Optimization of operation conditions for preventing sludge bulking and enhancing the stability of aerobic granular sludge in sequencing batch reactors

Jun Zhou, Hongyu Wang, Kai Yang, Fang Ma and Bin Lv

ABSTRACT

Sludge bulking caused by loss of stability is a major problem in aerobic granular sludge systems. This study investigated the feasibility of preventing sludge bulking and enhancing the stability of aerobic granular sludge in a sequencing batch reactor by optimizing operation conditions. Five operation parameters have been studied with the aim to understand their impact on sludge bulking. Increasing dissolved oxygen (DO) by raising aeration rates contributed to granule stability due to the competition advantage of non-filamentous bacteria and permeation of oxygen at high DO concentration. The ratio of polysaccharides to proteins was observed to increase as the hydraulic shear force increased. When provided with high/low organic loading rate (OLR) alternately, large and fluffy granules disintegrated, while denser round-shape granules formed. An increase of biomass concentration followed a decrease at the beginning, and stability of granules was improved. This indicated that aerobic granular sludge had the resistance of OLR. Synthetic wastewater combined highly and slowly biodegradable substrates, creating a high gradient, which inhibited the growth of filamentous bacteria and prevented granular sludge bulking. A lower chemical oxygen demand/N favored the hydrophobicity of granular sludge, which promoted with granule stability because of the lower diffusion rate of ammonia. The influence of temperature indicated a relatively low temperature was more suitable.

Key words | aerobic granular sludge, hydrophobicity, operation parameters, sequencing batch reactor, sludge bulking, stability

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INTRODUCTION

Aerobic granules are the spherical, self-immobilized aggregates of microbial cells and are considered to be a special case of biofilm (Beun *et al.* 1999). The granules were successfully cultivated without any carrier material in a sequencing batch reactor (SBR) (El-Mamouni *et al.* 1997). Compared to the conventional activated sludge, aerobic granular sludge has excellent settling ability, high biomass retention, high resistance to toxicity and organic loading, the capacity of simultaneous nitrogen and phosphorus removal, compact microbial structure and less production of sludge (De Kreuk *et al.* 2005; Liu *et al.* 2014). In response to these unique attributes, aerobic granulation technology has been successfully applied in the treatment of high-strength agro-based wastewater, rubber wastewater and textile wastewater (Muda *et al.* 2010; Abdullah *et al.* 2013; Rosman *et al.* 2013). However, a major barrier to practical engineering applications of aerobic granular sludge is that the sludge can easily become unstable gradually under long-term operation and disintegrates after prolonged operation (Wan *et al.* 2013). Thus, preventing granular sludge bulking and improving the stability of aerobic granular sludge are necessary, which would promote its engineering applications.

Many mechanisms have been proposed for the loss of granule stability for long-term operation (Lee *et al.* 2010). Among these mechanisms, outgrowth of filamentous organisms is one of the main reasons for granule instability. The limitation of mass transfer and diffusion has positive correlation with granule size. Other studies reported that extracellular polymeric substances (EPS) played a significant role in granule maintenance (Sheng *et al.* 2010). The EPS had high bundled ability, which favored the granule stability.

doi: 10.2166/wst.2014.406

In this study, different approaches focused on the operation conditions at mature period, such as dissolved oxygen (DO), organic loading rate (OLR), carbon source, chemical oxygen demand (COD)/N and temperature, were investigated to prevent sludge bulking and maintain stable granules. The influences of filamentous bacteria overgrowth and the stability of the granule core were taken into consideration to choose the parameters. DO and aeration rate are related to the competition advantage of filamentous bacteria. OLR determines the amount of carbon source that microorganisms can take up. Moreover, carbon source type, COD/N ratio and temperature play an important role in enhancement of granule stability. A strategy of operation conditions for preventing aerobic granular sludge bulking during operation was investigated, which provided theoretical support for the application of aerobic granular sludge.

MATERIALS AND METHODS

Reactor set-up and operation

The experiment was carried out in plexiglass SBRs, namely R1, R2 and R3. A schematic diagram of the reactor used in this study is shown in Figure 1. The working volume of the reactor was 9 L with a height of 80 cm and an internal diameter of 12 cm. Compressed air was supplied via a diffuser at the bottom of the reactor. The hydraulic retention time was 16 h, and the volume exchange ratio was 50%. These reactors were operated sequentially with a cycle time of 6 h, which included 2 min of influent filling, 120 min of anaerobic phase, 120 min oxic phase, increasing from 83 to 108 min anoxic phase gradually, decreasing settling time from 30 to 5 min and 5 min of effluent discharging. The initial DO concentration was about 5 mg l^{-1}. A slender agitator with a crescent-shape vane was placed at 30 cm above the bottom. The test was performed at the temperature of $18 \pm 2\,^{\circ}C$ and influent pH was 7.5 ± 0.2.

