The effect of organic loading rate on the aerobic granulation: the development of shear force theory

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Abstract The effect of organic loading rate (OLR) on aerobic granulation was studied by adopting three column-shaped, sequential aerobic sludge blanket reactors (SASBR). The reactors had been fed with laboratory prepared, synthetic dextrose-nutrient broth substrate. Experimental results showed clearly that the formation, characteristics and stability of aerobic granules had a close relationship with the strength of OLR applied. Aerobic granules appeared firstly under the OLR of 4 kg COD·$(m^3{\cdot}day)^{-1}$. The system stabilization was demonstrated by its little-changed amount and morphology of granules. The characteristics of the stabilized granules were: 5.4 mm in mean diameter, 1.29 in roundness, 118 mg $O_2{\cdot}(mg\ VSS{\cdot}hr)^{-1}$ in SPOUR. The respective biomass SVI was 50 mL·(g MLVSS)$^{-1}$ and the averaged COD removal rate was 95%. Under the OLR of 8 kg COD·$(m^3{\cdot}day)^{-1}$, granules appeared two days later than those for 4 kg COD·$(m^3{\cdot}day)^{-1}$ and they always coexisted with flocs. The formed granule bed was not as compact as that under 4 kg COD·$(m^3{\cdot}day)^{-1}$. There were no granules formed under the OLR of 1 kg COD·$(m^3{\cdot}day)^{-1}$. Instead, flocs with rather loose structure dominated reactor mixed-liquor. The respective SVI's were 65 and 138 mL·(g MLVSS)$^{-1}$ under OLR of 8 and 1 kg COD·$(m^3{\cdot}day)^{-1}$. It was proposed that the growth and maintenance of aerobic granules follow the shear force balance theory. Under the OLR of 4 kg COD·$(m^3{\cdot}day)^{-1}$, a balance was reached between the aeration shear force and organic loading rate. Under this favored condition aerobic granules formed quickly and, became stabilized with the experimental parameters remained unchanged.
Keywords Aerobic granulation; organic loading rate; sequencing batch reactor; shear force

Introduction

Suspended flocs and attached biofilm are two well-acknowledged natural occurring aggregates that could help to offer the function of biomass immobilization, that is, to separate the microorganisms from the effluent water while concurrently a high biomass concentration in the treatment is obtained. The studies about their mechanisms and applications have been carried out elsewhere (Rittman and McCarty, 1980a,b; Heijinen *et al*., 1992; Urbain *et al*., 1993; Barbusinski and Koscielniak, 1995).

As another immobilization idea, biogranulation has been widely studied and, successfully industrialized for the last two decades (Lettinga *et al*., 1980; van der Hoek, 1988; Hickey *et al*., 1991; Tijhuis *et al*., 1996; Van Benthum *et al*., 1996; Tay and Yan, 1996; Liu and Tay, 2001).

Recently the sequencing batch reactor (SBR) has been used to study the granulation under aerobic conditions (Morgenroth *et al*., 1997; Beun *et al*., 1999; Tay *et al*., 2001). Those researches have showed some promising characteristics for the granules over traditional activated sludge flocs. They included the good settling ability, regular in shape, high density, strong microbial structure, high biomass retention and good nutrient removing ability.

However, the effect of reactor organic loading rate (OLR) on the formation of aerobic granules has remained uninvestigated. In an activated sludge system, it was demonstrated that the size of flocs increased with organic loading (Barbusinski and Koscielniak, 1995). This would further influence other physical properties including floc shape, density and porosity, settling velocity, as well as coherence and specific surface area (Li and

Ganzarczyk, 1992; Námer and Ganczarczyk, 1993). The experimental data with biofilm airlift suspension (BAS) reactor disclosed that beside shear, the substrate loading rate plays an important role in the formation of smooth and well settling biofilm particles (Van Loosdrecht *et al.*, 1995; Kwok *et al.*, 1998).

It is this investigation's intention to test the effect of different OLRs on the aerobic granulation, including granule characteristics, and the results will be used for the better understanding of the aerobic granulation.

Methodology

Three acrylic column-shaped reactors of the same geometry were employed in the study. Each reactor was 600 mm in height and 60 mm in diameter with the working volume of 1.70 litres. Reactors were kept in a project room with temperature stabilized at 25°C. The OLR was controlled at 8, 4 and 1 kg COD·(m^3·day)$^{-1}$ from Reactor 1 to Reactor 3, respectively. Aerobic heterotrophic microorganisms were cultured within reactors which were operated under the same sequential batch mode. Each operation cycle consisted of 2 min for settling, 1 min for discharging, 2.5 min for feeding, and 177 min for aeration (the aeration started simultaneously with the feeding).

