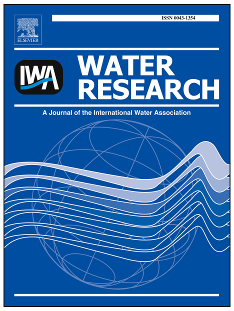

Fast formation of aerobic granules by combining strong hydraulic selection pressure with overstressed organic loading rate

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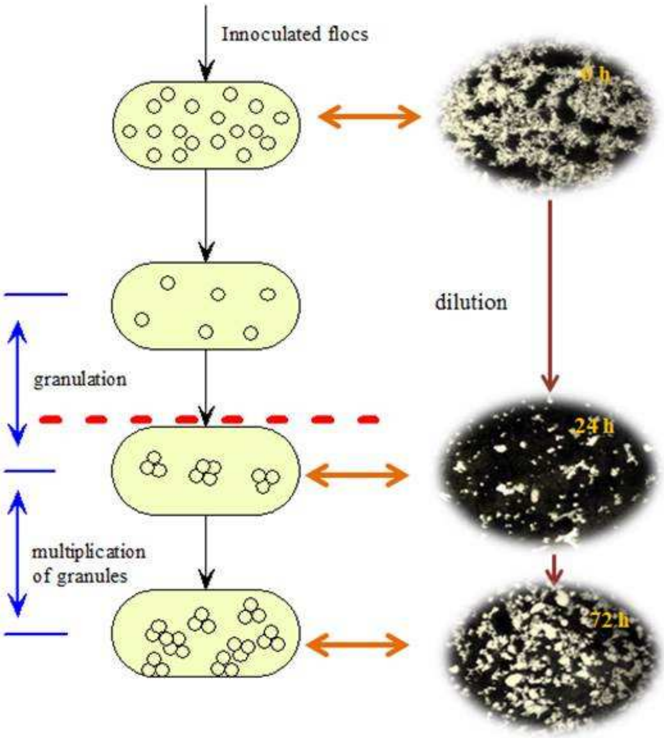
Innoculated flocs
0 h
dilution
granulation
24 h
multiplication of granules
72 h

Fast formation of aerobic granules by combining strong hydraulic selection pressure with overstressed organic loading rate

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Abstract

The combined strong hydraulic selection pressure (HSP) with overstressed organic loading rate (OLR) as a fast granulation strategy was used to enhance aerobic granulation. To investigate the wide applicability of this strategy to different scenarios and its relevant mechanism, different settling times, different inoculums, different exchange ratios, different reactor configurations, and different shear force were used in this study. It was found that clear granules with a size of 624 μm were formed at 72 h with steady state reached within three days when the fast granulation strategy was used in a lab-scale reactor seeded with well settled activated sludge (Reactor 2). However, granules appeared after 2-week operation and

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reached steady state after one month at the traditional step-wise decreased settling time from 20 to 2 min with OLR of 6 g COD/L·d (Reactor 1). With the fast granulation strategy, granules appeared within 24 h even with bulking sludge as seed to start up Reactor 3, but 6-day lag phase was observed compared with Reactor 2. Both Reactor 2 and Reactor 3 experienced sigmoidal growth curve in terms of biomass accumulation and granule size increase after granulation. In addition, the reproducible results in pilot-scale reactors (Reactor 5 and Reactor 6) with diameter of 20 cm and height/diameter ratio (H/D) of 4 further proved that reactor configuration and fluid flow pattern had no effect on the aerobic granulation when the fast granulation strategy was employed, but biomass accumulation experienced a short lag phase too in Reactor 5 and Reactor 6. Although overstressed OLR was favorable for fast granulation, it also led to the fluffy granules after around two-week operation. However, the stable 6-month operation of Reactor 3 demonstrated that the rapidly formed granules were able to maintain long-term stability by reducing OLR from 12 g COD/L·d to 6 g COD/L·d. A mechanism of fast granulation with the strategy of combined strong HSP and OLR was proposed to explain results and guide the operation with this fast strategy.

Keywords: aerobic granule; hydraulic selection pressure, organic loading rate, start-up; long-term stability; SBR

1. Introduction

Aerobic granule technology has been intensively studied almost for two decades, which has great potential in wastewater treatment due to the advantages of excellent settling ability of biomass, high biomass retention, and wide applications in treating various types of wastewater (Adav et al. 2008). However, the mechanism of aerobic granulation is still not fully understood, which impedes the optimization and application of this technology for real wastewater treatment. A distinct example is that aerobic granulation in pilot-scale reactor treating real wastewater took much longer time than in lab-scale reactor (Ni et al. 2009; Liu et al. 2010). Therefore, different strategies have been proposed to enhance the formation of aerobic granules.

Metal ions or metal were believed to be able to accelerate the start-up of aerobic granules by forming nucleus first for bacteria to attach (Jiang et al. 2003, Wang et al. 2012. Kong et al. 2014), but 16 days were still required for the formation of aerobic granules (Jiang et al., 2003). When pure culture with high self-aggregation and coaggregation ability was mixed with activated sludge to bioaugment granulation, it took 8 days to form aerobic granules with a mean diameter of 446 μm (Ivanov et al., 2006). Although bioaugmentation with specified pure culture speeds up the process of aerobic granulation, it is complex and expensive in the practice. In addition, aerobic granulation could be enhanced by seeding sludge with good settling ability such as crushed granular sludge (Verawaty et al. 2012), long-term stored aerobic granules (Liu et al. 2005), or activated sludge with excellent settling (Song et al. 2010). Adding external carrier media (Li et al. 2011, Liu et al. 2014) could also be helpful to provide nucleus for bacteria to attach just like the initial formation stage of biofilm. Although all of these abovementioned strategies could speed up aerobic granulation in a varied degree, granulation was still not improved drastically. Furthermore, all of these strategies require external auxiliary tools which increase the operational cost of aerobic granule reactor. Therefore, applying a simple operational approach to enhance aerobic granulation is desirable and promising for real application.

A great deal of effort has been put into enhancing granulation by just manipulating operational conditions. For example, by directly using 5-min settling time rather than step-wise decreased settling time to start up reactor, Qin et al. (2004) observed aerobic granules on day 7, which was much faster than others with step-wise decreased settling time. Alternating feed loading rate was also believed to be able to stimulate more EPS secretion for an enhanced aerobic granulation (Yang et al. 2014). Zhang et al. (2013) reported that by employing combined strong hydraulic selection pressure (HSP) such as short cycle time and short settling time with high OLR e.g. 24 g COD/L·d, aerobic granules were formed within 7 hours. This strategy led to not only the fastest granulation ever reported so far with the simplest operational strategy, but also a good alternative to study the mechanism of aerobic granulation. However, the operation with the fast strategy (Zhang et al. 2013) only lasted for 12 days in a lab-scale reactor. It is not sure if rapidly formed granules with the fast strategy could be maintained stably for the long term. In addition, the effects of many factors such as inoculums, reactor configuration, exchange ratio, shear force and comparison between traditional granulation and fast granulation were not fully investigated to see if this fast strategy is applicable for wider scenarios. Therefore, this study aims to systematically investigate the fast strategy proposed by Zhang et al. 2013 for aerobic granulation as well as long-term stability of granules formed by fast strategy.

2. Materials and methods

2.1. Reactor setup and operation

4 identical lab-scale reactors (R1, R2, R3 and R4) with a working volume of 2 L and 2 pilot-scale reactors (R5 and R6) with a working volume 25 L were used to cultivate aerobic granules. Both lab-scale reactors and pilot-scale reactors were bubble columns except that a stirrer with three-layer blades at different heights were installed to provide the strong mechanical shear force in R6. The main difference of reactor configuration between lab-scale reactor and pilot-scale is that diameter of pilot scale reactor was 20 cm with reactor height/diameter (H/D) ratio of 4 while the diameter of lab-scale reactor was only 5 cm with reactor H/D ratio of 20. The detailed reactor configurations are listed in Table 1.

Fine air bubbles for aeration were supplied through an air sparger at the reactor bottom with an airflow rate of 2.4 cm/s in R1, R2, R3 and R4 while membrane air diffuser was used in R5 and R6 with an airflow rate of 1.6 cm/s. In addition, the stirrer speed in R6 was maintained at 120 rpm during the aeration period. The flow diagram of reactor operation was same as those reported by Liu and Tay (2007b).

Reactors were operated in a sequencing batch mode and effluent was discharged from the middle port of the reactor with a volumetric exchange ratio of 50-70%. Both feeding time and discharging time were set as 5 min and no aeration was supplied to reactors during the feeding period. Detailed operational conditions are listed in Table 1 for the comparison between reactors with different settling time, different inoculums, different reactor configuration and different shear force. Fresh activated sludge collected from a local municipal wastewater treatment plant was first cultivated with the same synthetic wastewater as used in R1-R3 for 4 days in a barrel. Then the acclimated activated sludge was inoculated into the reactors for start-up except R3. For reactor R3, a bulking sludge from a deteriorated reactor in the lab was used as inoculums to study the effects of inoculums on fast granulation.