Seeding sludge and synthetic wastewater

The inoculated active sludge was taken from an urban wastewater treatment plant. The seeding sludge had a mixed liquor suspended solids (MLSS) concentration of 1.5 g l^{-1}, mixed liquor volatile suspended solids (MLVSS) concentration of 0.9 g L^{-1}, and sludge volume index (SVI) of 95.3 ml g^{-1}. A synthetic wastewater with the following composition was used in the experiments (per litre): sodium acetate 512.5 mg (400 mg COD), KH_2PO_4 43.9 mg,

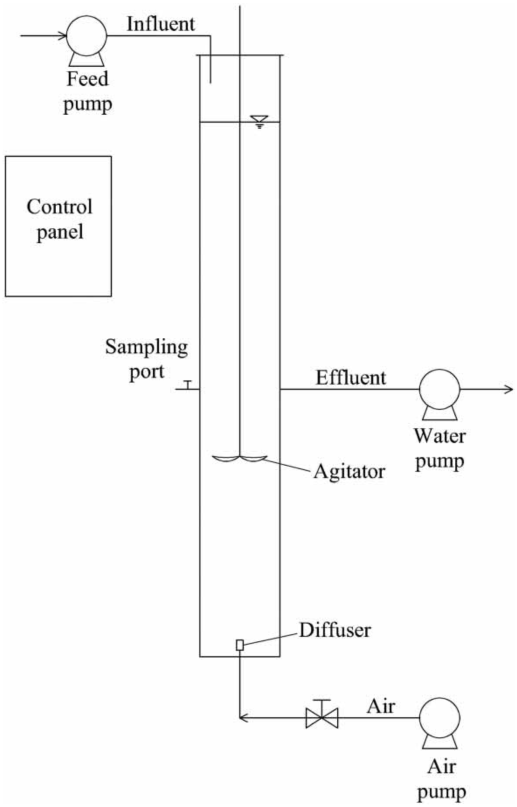

Figure 1 | Schematic diagram of aerobic granular sludge SBR.

NH_4Cl 38.2 mg, $NaNO_3$ 60.7 mg, $CaCl_2$ 111 mg and 0.07 ml of a microelements. The microelement mixture contained as follows (per litre): 3000 mg $FeSO_4 \cdot 7H_2O$, 26 mg $MnSO_4 \cdot 4H_2O$, 50 mg $CoCl \cdot 6H_2O$, 7 mg $CuCl_2 \cdot 2H_2O$, 24 mg $ZnCl_2$, 20 mg H_3BO_3, 36 mg $NiCl_3 \cdot 2H_2O$, 50 mg EDTA, 21 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Liu *et al.* 2009). This synthetic wastewater can simulate real wastewater, and the concentrations of constituents could provide enough necessary elements for the cultivation of aerobic granular sludge.

Analytical methods

COD, nitrate (NO_3^--N), ammonia (NH_4^+-N), total phosphorus, MLSS, MLVSS and SVI were measured according to *Standard Methods* (APHA 1998). DO and pH were determined by a DO meter (YSI 5000) and pH meter (828 Orion), respectively. The degree of filamentous overgrowth (Δ) was used to refer to the overgrowth of filamentous organisms. $\Delta = D/d$, where D is the diameter of granule with rough edge and d is the diameter of the granule without rough edge (Wang *et al.* 2008). Cell hydrophobicity was determined with the method described by Rosenberg *et al.* (Rosenberg *et al.* 1980), in which hexadecane was used as hydrophobic phase. Hydrophobicity is expressed as the percentage of cells adhering to the hexadecane after 15 min of partitioning. The carbohydrate content was measured by the anthrone method using glucose as standard. The content of protein in EPS was measured by the modified Lowry method using bovine serum albumin as the standard. Values are presented as means ($n = 3$).