A synthetic wastewater consisting of glucose, peptone and meat extract was used as system feeding substrate. Concentrated stock, solution I, composed of major organic and inorganic components, and concentrated stock solution II, composed of trace components, were separately prepared. The chemical compositions of these stock solutions were detailed elsewhere (Tay and Yan,1996).

Two major groups of parameters were measured during the experiment, i.e. routine and special tests. The major parameters and analytical methods used in the study are listed in Table 1.

Except for the effluent VSS that was based on a 24 hours composite sample, all the other parameters were analyzed using the grab samples. As for COD tests, the reactor influent samples taken from feed tanks were tested directly. While the effluent samples were pre-treated by passing them through a 0.45 μm filter paper, the tested soluble COD was reported as the final effluent COD results.

Table 1 Analytical methods employed

Parameters	Analytical methods	Sample tested
Routine tests		
COD, SS, VSS	Standard Method (APHA, 1995)	Influent/effluent
DO	DO meter model YSI-52	Mixed liquor
Special tests		
Morphology and structure of the granule (roundness, and averaged mean diameter)	Image analysis system	Granule sample
Specific gravity of granule	Standard Method (APHA, 1995)	Granule sample
Specific oxygen uptake rate	Standard Method (APHA, 1995)	Reactor mixed-liquor sample
Bacterial compositions	Scanning electron microscope (SEM)	Granule sample
Exopolysaccharide	Methodology developed by Urbain *et al.* (1993)	Reactor mixed liquor sludge
Hydrophobicity of granule	Nile Red staining followed by CLSM (Wolfaardt *et al.*, 1998)	Granule sample
Granule strength	Methodology developed by (Ghangrekar *et al.*, 1996)	Granule sample

Results and discussion

Activated sludge of the same biomass concentration was taken from the local Jurong West Sewage Treatment Plant and seeded in three reactors with the same concentrations. Figure 1 shows the reactor performance under different OLRs. The aerobic granules appeared first under the OLR of 4 kg COD·(m^3·day)$^{-1}$, four days after the reactor seeding. Whereas the appearance of granules under 8 kg COD·(m^3·day)$^{-1}$ was two days later, the granules had never been produced under 1 kg COD ·(m^3·d)$^{-1}$.

The appearance of aerobic granules had an obvious enhancement effect on reactor biomass concentration increment. Under the OLR of 4 kg COD·(m^3·day)$^{-1}$, the MLVSS was just 620 mg·L^{-1} when the aerobic granules appeared. The value increased sharply to 3,200 mg·L^{-1} five days later. Similarly, the MLVSS under 8 kg COD·(m^3·day)$^{-1}$ jumped from 890 to more than 5,000 mg·L^{-1} within five days since the granule appeared. However, only flocs had formed under the OLR of 1 kg COD·(m^3·day)$^{-1}$. As a result, the increasing rate of MLVSS was much slower than high OLR systems.

Compared with the OLR of 8 and 1 kg COD·(m^3·day)$^{-1}$, the reactor MLVSS was more stabilized under the OLR of 4 kg COD·(m^3·day)$^{-1}$. While the biomass mainly consisted of flocs under the OLR of 1 kg COD·(m^3·day)$^{-1}$, the mixed liquor was always a mixture of granules and flocs when the OLR was 8 kg kg COD·(m^3·day)$^{-1}$. During the very short settling period of 2 min, the existence of aerobic flocs provided a hindrance for the biomass to precipitate. As a result, too high or too low OLR appeared to be unfavorable for the formation of a compact sludge bed, and further, for maintaining the stability of reactor performance.

The biomass settleability also improved with the aerobic granulation. The SVI of the seed sludge was 243 mL·(g·MLVSS)$^{-1}$. After two weeks of operation, the average SVIs were 91 and 138 mL·(g·MLVSS)$^{-1}$ respectively, for the OLR of 8 and 1 kg COD·(m^3·day)$^{-1}$. The excellent biomass settleability was realized at the OLR of 4 kg COD ·(m^3·day)$^{-1}$, where the aerobic granules formed the major components of the biomass. The SVI value of 50 mL·(g·MLVSS)$^{-1}$, which was the lowest among the OLR applied.

The quality of reactor effluent varied with OLR applied. After two weeks of operation, the averaged COD removal rates were 84%, 95% and 88% with the best removal rate achieved under the OLR of 4 kg COD·(m^3·day)$^{-1}$. As shown by Figure 1, effluent VSS encountered the mass flush out at the start of the seeding. Compared with an OLR of 8 and 1 COD·(m^3·day)$^{-1}$, the effluent VSS was more stabilized under the OLR of 4 COD·(m^3·day)$^{-1}$. The average effluent VSSs after two weeks were 432, 155 and 71 mg·L^{-1} respectively, with regard to OLR of 8, 4 and 1 COD·(m^3·day)$^{-1}$.