Reactors R1, R2, R3, R4, R5 and R6 were operated for 60 days, 14 days, 171 days, 14 days, 10 days and 10 days, respectively. The operation of six reactors is summarized in Table 1 with the combined strong HSP and high OLR to study

i) Effects of initial settling time

ii) Effects of settling ability of seed sludge

iii) Effects of exchange ratio

iv) Effects of reactor H/D ratio and reactor diameter

v) Effects of mechanical shear force from stirrer

2.2. Medium

A synthetic wastewater with the following compositions was used as influent for cultivation of aerobic granules in the reactors: COD (sodium acetate), NH_4Cl, K_2HPO_4, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, H_3BO_3, $ZnCl_2$, $CuCl_2$, MnSO4·H_2O, $(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$, $AlCl_3$, $CoCl_2 \cdot 6H_2O$, $NiCl_2$. The COD concentrations in the influent were changed in different reactors to get different OLRs ranging from 8 g COD/L·d to 12 g COD/L·d, but COD: NH_4^+-N: P ratio was always kept at 100: 5: 1. The concentrations of other components were same as those reported by Liu and Tay (2007b).

2.3. Analytical methods

COD, sludge volume index (SVI_{30}), mixed liquor suspended sludge (MLSS), and mixed liquor suspended sludge (MLVSS) were analyzed in accordance to the standard methods (APHA, 1998). SVI_5 was measured in a similar manner as SVI_{30} by modifying the settling time from 30 minutes to 5 minutes. Average particle size was measured by a laser particle size analysis system (Malvern MasterSizer Series 2600, Malvern Instruments Ltd, Malvern, UK). The morphology of sludge was observed, and sludge photos were taken by the image analysis system (Image-Pro Plus, V4.0, Media Cybernetics) with an Olympus SZX9 microscope. Microbial compositions of granules were observed qualitatively with scanning electron microscope (SEM) (Stereosan 420, Leica Cambridge Instruments), with the same method reported by Ivanov et al. (2006).

2.4. Calculations of the specific biomass accumulation rate and the specific biomass growth rate by size

The biomass accumulation in reactor depends on both biomass growth and biomass wash-out. The specific biomass accumulation rate (d^{-1}) in the reactor can be described as $q = dx/xdt$ in the exponential phase, in which x is the biomass concentration at time t in the reactor. $s = dD/dt$ can be used to describe the biomass growth rate by size ($\mu m\ h^{-1}$) in the exponential phase, in which D is the mean size of sludge at time t.

3. Results

3.1 Effects of the step-wise decreased settling time and the fixed settling time on the formation of aerobic granules

Fig. 1 shows the sludge morphology in R1 at the step-wise decreased settling time and in R2 at the fixed settling time of 2 min. Compact granules with clear boundary appeared in 24 h in R2 while sludge was still in suspended state in R1 after 336 h. In addition, it can be seen from Fig. 2 that the sludge size increased to 624 μm after 72-h operation in R2 while the sludge size was only 230 μm even after a 336-h operation in R1. SVI_5 of the sludge in R2 maintained at lower values than that in R1 during more than a 10-day operation as shown in Fig. 2 although there was a transient rise of SVI_5 at 24 h. SVI_5 and size of sludge combined with sludge morphology are usually used to judge the formation of aerobic granules. Fig. 1 and Fig. 2 clearly show that sludge in R1 did not transform from suspended state to granule form even after 336-h operation in terms of SVI_5, sludge size and sludge morphology. Actually, small granules were observed after 2-week operation in R1 and the SVI_5 of sludge in R1 decreased to less than 100 mL/g after 18 days of cultivation (data not shown). Mean size of sludge increased greatly after the settling time was decreased gradually. It took about 4 weeks for granular sludge to be mature in R1. Since the step-wise decreased settling time was widely used in literature, the changes of SVI_5, MLVSS and sludge size in the whole process were not repeated here. In terms of granule size, sludge SVI_5 and MLVSS, reactor R2 actually reached steady state after 72 hours. To the best of our knowledge, this is the fastest time reported ever to reach a steady state in a lab-scale reactor for aerobic granules.

The specific biomass accumulation rate in the reactors and the specific biomass growth rate by size are depicted in the Fig. 3. It can be seen that the growth rate of sludge size in R2 is almost 3.0-fold of that in R1 and the specific biomass accumulation rate in R2 is 2.9-fold of that in R1 in the exponential period. In addition, no lag phase for biomass accumulation and size increase of sludge was observed in R2. Since all the other operational conditions were same except for the mode of settling time, it was thought that the evident difference of granulation speed in R1 and R2 was due to the operational strategy of settling time, which influenced the granulation speed significantly.

3.2 *Effects of inoculums and exchange ratios on the formation of aerobic granules with the combined strong HSP and overstressed OLR*

Well settled sludge is usually employed to seed reactor for granulation. In this study, well settled sludge with SVI_5 of 108 mL/g and bulking sludge with SVI_5 of 484 mL/g from a deteriorated reactor were inoculated to start up reactors R2 and R3, respectively. Since the settling ability of seed sludge to R3 was very poor and hydraulic retention time was further shortened to 2 h to increase hydraulic selection pressure, the vast majority of sludge was immediately washed out of the reactor with only around 0.5 g/L of biomass was retained in the reactor after 24 h, which was much lower than 1.61 g/L at 24 h in R2 (Fig. 2). Even in this case, however, it is quite interesting to note that small granules appeared at 24 h in R3 just like in R2 and no flocs were observed after 24 h. Since little biomass was retained in R3, SVI was not measured from 0 to 96 hr. At 96 h, SVI_5 in R3 was only 68 mL/g, which was similar to that in R2. Obviously, granules were formed very quickly in R3 too even with poorly settled inoculums containing large amount of filamentous bacteria. The big difference in terms of aerobic granulation in R2 and R3 is only the appearance of evident lag phase in R3 due to the very low biomass concentration from 24 h. As both short settling time and short cycle time were used in this study, the immediate biomass wash-out within 24 h led to the low inoculation ratio if the only retained biomass after 24 h was considered as the actual inoculated sludge, which was only 10% in R3 while 36% in R2 compared with the steady state biomass concentration. The biomass concentration change with 10% actual inoculation ratio in R3 had the exactly same sigmoidal growth curve of typical bacteria (Zwietering et al. 1990) with lag phase, exponential phase and steady phase as shown in Fig. 2. But 36% actual inoculation ratio in R2 eliminated completely lag phase with immediate exponential growth of biomass. Interestingly, granule size experienced the same changing trend with biomass concentration in R3 experiencing typical sigmoidal growth curve while in R2 having exponential growth immediately. Due to the lag phase and higher OLR in R3, the reactor reached the steady state after 240 h in terms of biomass concentration, which was one week slower than R2, but still much faster than the reports with other fast start-up strategies. In addition, it was noted that granule growth rate by size in R3 was 6.99μm/h while it was 4.67 μm/h in R2 although the specific biomass accumulation rates in two reactors were similar.

As reported before (Liu and Tay, 2007a), starvation is not a prerequisite for granulation. In this study, although aerobic granules appeared within 24 hours, effluent COD in R3 was ranging from 600 to 900 mg/L during the first 120 hours because the biomass concentration was only around 0.5 g/L. However, once the lag phase in terms of biomass concentration ended, effluent COD dropped significantly to less than 50 mg/L at 192 h. During the subsequent operation period for the long-term stability study, effluent COD in R3 was always maintained at around 50 mg/L due to high biomass concentration. For R2, effluent COD was around 50 mg/L due to relatively high biomass concentration e.g. around 2 g/L, even at the initial stage of reactor operation. This demonstrated again that microbial aggregation for granulation was independent on starvation or if COD could be fully removed. R2 with exchange ratio of 50% and R4 with exchange ratio of 70% did not present any difference (data in R4 not shown) in terms of aerobic granulation at the same OLR, which indicates that exchange ratio between 50% and 70% had no influence when the strong hydraulic selection pressure from short cycle time and short settling time was used in this study.

3.3 Effects of reactor configurations and mechanical shear force on the formation of aerobic granules with the combined strong HSP and overstressed OLR

Similar to lab-scale reactor, the vast majority of biomass in R5 and R6 was washed out in the first several cycles after start-up, and only little biomass was retained due to strong hydraulic selection pressure. However, white granules were observed after 24-h operation as shown in Fig. 4. During the first 96-h operation, biomass volume in R5 after 30-min settling was below 0.28 L. However, the newly formed granules multiplied rapidly after 96-h operation and biomass volume increased significantly, and then reached 4.8 L after 216-h operation as showed in Fig. 5. Therefore, it can be said that the low biomass concentration during the first 96 hours due to the strong hydraulic selection pressure can be increased rapidly after granules were formed in the 20-cm diameter reactor. The granulation of sludge in R5 was similar to that in R3, which indicated that reactor diameter and reactor H/D ratio did not influence granulation speed with the strong hydraulic selection pressure. However, even with the inoculation of well settled sludge in the pilot-scale reactor R5, the biomass washout was much more serious with an obvious lag phase in terms of the increase in biomass concentration than in the lab-scale reactor R2. It is well known that reactor with a diameter smaller than 10 cm has serious reactor wall effect, which could retain biomass with high density at the bottom part of the reactor to avoid wash-out at strong hydraulic selection pressure. Therefore, the lag phase in pilot-scale reactor R5 is probably due to the poor retention of biomass in the reactor with a diameter of 20 cm lacking of reactor wall effect. Fig. 6 shows the concentration of granular sludge along the reactor height in 5-cm diameter reactor and 20-cm diameter reactor. Obviously, the reactors used in this study with 5-cm diameter themselves possess the capability to retain biomass, which also play a role for hydraulic selection pressure and retain more biomass with shorter lag phase.