Single-factor experiment design

When mild sludge bulking occurred, several operation parameters were changed as follows, respectively:

(1) DO: 4 mg l^{-1} - R1, 5 mg l^{-1} - R2, 6 mg L^{-1} - R3,
(2) OLR: 0.3 kg COD m^{-3} d^{-1} - R1, 0.6 kg COD m^{-3} d^{-1} - R2, alternation of 0.3 and 0.6 kg COD m^{-3} d^{-1} - R3,
(3) carbon source: 512.5 mg l^{-1} sodium acetate - R1; 82 mg l^{-1} sodium acetate, 288 mg l^{-1} sodium propionate - R3; 41 mg l^{-1} sodium acetate, 144 mg l^{-1} sodium propionate, 179.2 mg l^{-1} particulate starch - R3,
(4) COD/N: 100:5 - R1, 100:10 - R2, 100:15 - R3,
(5) temperature: 26 °C - R1, 22 °C - R2, 18 °C - R3.

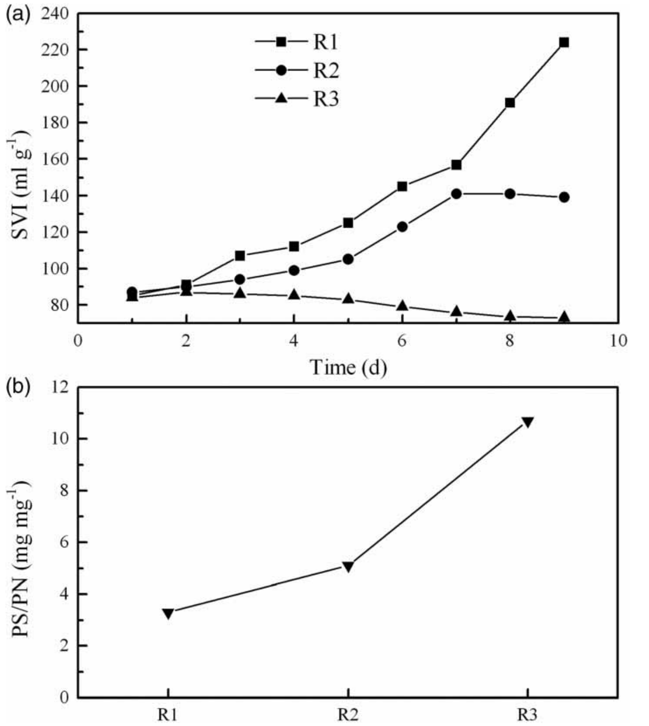

Figure 2 | Evolution of (a) SVI profiles and (b) ratio of PS to PN in reactors during the study of DO.

RESULTS AND DISCUSSION

Effects of dissolved oxygen (DO) and shear force

The evolution of SVI profiles in three reactors are shown in Figure 2(a). The SVI of granular sludge increased to a value of 224 and 139 ml g^{-1} in R1 and R2 respectively, whereas the SVI value decreased to 76 ml g^{-1} in R3. Figure 2(b) shows the ratio of sludge polysaccharides (PS) to sludge proteins (PN). The PS/PN ratio reached 3.3, 5.1 and 10.7 in R1, R2 and R3 respectively.

The result indicated that DO concentration had an extremely strong effect on granular sludge settleability and stability. Low DO concentration had a negative effect on sludge settleability (Figure 1(a)) (Martins *et al.* 2003). Low DO concentration limited the penetration of oxygen in granules, which produced an anaerobic core to promote the activity of anaerobic bacteria, and then disintegrated granules. The granules grew in size with a loose structure due to the propagation of filamentous bacteria. It has been reported that overgrowth of filamentous bacteria was favored by low DO concentration (Martins *et al.* 2004). Rapid proliferation of filamentous bacteria occurred when DO deficiency existed. A relatively high DO concentration inhibited the competitive advantage of filamentous bacteria and favored the DO penetration in granules.

Moreover, as aeration rates adjusted to DO, variation of hydraulic shear force accompanied the changes of DO concentration. Hydraulic shear force strengthened the interaction between the gas phase, liquid phase and particles and favored the aerobic granulation (Di Iaconi *et al.* 2006). The result shown in Figure 2(b) indicates that the PS/PN ratio significantly increases with the shear force. Both cohesion and aggregation are mediated by cell PS, which has been generally approved. It appeared that cell PS played a crucial role in maintaining the granular sludge. Higher shear force seemed to induce the production of more PS compared to protein, as Figure 2(b) shows. As reported, cell PS were conducive to the compact structure and stability of granular sludge (Liu & Tay 2006). Hence, intermediate aeration intensity neither satisfied oxygen supply nor prevented overgrowth of filaments.