Table 2 summarizes the characteristics of aerobic granule/aggregate obtained from different OLR. While it shows that the granule size decreased with the OLR applied, the roundness of granule was the smallest at the OLR of 4 kg COD·(m^3·d)$^{-1}$. According to the definition by Image Analysis, the value of roundness can not be smaller than one. The smaller the value is, the more regular the detected object's shape is in terms of roundness.

Figure 2 shows the stereomicroscopic pictures for typical aerobic granules/aggregates under different OLRs. Under the OLR of 8 and 4 kg COD·(m^3·d)$^{-1}$, the surface of the aerobic granules appeared to be smooth. With the formed compact granule structure, their specific gravity was 1.024 and 1.034 kg·L^{-1} respectively. However, it is totally different under OLR of 1 kg COD ·(m^3·d)$^{-1}$. Figure 2C shows that the aggregate has a loose structure with a small core surrounded by a coat of fluffy filaments. This loose structure made the settling of biomass and formation of compressed sludge bed difficult. As a result, the lowest aggregate specific gravity of 1.011 kg·L^{-1} and respective biomass highest SVI 138 mL·(g VSS)$^{-1}$ were observed.

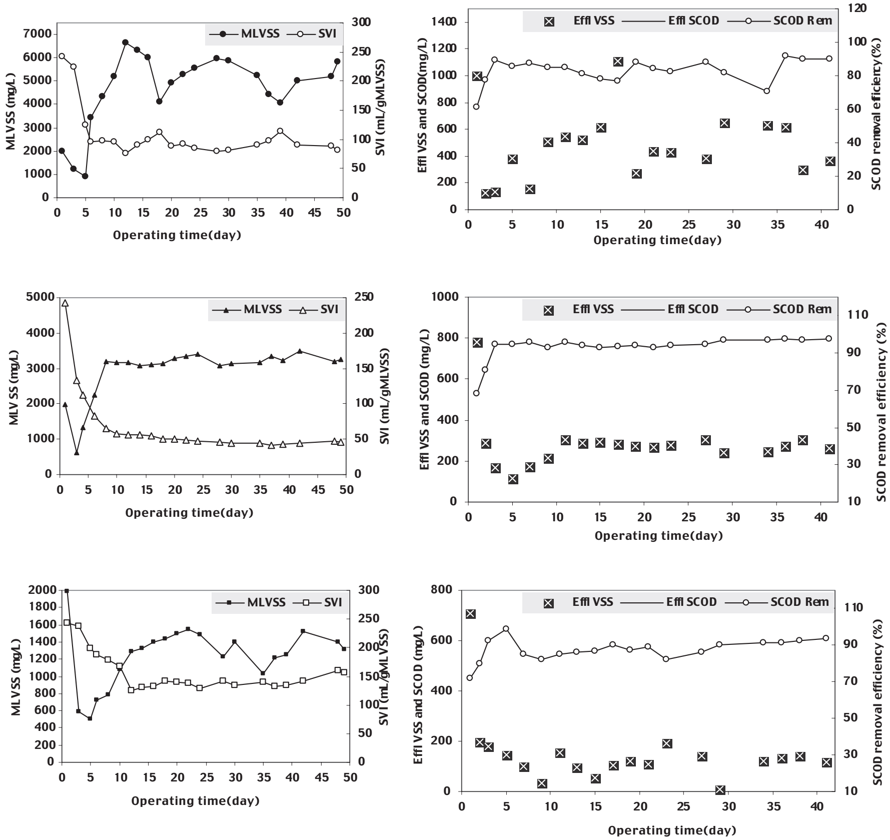

Figure 1 Reactor performance under different OLR. Top, middle and bottom, the applied OLR are 8, 4 and 2 kg COD·$(m^3·d)^{-1}$ respectively

Table 2 The characteristics of aerobic granules/aggregates

Reactor	R1	R2	R3	Seed sludge
Organic loading rate, kg COD˙$(m^3$˙$day)^{-1}$	8	4	1	
SPOUR, mg O_2˙(mgVSS˙$hr)^{-1}$	148	131	82	122
Mean diameter by number, mm	8.8	5.4	4.0	0.03
Granule roundness	1.49	1.29	2.23	
Specific gravity, kg˙L^{-1}	1.024	1.034	1.011	
SVI, mL˙(g $VSS)^{-1}$	65	50	138	234
Integrated co-efficient	0.79	0.985	0.952	
VSS to SS ratio, %	0.91	0.87	0.88	0.92
EPS:VSS ($\times 10^{-3}$)	0.17	0.87	1.34	

The strength of the mixed liquor was tested after two weeks of operation. The tested integrated coefficients (IC) are, from high to low OLR, 0.79, 0.985 and 0.952 respectively. According to the definition, the higher integrity coefficient stands for the higher strength of the sample (Ghangrekar *et al.*, 1996). Under high OLR of 8 kg COD·$(m^3·day)^{-1}$, the mixed liquor was a mixture of granules and flocs. The lowest IC value reveals the weak capacity of the granule-floc mixture to resist the outside shear force. However, the tested IC of biomass under the OLR of 4 and 1 COD·$(m^3·day)^{-1}$ was 0.985 and 0.952, showing their relatively high strength against the external shear.