R6 with the stirrer had the similar granulation with R5, but fluffy granules and smooth granules coexisted in the reactor even at the very beginning i.e. 24 hours as shown in Fig. 4. This is beyond the expectation as it is commonly believed that high shear force could lead to the smaller but more compact granules (Liu et al. 2002). Compared with R5, the high share force from mechanical stirrer in R6 resulted in more fluffy granules and only fluffy granules were observed after 216 h as seen in Fig. 4. SEM photos of the granules in R5 and R6 at 240 h showed that filamentous bacteria growing from the interior of granules and coexisted with rod bacteria. Due to the overgrowth of filamentous bacteria, settling ability of granules in both R5 and R6 deteriorated leading to the biomass loss as shown in Fig. 5. The operation of

R5 and R6 were collapsed after 10-day operation. High mixed mechanical/pneumatical shear force in R6 alone was not able to maintain the stability of granules. This is likely that high shear force is not a critical factor for granule stability or high shear force alone is not enough to maintain granule stability. The results here indicate clearly that the factors which inducethe formation of aerobic granules are not same with those which could maintain the stability of the newly formed granules.

3.4 The operational strategy to maintain the long-term stability of the rapidly formed aerobic granules

Based on the aforementioned results, combined strong HSP with overstressed OLR enhanced aerobic granulation significantly. To investigate the long-term stability of these rapidly formed granules, reactor R3 started up with bulking sludge was operated more than 180 days. Although high OLR is beneficial to accumulate enough biomass in reactors and shorten time required to reach the steady state, it has been reported that high OLR led to fluffy granules and the instability of reactor operation (Zheng et al. 2006). To maintain the long-term stability of rapidly formed granules, the cycle time in R3 was extended from 1 h to 2 h to reduce the OLR from 12 to 6 g COD/L·d after 2-week operation. During the following 5.5-month operation, granules in R3 always maintained good settling ability, constant granule size and biomass concentration as shown in Fig. 7, which demonstrated that it was feasible to use 12 g COD/L·d OLR combined with strong HSP to start up reactor within 1-2 weeks and maintain the long-term operational stability of granules with reduced OLR such as 6 g COD/L·d.

4. Discussion

4.1. Fast granulation using the combined strong HSP with overstressed OLR

Aerobic granules appeared within 24 hours in all reactors with different inoculums, different exchange ratios, different reactor configurations, different shear force by using the combined aeration and mechanical stirrer, which highly underlined that the combined strong HSP with overstressed OLR are crucial factors for fast granulation. Table 2 summarizes all fast granulation strategies reported so far and the comparison between them. For a fair comparison, the time for appearance of aerobic granules and the time taken for reaching the steady state in terms of granule size and biomass concentration were used. It can be seen from Table 2 that the strategy using combined HSP and overstressed OLR in this study achieved e the fastest granulation with granules appeared within one day and steady state reached within three days.

Aerobic granulation is a process that bacteria could be self-aggregated and form compact structure with a size of 0.1-5 mm. It has been thought that the basis for aerobic granulation is a repetitive selection for sludge particles cultivated in the SBR such that denser components are retained in the system while lighter and dispersed particles are washed out (Qin et al. 2004b). Settling time and HRT controlled by cycle time and exchange ratio in sequencing batch reactor were thought to be the most important hydraulic selection pressure for aerobic granulation (Qin et al., 2004a; Wang et al., 2006). However, much biomass would be washed out and incomplete COD degradation would happen if strong hydraulic selection pressure was used e.g. settling time and HRT were too short. The operational strategy with step-wise decreased settling time is thus usually used to avoid the biomass wash-out during the start-up period for aerobic granular sludge reactor. Meanwhile, cycle time longer than 3 h is usually used to provide a periodical feast-famine phase in a single cycle, which was considered as a critical factor for the granulation (Tay et al., 2001, Li et al. 2006). Generally, 2 weeks were required to grow compact aggregates and more than one month were taken to form mature granules at step-wise decreased settling time in a lab-scale reactor. However, it can be seen very clearly from Fig. 2 that although the changing trends of biomass concentration and sludge SVI with the traditional step-wise decreased settling time at the beginning of reactor operation were similar to those in R2 and R3 during the first 24 hours, the changes in R1 during 336 h were far more mild with much smaller slopes compared with R2 and R3. To enhance aerobic granulation, these changes have to become drastically by strong HSP.

In addition, it has been reported that starvation is not prerequisite for aerobic granulation (Liu et al. 2007a). Overstressed OLR has been proved to induce more EPS production which is favorable for granulation (Yang et al. 2014). Accelerated start-up and granulation processes by the application of stressing OLR without achieving complete substrate removal was also reported in UASB (Francese et al., 1998; Show et al., 2004).Therefore, OLR of 8 and 12 g COD/L·d combined with strong hydraulic selection pressure was used for the fast start-up of aerobic granular sludge reactor in this study. High OLR is crucial not only for changing the aggregation ability of flocs by secreting more EPS or changing cell surface properties but also for the rapid biomass accumulation as shown in Fig. 3.

4.2 Uncoupled optimal conditions for fast granulation and maintaining long-term stability of granules

Although granules appeared within 24 hours in all reactors, compact granules turned into fluffy leading to the biomass washout and the collapse of reactor operation after a certain period in R5 and R6. Obviously, the optimal conditions for aerobic granulation and maintaining the long-term stability are different due to the changes of sludge size, biomass concentration and fluid mixing in reactor. In this study, OLR of 8 and 12 g COD/L·d were used for accelerating aerobic granulation. However, it has been reported that high OLR such as OLR of 8 g COD/L·d could lead to the instability of aerobic granules (Zheng et al. 2006). It was believed that a high COD loading easily led to outgrowth of filaments and unstable reactor operation (Beun et al., 1999; de Kreuk, 2005). The results by Chen et al. (2008) showed that aerobic granules could maintain a long-term stability even at the OLR of 15 g COD/L·d when high aeration rate was used. In addition, the low aeration rate such as 0.3

cm/s in famine period did not affect the long-term stability of aerobic granules (Liu and Tay, 2006). Thus, the instability of aerobic granules under high OLR might mainly come from the DO limitation in the feast period. In this study, since COD was ranging from 600-900 mg/L during the first 120 h in R3 due to low biomass concentration, DO was still as high as 6 mg/L even with OLR of 12 g COD/L·d. DO limitation in R3 with high OLR during granulation period was thus reduced by low biomass concentration. To maintain the stability of rapidly formed granules by exerting high OLR and strong HSP, increasing aeration rate or reducing OLR should be adopted after granules are formed. The cycle time of reactor R4 was extended to 2 h with OLR reduced accordingly to 6 g COD/L·d after 2-week operation. The results in R4 indicated that rapidly formed granules were able to maintain the good stability during 6-month operation period. In addition, it was thought that the rapid growth of bacteria in granules was likely to result in the instability of aerobic granules. Thus, slow-growing bacteria were selected to enhance the long-term stability of aerobic granules (de Kreuk et al., 2004). However, R3 demonstrated that granulation speed and bacteria growth rate in the start-up period does not affect the long-term stability of granules as long as the optimal conditions are used respectively. Therefore, it is very efficient to start up aerobic granular sludge reactor with the strong HSP and high OLR for the rapid granulation while reduced OLR or improved aeration rate are used to maintain the long-term stability of aerobic granules.

4.3 Mechanism of fast granulation using the combined hydraulic selection pressure with overstressed OLR

Different hypothesis has been proposed to try to explain the mechanism of aerobic granulation. However, the most hypotheses proposed were based on certain specific experiments within a certain limits, which was thus easily contradictory to the experimental data reported later with different operational conditions. For example, starvation was believed to be as the most important factor for aerobic granulation (Tay et al. 2001; Li et al. 2006), but granules were cultivated successfully without starvation (Liu et al. 2007a). Short settling time was thought to be crucial for the formation of aerobic granules (Qin et al. 2004), but aerobic granules were still formed with long settling time such as 30 min (Wan et al. 2011). In this study, various factors such as inoculums, reactor configuration, mechanical shear force, exchange ratio have been investigated with the combined strong HSP and overstressed OLR strategy for fast granulation. The successful granulation under various conditions indicates that there are common crucial factors for fast granulation and a mechanism could thus be proposed to explain this phenomenon. Fig. 8 depicted the mechanism of fast granulation with the combined strong HSP and overstressed OLR strategy. In the granulation phase, strong hydraulic selection pressure leads to slightly diluted (when seeded with well settled sludge in slim reactors) or significantly diluted (when seeded with poorly settled sludge or in a evenly mixed reactor) flocs, which experience overstressed OLR as well as strong hydraulic selection to stimulate EPS production (Yang et al. 2014) and cell surface property change for easy aggregation. Due to the strong hydraulic selection pressure, the flocs which do not aggregate to form granules are washed out leaving only granules in reactor. In the second phase, granules multiply very quickly under overstressed OLR to reach steady state. The rapid rise of biomass concentration involves the increase in both granule size and granule number, which follows the sigmoidal growth curve, a typical growth characteristic of pure bacteria. In this sense, we could consider the newly formed granules as granule inoculums to grow and multiply. Since granule cannot compete with flocs in terms of substrate utilization and biomass growth (Liu et al. 2012), the wash-out of enough flocs from reactors immediately by strong hydraulic selection pressure is a prerequisite for newly formed granules to grow exclusively. Weaker hydraulic selection pressure sill could lead to granulation, but the granule percentage in the mixed sludge would decrease with the reduction in strength of hydraulic selection pressure (Qin et al. 2004a).