Effect of OLR

As depicted in Figure 3, the value of SVI increased obviously from 77 to 368 ml g^{-1} and floc-like granules emerged in R2. The SVI in R1 increased to 152 ml g^{-1}. Nevertheless, the sludge bulking was not observed and the SVI reached 63 ml g^{-1} in R3.

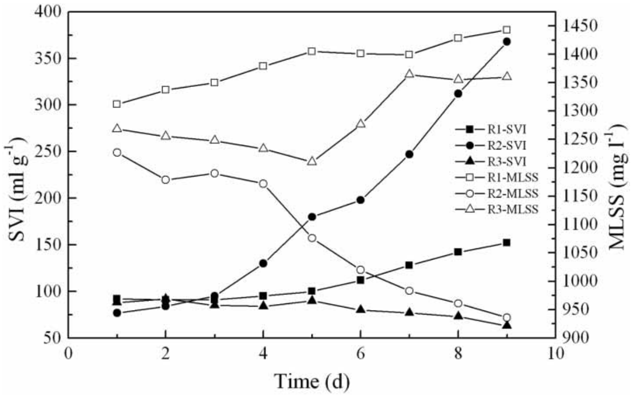

Figure 3 | Evolution of the SVI profiles and MLSS during the study of OLR.

At high OLR, the size of granules could increase and mass transfer was limited gradually. The mass transfer limitation of large granules could produce an anaerobic core to stimulate activities of anaerobic strains, weaken granule structure and make the granules unsteady, hence leading to disintegrated granules (Zheng *et al.* 2006). Moreover, with the high COD concentration, the substrate supplied enough carbon source to the growth of filamentous bacteria in the oxic phase.

On the other hand, the substrate transmission to the interior of the granule was inhibited at low substrate concentration, which made the surface of granules fluffy. The growth of bacteria is assumed to follow the Monod equation. The maximum specific growth rate and half-saturation constant of filamentous bacteria are smaller than those of zoogloea bacteria. When the substrate concentration is relatively low, the specific growth rate of filamentous bacteria is higher, and then the large granules disintegrate into small debris which has poor settling performance. The granules became weak in terms of structural integrity under an extended starvation.

When the COD concentration was low, fluffy granules were washed out, while the smaller aerobic granules were retained. The filamentous bacteria with high area to volume (A/V) ratio had advantages for the mass transfer to the cell (Peyong *et al.* 2012); thus, the remaining small granules were covered with filamentous bacteria. When fed the substrate of relatively high COD concentration, the non-filamentous bacteria had higher specific growth rate than filamentous bacteria according to the Monod equation. The relatively high OLR favored the growth of non-filamentous bacteria and the filamentous bacteria dropped off from the granule surface. Consequently, denser and smoother granules grew and sludge bulking was prevented. Meanwhile, the evolution of biomass retention is shown in Figure 3, which corresponded to the result. Figure 3 suggests that alternation of high/low OLR led to a biomass concentration increase after an initial decrease.

Effect of carbon source types

Filamentous overgrowth happened at the outer aerobic granules, and the degree of filamentous overgrowth (Δ) is shown in Figure 4(a). The filamentous overgrowth in R3 was the least among the three reactors, which indicated the prevention of granular sludge bulking. Sludge bulking occurred in R1 and R2 and filamentous overgrowth in R3 was prevented.

It can be concluded from Figure 4(a) that feeding wastewater containing both easily degradable and slowly biodegradable substrates prevents granular sludge bulking. Actually, the substrates in wastewater affected the growth of non-filamentous and filamentous microorganisms. It is generally accepted that soluble and readily degradable substrates such as acetate and glucose trigger the growth of filamentous microorganisms and favor bulking. Meanwhile, it was hypothesized that most of the slowly biodegradable substrate

