Modeling and verification of selective sludge discharge as the controlling factor for aerobic granulation

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ABSTRACT

Mathematical simulation and laboratory experiments were conducted to investigate the controlling factor for aerobic sludge granulation. A model was used to describe the biomass dynamics during the granulation process. The simulation results indicate that the selective discharge of small and loose sludge flocs is the key controlling factor for granulation. In the experimental studies, tests were conducted with four batch column reactors (BCR) that were seeded with both activated sludge flocs and mature granules. Three different sludge discharge methods were tested, including unselective discharge of mixed sludge, selective discharge of small and slow-settling flocs, and selective discharge of settled dense sludge. The results show that mixed sludge discharge and discharge of dense sludge resulted in disappearance of granules from the reactors. Only selective discharge of small and slow-settling sludge flocs led to complete granulation. Small and loose sludge flocs were found to have a clear advantage over large and dense granules in substrate uptake. It can be concluded that selective discharge of loose flocs removes these competitors in suspended-growth mode from the reactors and makes the substrate more available for uptake and utilization by the biomass in attached-growth form, leading to granulation.

Key words | activated sludge, aerobic granulation, biological wastewater treatment

INTRODUCTION

Sludge granules are a special form of microbial self-immobilization in a bioreactor. Aerobic granulation may completely eliminate the biomass-effluent separation problems that are frequently encountered in conventional biological wastewater treatment processes. Although a number of factors have been found to affect sludge granulation in sequencing batch reactors (SBR), there is no consensus on the crucial mechanism of granule formation (Beun et al. 2002; Liu & Tay 2002; Liu & Tay 2004; McSwain et al. 2004; Yang et al. 2008). It has been realized that the growth of aerobic granules after the initial cell-to-cell attachment is similar to the growth of biofilms without the addition of foreign carriers (Beun et al. 2002; Liu & Tay 2002; Yang et al. 2004). Cell-cell signaling is considered to be essential to the formation of biofilms and the spatial distribution of bacteria in a biofilm (Davies et al. 1998). However, the controlling factor for granulation in SBR operation that shifts the biomass from the suspended-growth mode to the attached-growth mode has not yet been identified. Theoretical models are lacking to describe the influences of important operating factors on the growth and accumulation of granules. Mathematical simulations are also needed to actually demonstrate the granulation process under different process conditions. In relation to the modeling work, experimental studies need to be carried out systematically to verify the simulation results. A combination of theoretical modeling and experimental tests is essential to the identification of the controlling factor for aerobic granulation and its underlying mechanism.

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In this study, a mathematical model was used to describe the biomass dynamics during the early phase of granule formation and accumulation in a batch reactor. The results of the mathematical simulation indicate that selective discharge of small and loose sludge flocs from the reactor is the key factor to aerobic granulation. To validate the simulation result, laboratory experiments were conducted with four batch column reactors (BCR), using different types of seed sludge combinations and different methods of sludge discharge. The aim of the study was to determine the controlling factor for sludge granulation and its underlying mechanism.

MATERIALS AND METHODS

Theoretical modeling of the biomass dynamics during granule formation

Biological aggregates in activated sludge reactors span a wide range of sizes, densities and settling velocities (Li & Ganczarczyk 1991; Li et al. 2003; Sears et al. 2006). Conceptually, sludge flocs can be divided into a number of sections, or classes, such as small and loose flocs, regular flocs, dense flocs and compact granule precursors. In fact, bacterial aggregates may be classified into two types in terms of the form of growth: a suspended-growth mode and an attached-growth mode (Rittmann & McCarty 2001). Loose sludge flocs are formed by bacteria in a suspended-growth mode, while granules are believed to be formed by bacteria in an attached-growth mode (Beun et al. 2002; Liu & Tay 2002; Yang et al. 2004). Based on this sectional concept, the change in the biomass concentration in a sludge class, \( X_i \), in a bioreactor can be written (Li & Li 2009) as

\[
\frac{dX_i}{dt} = Y_i \Delta S_i - f_i X_i,
\]

where \( \Delta S_i \) is the amount of organic substrate utilized by the biomass in class \( i \), \( Y_i \) is the corresponding observed yield coefficient, and \( f_i \) is the proportion of biomass discharging for the sludge class. Different types of sludge flocs have different substrate uptake capabilities. In general, we have \( \Delta S_i = p_i \Delta S X_i \), where \( \Delta S \) is the total amount of organic utilization and \( p_i \) is the actual substrate uptake factor of the biomass in class \( i \). In bioreactor operation, the sludge discharge ratios may differ between sludge classes due to their different settling velocities. Hence, \( f_i = q_i f \), where \( f \) is the overall sludge discharge factor and \( q_i \) is the effective sludge discharge factor for class \( i \). Assuming the same observed yield coefficient \( Y_{obs} \) for all sludge classes, the biomass growth dynamics for a sludge class in a bioreactor can be written as

\[
\frac{dX_i}{dt} = Y_{obs} p_i \frac{\Delta S}{X} X_i - Q_i X_i.
\]  

(2)

It is assumed that healthy growth conditions are provided for the sludge mixture in the bioreactor, such as a sufficient substrate feeding load and aeration intensity. Using the section-based mathematical model, the biomass dynamics during the start-up of a bioreactor for granulation can be simulated. The model suggests that the dominance of different types of biomass sludge can be achieved by adjusting the \( q_i \) factor.