5. Conclusions

The strategy with combined strong HSP and overstressed OLR to speed up aerobic granulation was applicable to wider scenarios regardless of settling ability of seed sludge, exchange ratio between 50% and70%, reactor configuration with different diameter and reactor H/D ratio, and mechanical/pneumatic shear force. Although high OLR may lead to fluffy granules and collapse of reactor operation, the rapidly formed granules with the fast start-up strategy were able to maintain long-term stability by reducing OLR to a suitable level after the steady state was reached. The results in this study indicates that the crucial conditions for aerobic granulation and maintaining long-term stability of aerobic granules are different, which could be well controlled to achieve both fast granulation and long-term stability.

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Fast formation of aerobic granules by combining strong hydraulic selection pressure with overstressed organic loading rate

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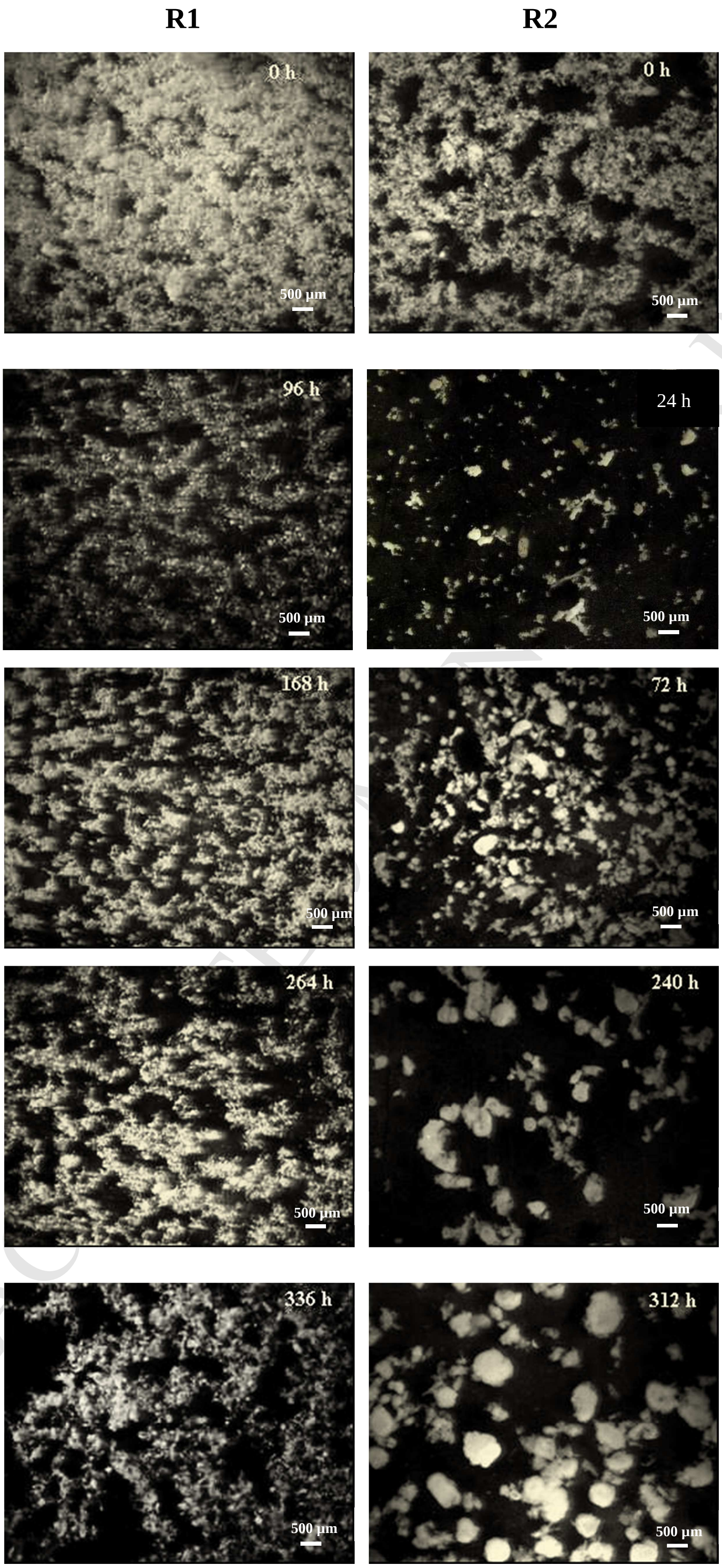


Fig. 1 Morphology of sludge in R1 and R2 in the first two weeks with a multiplication of 6.3

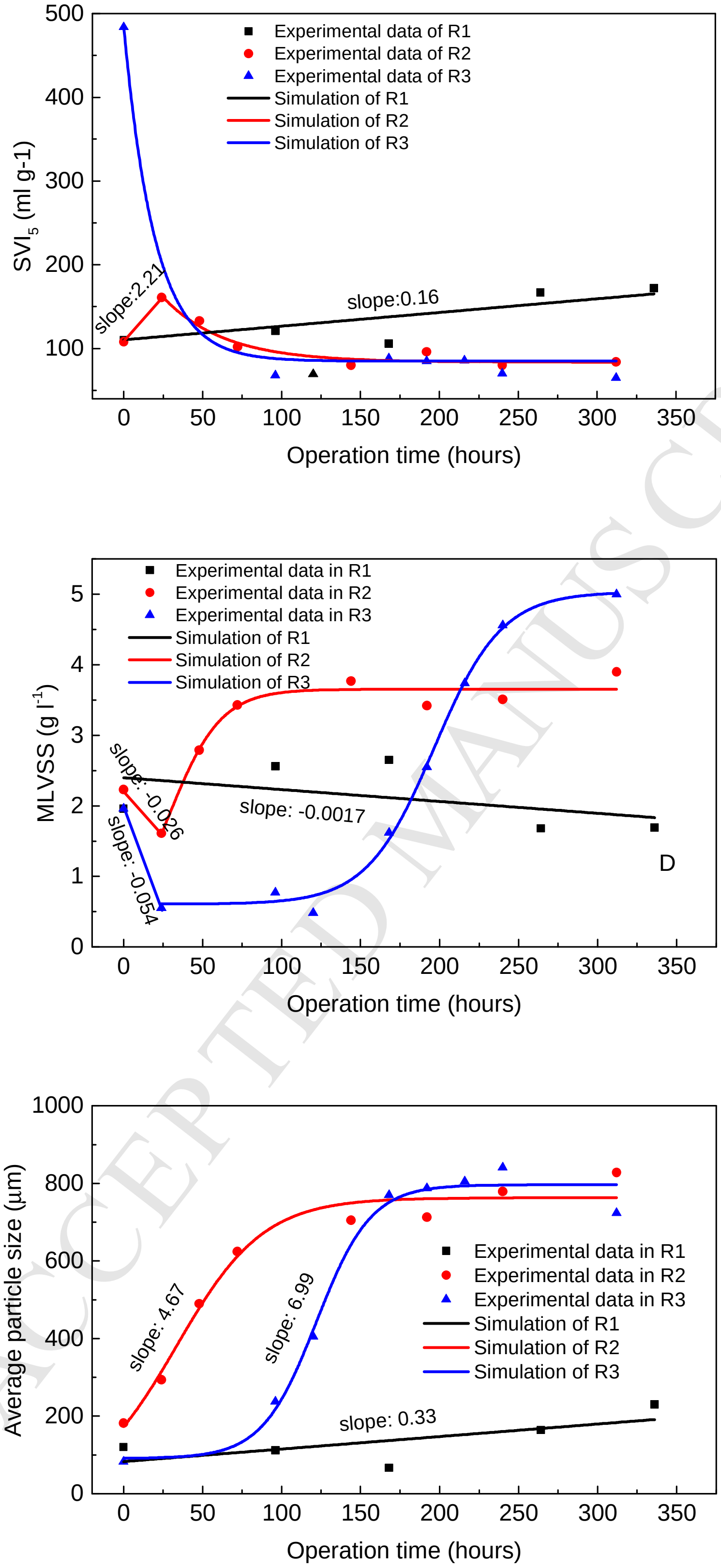


Fig. 2 Evolution of SVI5, MLVSS and average particle size of sludge in R1, R2 and R3 in the first two weeks