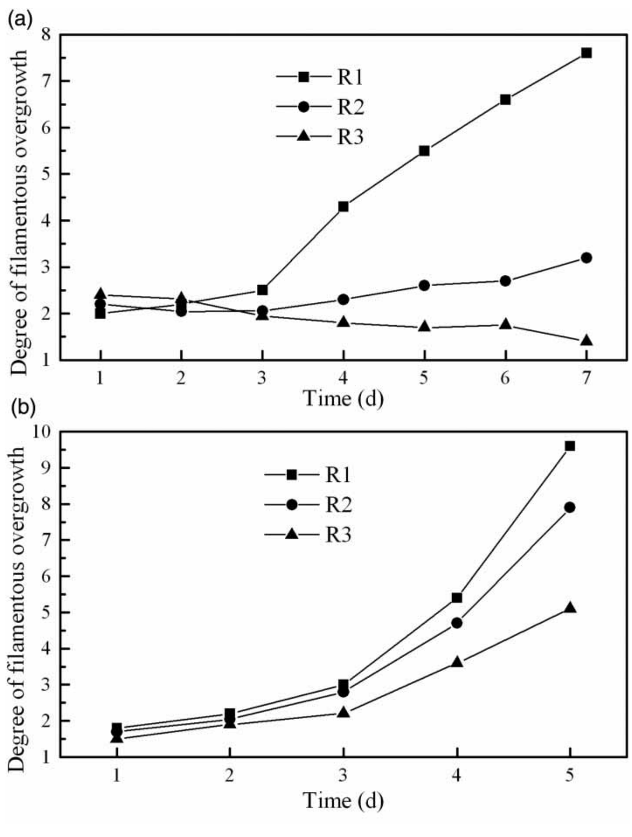

Figure 4 | Variation of the degree of filamentous overgrowth $\triangle$ during the study of (a) carbon source type, (b) temperature.

was removed by adsorption at the granule surface (De Kreuk et al. 2010). The substrate hydrolyzed, and hydrolysis products were consumed by the bacteria, which resulted in the substrate gradients causing irregular filamentous microorganism overgrowth. However, according to the kinetic selection theory, a high substrate gradient in the bulk fluid can suppress excessive growth of filamentous bacteria (Liu & Liu 2006). In addition, Wang et al. (2008) showed that the overgrowth of filamentous bacteria was under control after feeding vitamin C wastewater containing slowly biodegradable substrate such as long carbon chain organic matter and benzodiazepines instead of glucose, which conformed to this study. The reason for this phenomenon was that the maximum growth rate of both filamentous and non-filamentous bacteria was smaller when used slowly biodegradable substrate instead of highly biodegradable substrate, and the advantage of high A/V ratio filamentous bacteria was not apparent due to the mass transfer limitation at low substrate rate. Consequently, the influent consisted of both easily and slowly biodegradable substrate, which promoted growth of non-filamentous bacteria and suppressed the overgrowth of filamentous bacteria. The accumulation of P-accumulating or nitrifying bacteria also exhibited the ability of preventing granular sludge bulking.

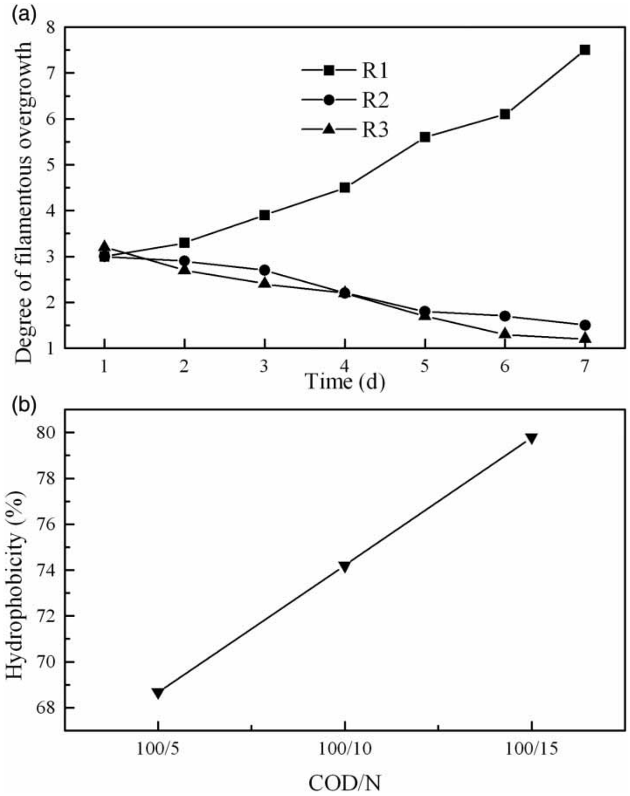

Figure 5 | (a) Variation of the degree of filamentous overgrowth △ and (b) cell hydrophobicity in reactors during the experiment of COD/N ratio.