Using the extraction and detecting methodologies specified by Urbain *et al.* (1993),

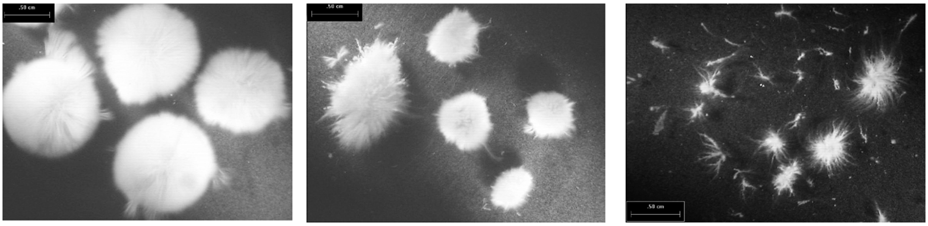

Figure 2 Stereomicroscopic pictures of aerobic granules. From A to C, the applied OLR are 8, 4 and 1 kg COD $\cdot(m^3\cdot d)^{-1}$ respectively

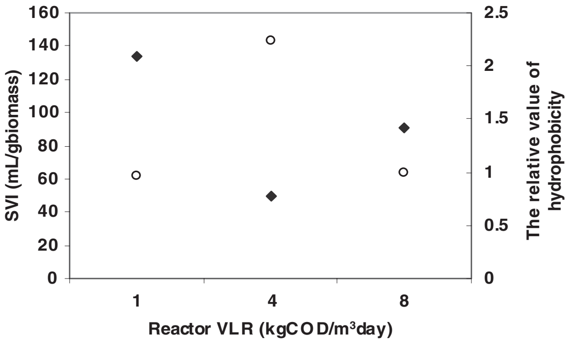

Figure 3 Hydrophobicity (open point) and biomass SVI (closed point) under various OLR

granule/aggregate samples from different OLR were subjected to sonication and the extraction was tested for its exopolysaccharides (EPS) concentration. The results are represented as the relative amount of EPS in the granule/aggregate samples (EPS:VSS). Experiments showed that with OLR decreased, the relative amount of EPS in the biomass increased. EPS is considered as one of the important components of extracellular polymer (ECP). The increased amount of EPS in the granule/aggregate samples indicates that the higher ingredients of EPS might play a positive role in the strengthening granule/aggregate structure.

By using the confocal laser scanning microscope, the surface hydrophobicity of the granule was conventionally characterized as the ratio of the sum of red and green dots on XY, XZ or XYZ extended focus view image (Wolfaardt *et al.*, 1998). It is expressed as the ratio between the number of hydrophobic sites reacted with nile red (red fluorescence) and the number of hydrophilic sites of exopolysaccharides reacted with concanavalin A (green fluorescence). The detected granule hydrophobicity and their respective reactor OLR are shown in Figure 3. The surface hydrophobicity was highest under the OLR of 4 kg COD$\cdot(m^3\cdot day)^{-1}$, where the well formed, compact and stabilized granules with lowest biomass SVI had been obtained.

Conclusions

During the study, shear force was introduced by the aeration air at a superficial velocity of 0.041 $m\cdot s^{-1}$. When the relatively high OLR of 8 kg COD$\cdot(m^3\cdot day)^{-1}$ was applied, the growth rate of biomass was high and the aerobic granules always coexisted with the fluffy flocs with pores and filament. It contained a relatively smaller amount of EPS and its strength was rather weaker.

Under the OLR of 1 kg COD$\cdot(m^3\cdot day)^{-1}$, only the patchy flocs were produced. The long starvation period during a reactor sequential cycle (data not shown) triggered the production and accumulation of biomass EPS. The relatively high EPS amount made it display a high strength value, i.e. high capacity to resist external shear force.

The aeration shear force became balanced with the OLR of 4 kg COD·$(m^3·day)^{-1}$ in Reactor 2. After two weeks of operation, biomass converted to compact, high strengthening granules with excellent settleability. Its surface hydrophobicity was the highest, which was believed to play a positive role during aerobic granulation.

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