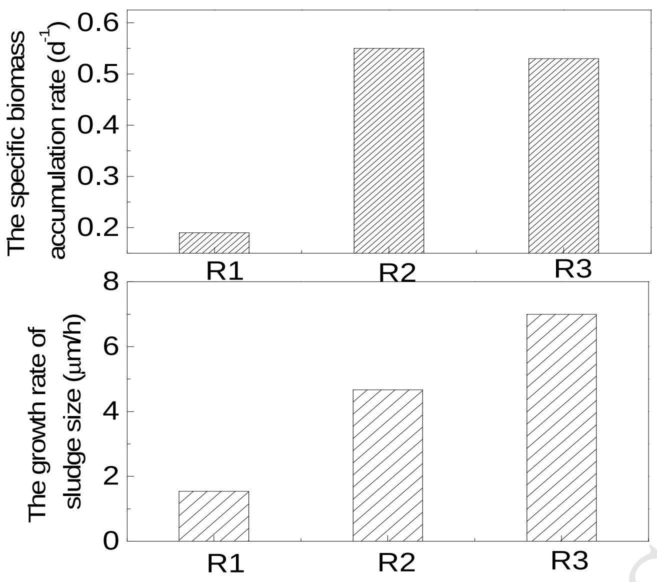


Fig. 3 The specific biomass growth rate and the particle size growth rate of sludge in R1, R2 and R3 during the exponential growth period

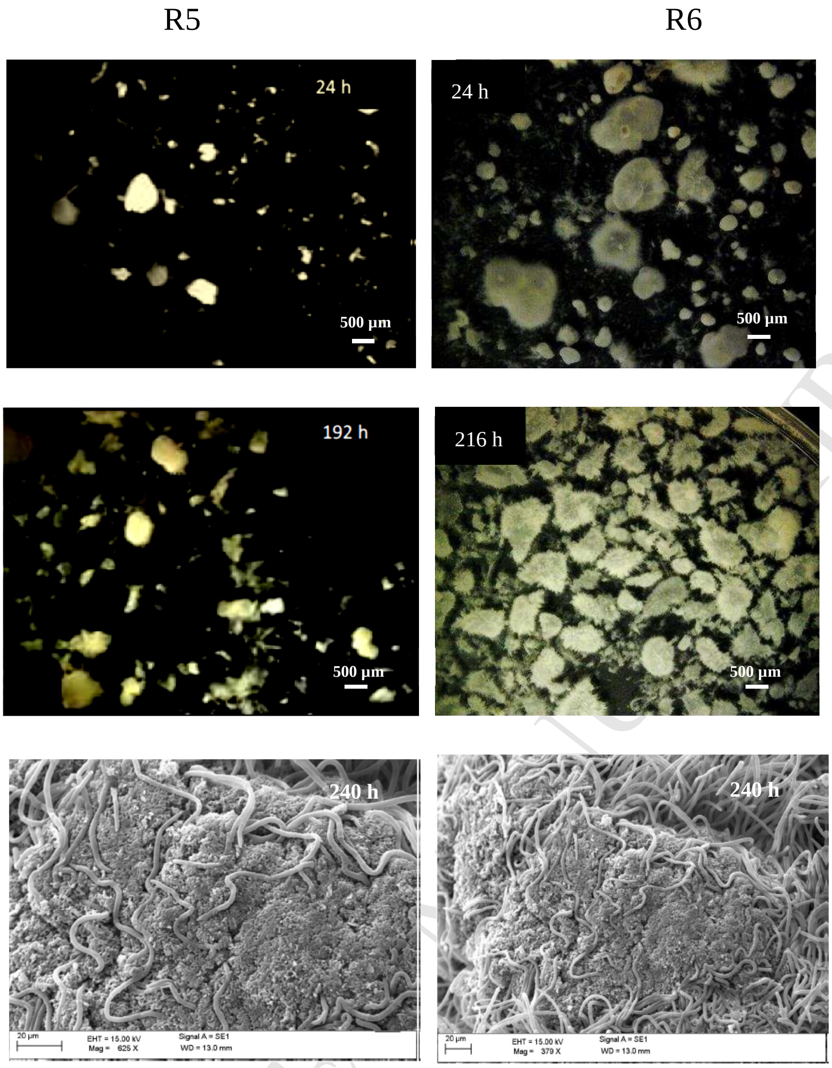


Fig. 4 Morphology of sludge in R5 and R6 in the first ten days observed by Image Analyzer and SEM

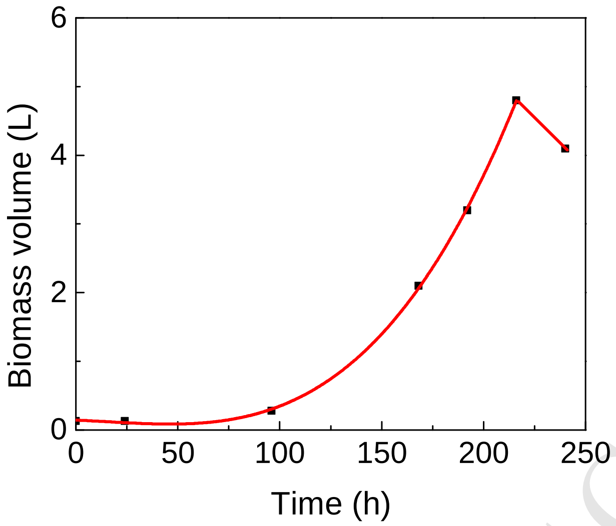


Fig. 5 Biomass accumulation in reactor R5 over operation time

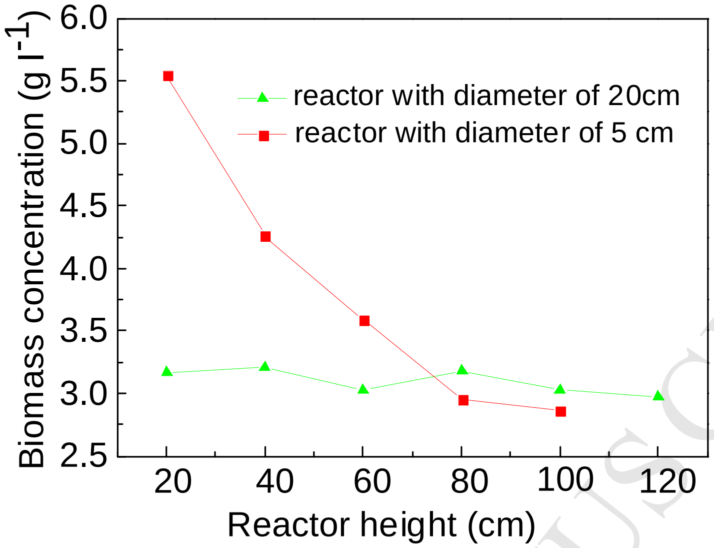


Fig. 6 Concentrations of aerobic granular sludge along reactor height in 5-cm diameter and 20-cm diameter reactors

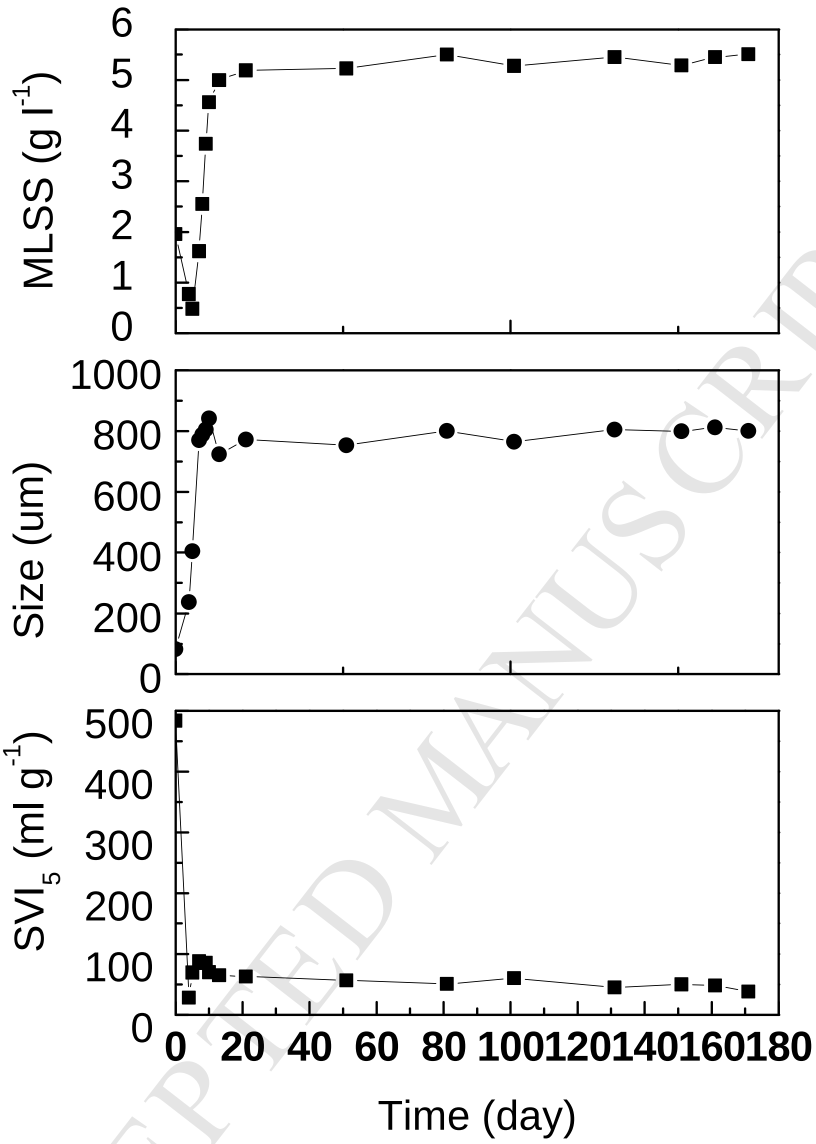


Fig. 7 Evolution of biomass concentration, average particle size and SVI$_5$ of sludge in reactor in R3 with long-term operation