Effect of COD/N ratio

As in Figure 5(a), the degree of filamentous overgrowth Δ_{R1} increased to 7.5 when the days went on; conversely, Δ_{R2} and Δ_{R3} decreased to 1.5 and 1.2, respectively. Aerobic granular sludge bulking was observed in R1 and did not appear in R2 and R3. Most research on aerobic granular sludge used acetate synthetic wastewater at a COD/N ratio of 100:5, which was a commonly used ratio in conventional activated sludge processes. Nevertheless, the situation in granule sludge is probably more complex than in activated sludge due to the diffusion limitations in the aerobic granules. The values of diffusion coefficients used here to compare diffusion rates are $2.5 \times 10^{-9}\,m^2\,s^{-1}$ for acetate, $1.67 \times 10^{-9}\,m^2\,s^{-1}$ for DO and $1.01 \times 10^{-9}\,m^2\,s^{-1}$ for ammonia (Liu & Liu 2006). This indicates that the localized COD/N ratio within aerobic granules is much lower than that in bulk fluid because of the lowest diffusivity that ammonia has. Namely, a nitrogen deficiency would be encountered inside aerobic granules. A high COD/N ratio or nitrogen deficiency was considered to be the main cause of bulking, which coincided exactly with the result shown in Figure 5(a).

Microorganisms produce significant amounts of extracellular PS in the absence of sufficient nitrogen (Aquino & Stuckey 2003). PS are an important part of EPS. Cell surface hydrophobicity is an important property of the EPS and supposed to be a reason for triggering microbial aggregation (Liu et al. 2004b). The presence of fibroid structures on the cell surface is usually associated with high cell surface hydrophobicity. Figure 5(b) shows the relationship between COD/N ratio and hydrophobicity. It can be concluded from Figure 5(b) that the overproduction of extracellular PS due to the nitrogen deficiency inside the granules decreased the cell surface hydrophobicity of the granules. It appears that high cell surface hydrophobicity promotes cell-to-cell interaction, which keeps the bacteria tightly together and favors a more stable structure of granular sludge (Liu et al. 2004a). Hence, extracellular PS -rich aerobic granules have settling and stability problems. On the other hand, filamentous bacteria have higher A/V ratio than non-filamentous bacteria, which enabled the uptake of nitrogen from media when COD/N was high. In addition, the slow-growing nitrifying bacteria enriched significantly at low substrate COD/N ratio (Liu et al. 2004a). High stability in terms of cell hydrophobicity was observed in aerobic granular sludge with slow-growing bacteria (shown in Figure 5(b)).

Effect of temperature

Figure 4(b) shows the variations of the degree of filamentous overgrowth Δ in R1, R2 and R3 over the operational period. From the figure, it can be seen that Δ increased slower at 18 °C than at 22 °C and at 26 °C. The growth of filamentous bacteria is favored at a high temperature (Liu & Liu 2006). Moreover, a higher temperature resulted in a decrease of DO concentration in reactors. The decrease in DO promoted the overgrowth of filamentous bacteria, which was discussed in a previous section. However, the degree of filamentous overgrowth Δ still increased and increased slower at 18 °C. This implied that decreasing temperature could control the aerobic granular sludge bulking to some extent.

CONCLUSION

Aerobic granules lost stability after a long-term operation. Increasing aeration rate provided sufficient DO to the cell, suppressed the overgrowth of filamentous bacteria and promoted the cell aggregation. On the other hand, high shear force accompanied the increase of DO, which resulted in increase of PS/PN. High PS/PN led to compact structure of granules and enhanced stability. Feeding synthetic wastewater containing alternately high/low COD concentration washed out fluffy granules, and denser round-shape granules still remained due to the high A/V ratio of filamentous, hence prevented the sludge bulking. In addition, feed containing both quickly and slowly biodegrading substrates provided a high gradient that promoted non-filamentous and suppressed filamentous bacteria. Low COD/N ratio offered sufficient nitrogen inside granules and steadied hydrophobicity, which prevented granules from losing stability. The granule SBR run at 26 °C was more impressionable to filamentous bacteria growth compared to a, identical reactor operated at 18 °C. Furthermore the nutrient removal was effective for previous operations.

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China (NSFC) (NO. 51008239 and 51378400), the Natural Science Foundation of Hubei Province, China (NO. 2013CFB289 and 2013CFB308) and the opened fund of State Key Laboratory of Urban Water Resources and Environment (HIT) (NO. QAK201014).

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First received 16 June 2014; accepted in revised form 10 September 2014. Available online 24 September 2014