophobicity under long aerobic starvation and high shear force, and cell-to-cell aggregation would occur when bacterial cell surface was highly hydrophobic [10,13]. Thus, aerobic granules developed with high shear force and long starvation time had relatively high integrity and, thereby high physical strength. In addition, it was reported that the physical strength of aerobic granules decreased with the increase in organic loading rate, and the enlarged granular size [39,40]. This is consistent with the results of the granules in R1. On the other hand, the physical strength of the granules in R1 was still much higher than those in R2. This is likely attributed to the supplementation of Ca^{2+}. Previous reports indicated that calcium-rich granules had rigid structure and high strength [17–19].

The results of storage stability test indicated that the integrity coefficient of the granules was as high as 99% for R2, and 97.7, 95.9 and 94.4% for R1, R3 and R4 respectively. This indicated that the granules had higher EPS content, especially higher protein content (R2 and R1) showed a higher storage stability than those had less content (R3 and R4). Recent report indicated that the de-granulation of aerobic granules was due to hydrolysis of extracellular proteins of stored granules by enzymes secreted by proteolytic bacteria and degradation of the hydrolyzed products by nearby anaerobic strains [41]. The granules had higher EPS content the granular structure likely lasted longer period.

3.2.6. The kinetics of substrate degradation

The substrate degradation kinetics by the granules was assumed to follow Monod kinetics (Fig. 8). q_{max} is the maximum specific substrate degradation rate, and K is apparent half rate constant (mg COD/L). It indicated that q_{max} of the granules in R1 (0.16 g COD/g VSS-h) was slightly higher than those in other reactor but was still at a similar level. However, the K values of the granules in R1 and R2 were higher than those in R3 and R4. Mass transfer resistance can significantly increase K values for microorganisms in aggregates or biofilm [42]. Research on anaerobic granular sludge indicated that substrate degradation by the granular sludge is influenced by mass

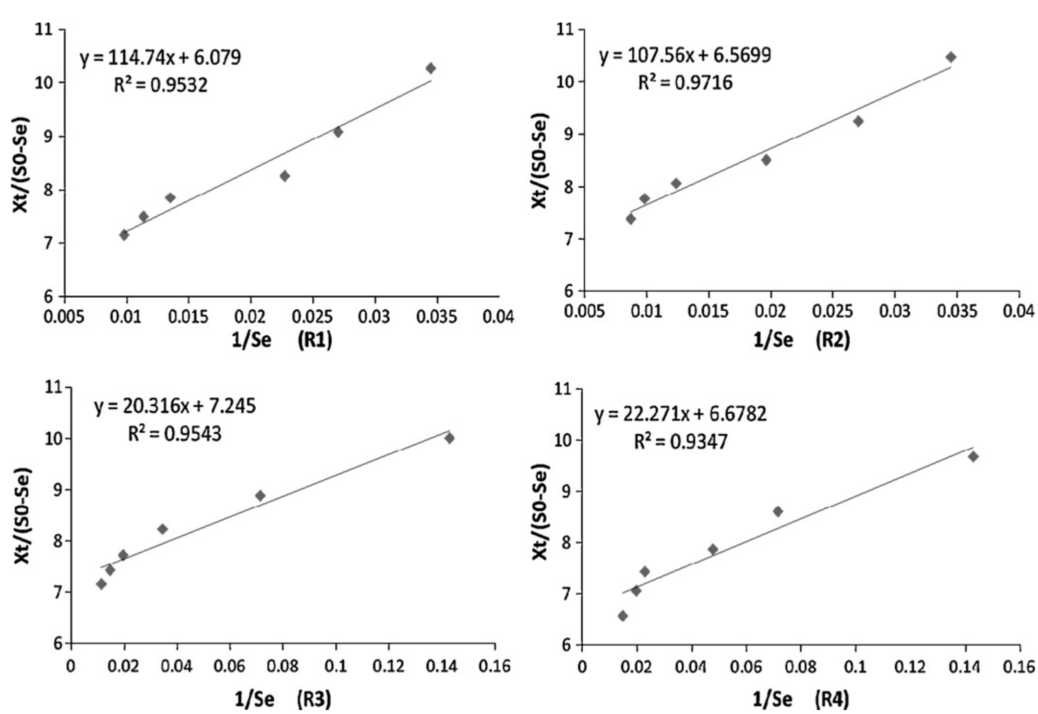

Fig. 8. The kinetics curve of substrate degradation in R1–R4.

Table 3
Characteristics of granules developed with different strategies.

	Reactor			
	R1 Loading rate + Ca[a]	R2 Settling time[a]	R3 Starvation[a]	R4 Shear force[a]
Granulation progress				
Granule appearance	Day 21	Day 11	Day 16	Day 16
Full granulation	Day 70	Day 54	Day 75	Day 72
Physical properties				
Average diameter (mm)	1.0	0.8	0.6	0.6
Physical strength (%)	93.1	86.8	95.6	96.2
Storage stability (%)	97.7	99	95.9	94.4
SVI (mL/g)	20	19	24	22
Settling velocity (m/h)	34–90	32–95	22–85	25–86
Chemical properties				
Total EPS (mg/g SS)	96.5	100.8	89.4	85.6
Proteins in EPS (mg/g SS)	58.1	65.2	57.3	56.2
Polysaccharides in EPS (mg/g SS)	38.4	35.6	32.1	29.4
PS/PN ratio (%)	66	56	55	52
Kinetics				
K (mg COD/L)	18.87	16.37	2.8	3.33
q_{max} (g COD/g VSS-h)	0.16	0.15	0.13	0.14

[a] Granulation strategy.

transfer of substrate into granules and the K value depends on both the K value of bacteria for the substrate, the thickness of biolayer and the maximum specific degradation rate of the biolayer [43]. The K values of R1 (18 mg/L) and R2 (16 mg/L) were much higher than R3 (2.8 mg/L) and R4 (3.3 mg/L) due to the size of the granules in R1 and R2 were greater than those in R3 and R4 (Fig. 5).

The effluent COD concentration of the SBR was likely controlled by the K of the granules as the q_{max} of the granules in the four reactors was at a similar level. The higher K resulted in relatively high effluent COD concentrations in R1 and R2 (Table 2). The results suggested that large-sized granules may not be considered as a target for granulation if effluent quality is considered.

4. Conclusions

Aerobic granules were successfully developed in SBR reactors by using four different strategies for granulation enhancement i.e. (a) controlling organic loading rate and supplementation of Ca^{2+}, (b) short settling time, (c) long aerobic starvation time, and (d) increased shear force. The effect of four enhancement strategies on the characteristics of aerobic granules developed in the four SBRs is summarized in Table 3.

The results of this study indicated that rapid granulation was observed by using short settling time in SBR, and the granules showed a better settling property and higher storage stability, and had higher EPS and protein content than those developed under other conditions. The granules developed by extended aerobic starvation time and enhanced shear force had small size, compact structure, higher physical strength and low K value. The granules developed under higher organic loading rate had larger particle size. In addition, aerobic granules developed under different conditions had a similar level of maximum COD removal rate but the apparent half rate constant (K) was dependent on average size of granules.

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