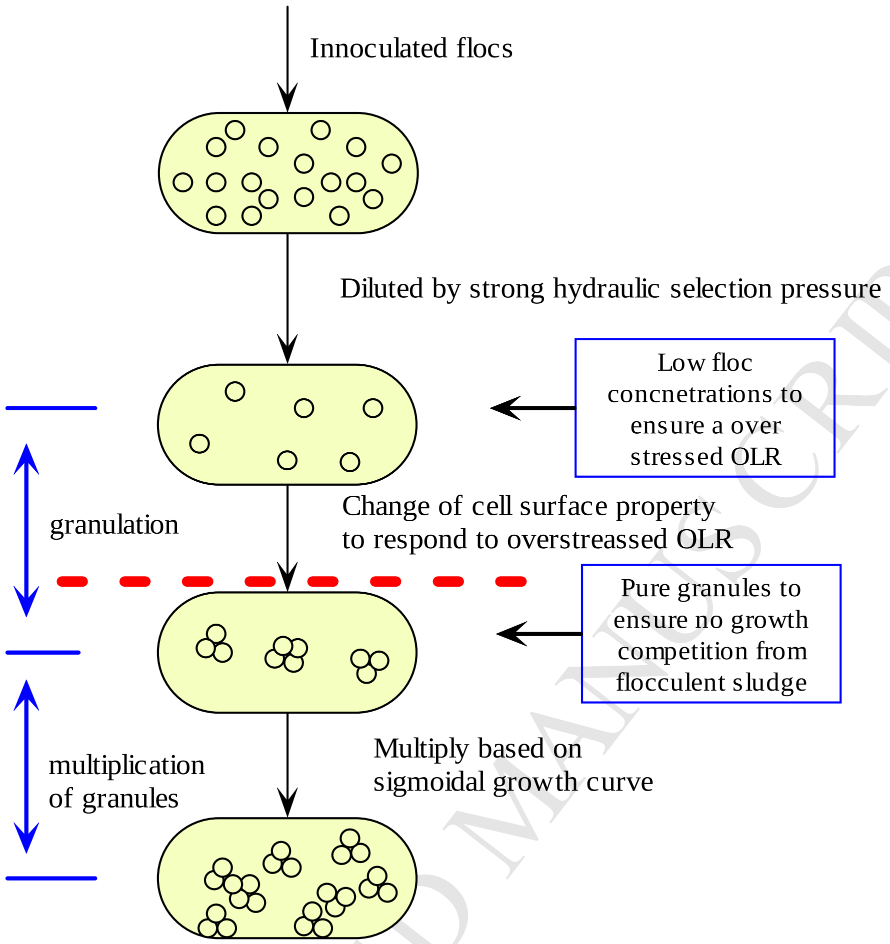


Fig. 8 Mechanism of fast aerobic granulation with the combined strong HSP and overstressed OLR

Table 1 Reactor characteristics and detailed experimental conditions of the sequencing batch reactors

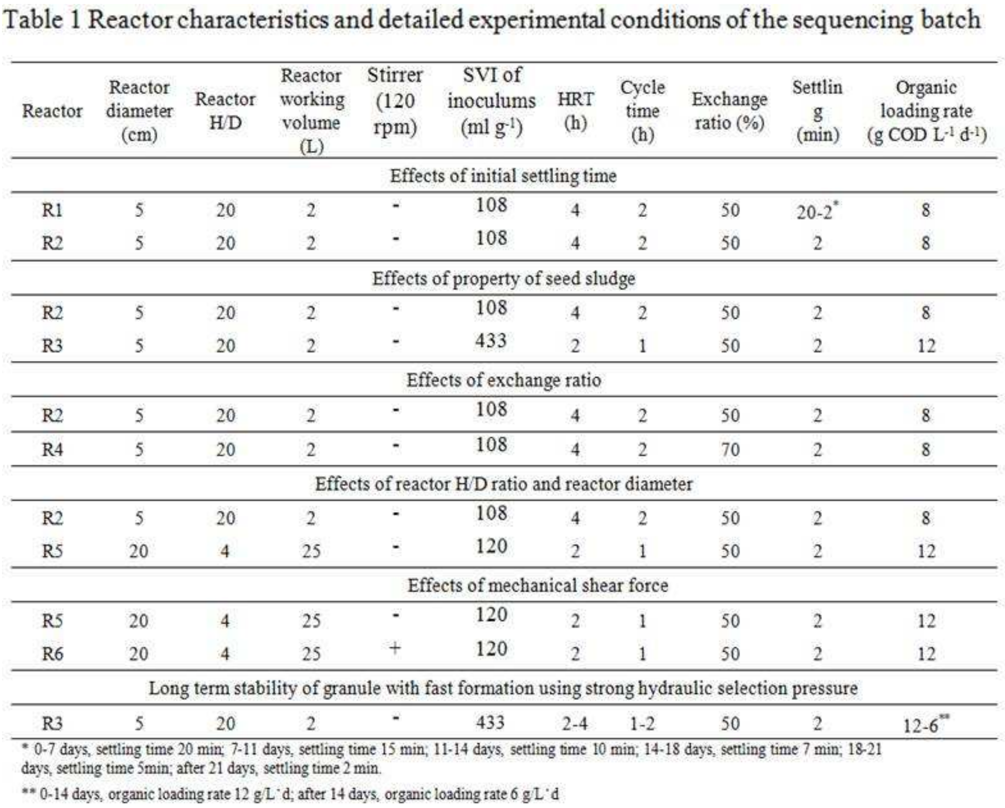
Reactor	Reactor diameter (cm)	Reactor H/D	Reactor working volume (L)	Stirrer (120 rpm)	SVI of inoculums (ml g^{-1})	HRT (h)	Cycle time (h)	Exchange ratio (%)	Settling (min)	Organic loading rate (g COD L^{-1} d^{-1})
Effects of initial settling time										
R1	5	20	2	-	108	4	2	50	20-2*	8
R2	5	20	2	-	108	4	2	50	2	8
Effects of property of seed sludge										
R2	5	20	2	-	108	4	2	50	2	8
R3	5	20	2	-	433	2	1	50	2	12
Effects of exchange ratio										
R2	5	20	2	-	108	4	2	50	2	8
R4	5	20	2	-	108	4	2	70	2	8
Effects of reactor H/D ratio and reactor diameter										
R2	5	20	2	-	108	4	2	50	2	8
R5	20	4	25	-	120	2	1	50	2	12
Effects of mechanical shear force										
R5	20	4	25	-	120	2	1	50	2	12
R6	20	4	25	+	120	2	1	50	2	12
Long term stability of granule with fast formation using strong hydraulic selection pressure										
R3	5	20	2	-	433	2-4	1-2	50	2	12-6**

* 0-7 days, settling time 20 min; 7-11 days, settling time 15 min; 11-14 days, settling time 10 min; 14-18 days, settling time 7 min; 18-21 days, settling time 5min; after 21 days, settling time 2 min.

** 0-14 days, organic loading rate 12 g/L·d; after 14 days, organic loading rate 6 g/L·d

Table 2 Comparison of aerobic granulation speed with different strategies for enhancement

Strategizes for improving aerobic granulation		Wastewater treatment	Time taken for the appearance of granules (days)	Time taken to achieve steady state (days)	References
Addition of metal ions or metal	Ca^{2+}	COD	16	50	Jiang et al. 2003
	Mg^{2+}, Al^{3+}	COD and N removal	-	43	Wang et al. 2012
	zero-valent Fe	COD and N removal	-	43	Kong et al. 2014
Bioaugmentation by pure culture/sludge with high aggregation ability	pure culture isolated from aerobic granules	COD	2	8	Ivanov et al. 2006
	crushed granules	COD and N removal	-	60	Verawaty et al. 2012
Selection of good seed sludge	Stored aerobic granules	COD	-	20	Liu et al. 2005
	sludge from beer wastewater treatment plant	-	7	35	Song et al. 2010
Addition of carrier media	granular activated carbon	Low strength COD	-	100	Li et al. 2011
	poly aluminum chloride	COD	7	30	Liu et al. 2014
Manipulation of operational conditions	Shortening settling time	COD	7	21	Qin et al. 2004
	Extending starvation period	COD	13	-	Gao et al. 2011
	Increasing OLR and Ca2+ addition	COD	21	-	Gao et al. 2011
	Alternating feed loadings	COD	8	-	Yang et al. 2014
	Combined strong HSP and overstressed OLR	COD	0.29	12	Zhang et al. 2013
	Combined strong HSP and overstressed OLR with bulking sludge as seed	COD	1	10	This study
	Combined strong HSP and overstressed OLR with well settled sludge as seed	COD	1	3	This study



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Effects of reactor H/D ratio and reactor diameter										
R2	5	20	2	-	108	4	2	50	2	8
R5	20	4	25	-	120	2	1	50	2	12
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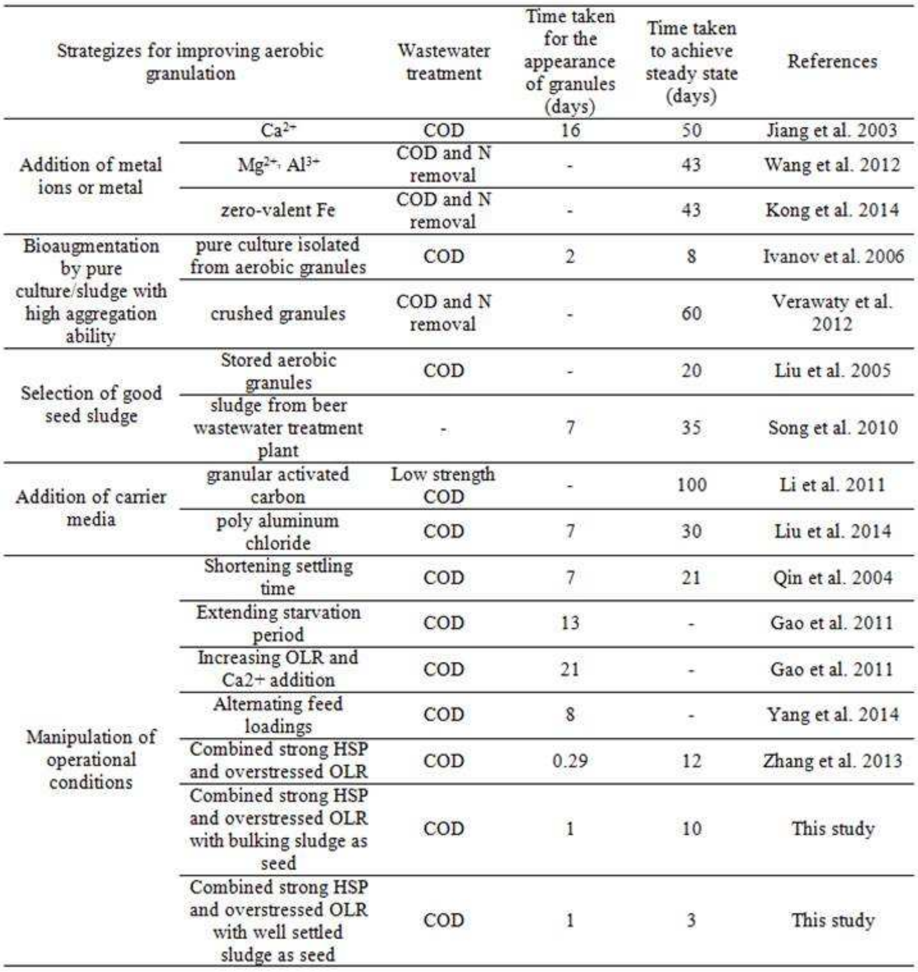

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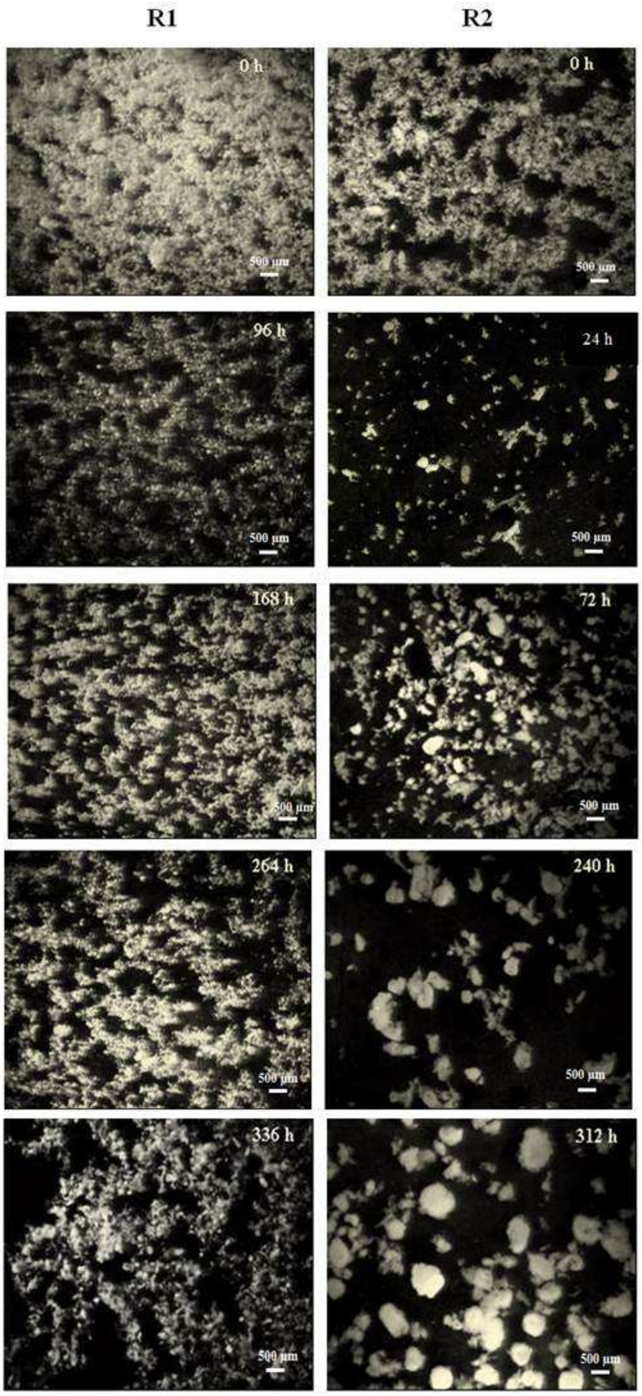

Fig. 1 Morphology of sludge in R1 and R2 in the first two weeks with a multiplication of 6.3

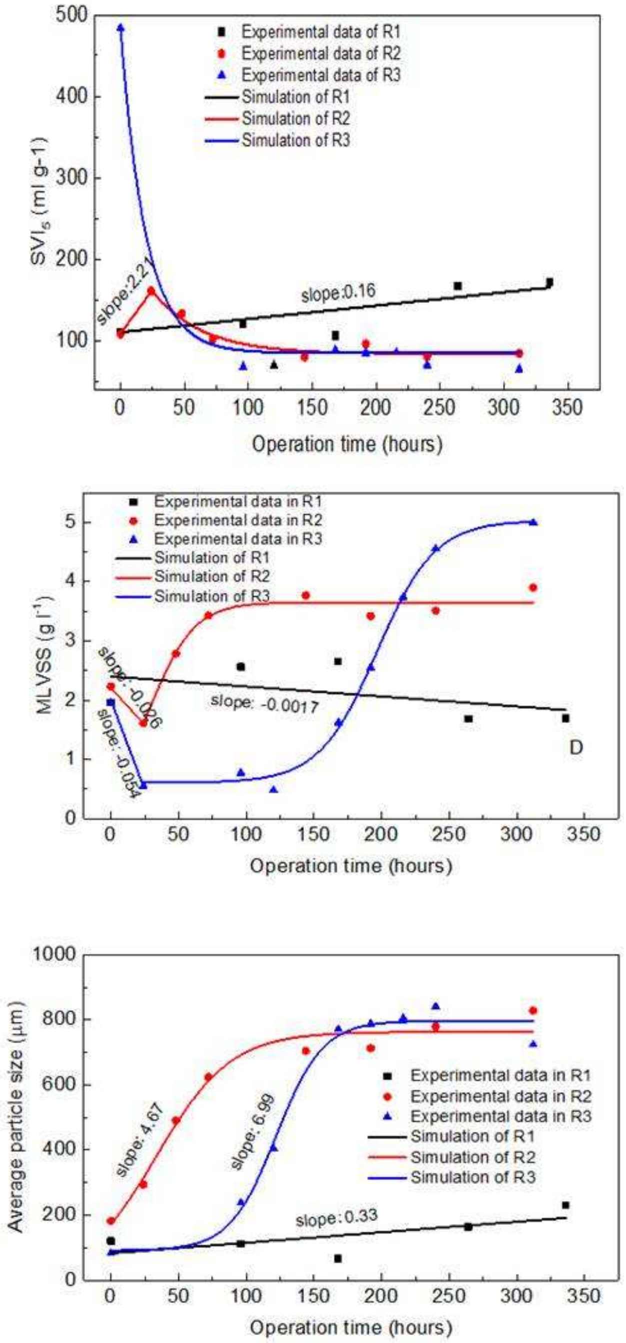

Fig. 2 Evolution of SVI5, MLVSS and average particle size of sludge in R1, R2 and R3 in the first two weeks

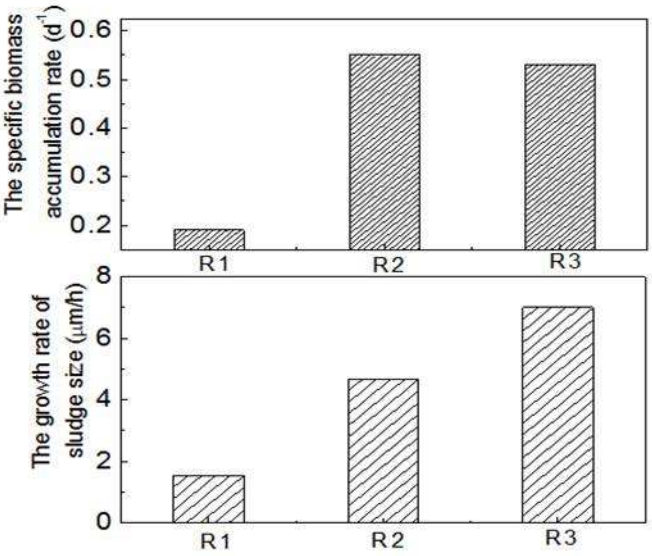

Fig. 3 The specific biomass growth rate and the particle size growth rate of sludge in R1, R2 and R3 during the exponential growth period

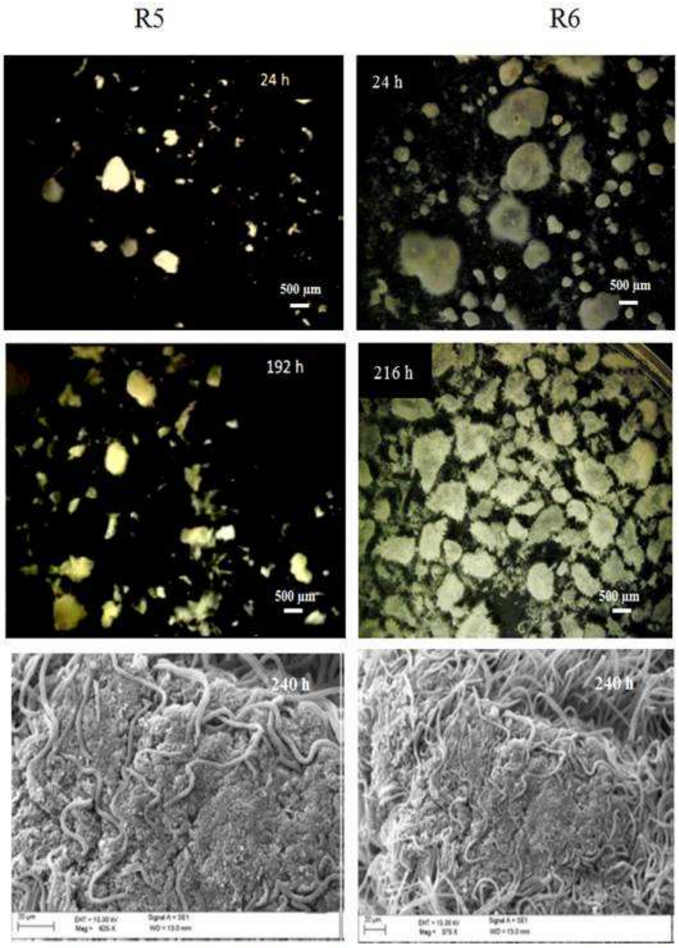

Fig. 4 Morphology of sludge in R5 and R6 in the first ten days observed by Image Analyzer and SEM

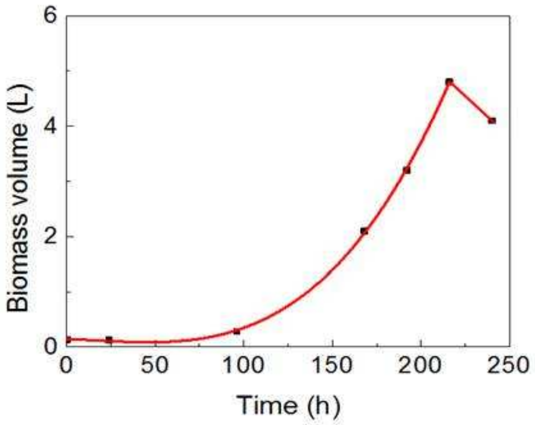

Fig. 5 Biomass accumulation in reactor R5 over operation time

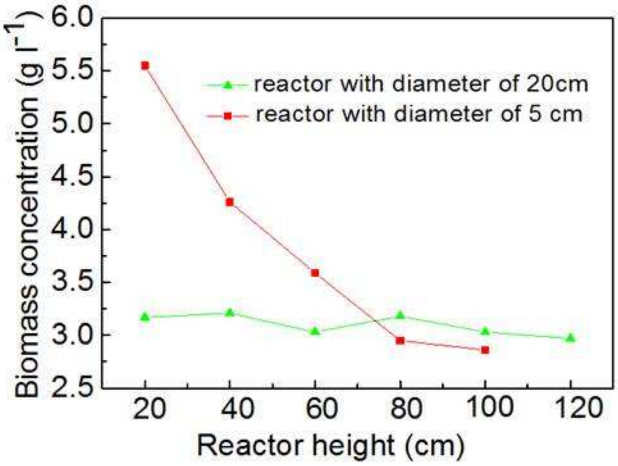

Fig. 6 Concentrations of aerobic granular sludge along reactor height in 5-cm diameter and 20-cm diameter reactors

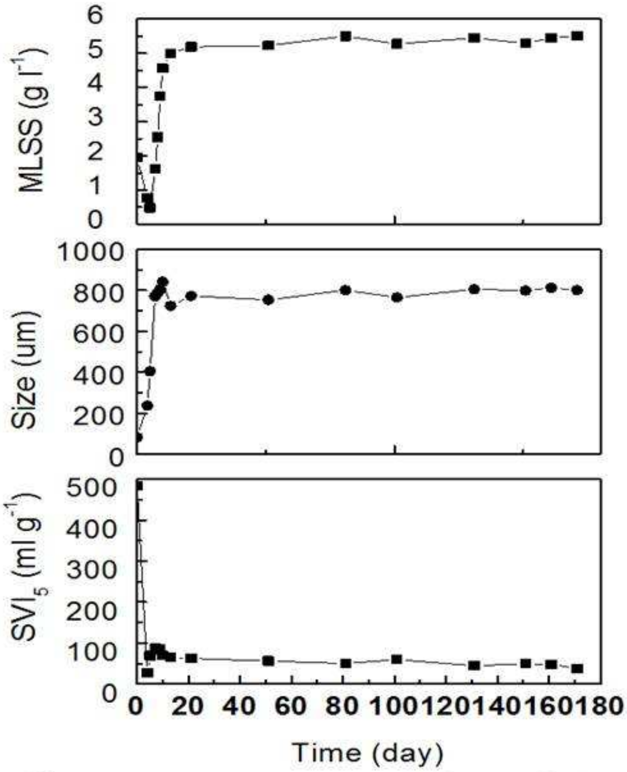

Fig. 7 Evolution of biomass concentration, average particle size and SVI₅ of sludge in reacto
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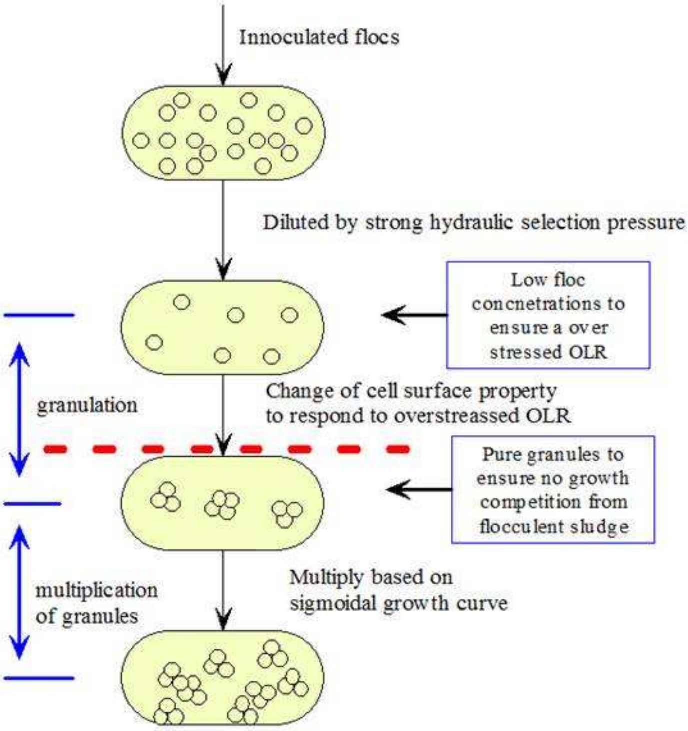

Fig. 8 Mechanism of fast aerobic granulation with the combined strong HSP and overstressed OLR

Highlights:

- A new operational strategy was used to speed up aerobic granulation
- Suspended sludge turned into aerobic granules within 24 hours
- Steady state of aerobic granule reactor was reached within 3 days
- Seed sludge, exchange ratio, reactor configuration and shear force did not affect granulation
- Rapidly formed granules maintained long-term stability at reduced organic loading rate