Bioreactors and their operation

Four identical columns (H 22 cm \( \times \) D 5.2 cm) with a working volume of 0.4 L each were used as the batch column reactors (BCR) for the sludge granulation study. The four reactors were operated in a fixed sequential mode for a 24-hr cycle with 2 min of feeding, 22 hr and 56 min of aeration, 1 hr of sludge settling and 2 min of effluent withdrawal. The feeding synthetic wastewater consisted of glucose and other nutrients according to the chemical composition given by Tay et al. (2002). The influent had an organic concentration of 1,000 mg/L in terms of the chemical oxygen demand (COD), resulting in a COD loading rate of 1.0 kg/m³ d in the four reactors. Aeration was conducted from the bottom of each reactor at an air flow rate of 1.0 L/min during the aeration phase. The experiments were performed at room temperature, and the water temperature was 20–22°C. NaHCO₃ was dosed into the feed solution to maintain the pH of the reactors between 7.0 and 7.5, and the dissolved oxygen (DO) concentration in the sludge suspension was in a range of 2–5 mg/L.

Sludge was added as the seed biomass into each of the batch reactors at an initial mixed liquor suspended solids (MLSS) concentration of 2,000 mg/L. The seed sludge included activated sludge flocs and mature granules.
Activated sludge (Figure 1(a)) was collected from a full-scale sewage treatment plant (Stanley Sewage Treatment Works, Hong Kong). Aerobic granules (Figure 1(b)) had been cultivated from activated sludge in a sequencing batch reactor (Li & Li 2009). The mature granules sized around 2 mm and had a dense structure with a clear and smooth surface. The activated sludge (AS) flocs also had been acclimated for one month with the glucose-based synthetic wastewater before seeding into the reactors for the granulation study. The four batch reactors, R1, R2, R3 and R4, were seeded with different combinations of the AS flocs and mature granular sludge (GS) and operated with different sludge discharge methods.

In reactor R1, only AS was used as the seed sludge without adding granules. The daily sludge discharge was conducted by means of the selective discharge from the top after a few minutes of settling. For R2, R3 and R4, the seed sludge MLSS consisted of the same amount, i.e. 1,000 mg/L, of both AS and GS. During the reactor operation, sludge was also discharged once a day at a predetermined rate by different methods—selective discharge from the bottom for R2, unselective discharge for R3 and selective discharge from the top for R4. Hence, it was expected that, in comparison, the sludge discharged from R1 and R4 contained a higher fraction of small and slow-settling sludge flocs, the sludge discharged from R2 contained a higher fraction of dense and fast-settling sludge, and the sludge discharged from R3 was fully mixed sludge liquor. For reactor R2, after 40 days with the selective sludge discharge from the bottom, the sludge removal method was changed to the selective discharge from the top. The ratio of the daily sludge discharge from each reactor was adjusted slightly in order to maintain the MLSS concentration at around 2,000 mg/L.

For the method of unselective or mixed sludge discharge, a certain amount of the sludge mixture was withdrawn from the reactor column, while the sludge suspension was well mixed by aeration. For selective sludge discharge from the top, the sludge was allowed to settle in the column without aeration. During the settling phase, a certain amount of the sludge suspension was withdrawn from the surface down, and the slow-settling sludge in the suspension was therefore removed from the reactor. For selective sludge discharge from the bottom, also during the settling phase without aeration, a certain amount of the sludge mixture was withdrawn from the bottom of the reactor column. The purpose of this operation was to remove the fast-settling sludge from the mixture. The amount of the sludge discharged and the sludge concentration in each BCR were measured. Accordingly, the rate of daily sludge discharge was determined for each reactor with an intention to maintain the same biomass concentration and a constant F/M (food-to-microorganisms) ratio for all of the four batch reactors.

**Analytical methods**

The COD concentration, sludge MLSS concentration and effluent suspended solids (ESS) were measured according to the Standard Methods (APHA-AWWA-WEF 1998). The total organic carbon (TOC) concentration was measured with a TOC analyzer (IL550, HACH-Lachat, Milwaukee, WI, USA). The morphology of the sludge flocs and granules was observed under a stereomicroscope (S8 APO, Leica, Cambridge, UK) equipped with a digital camera (EC3, Leica, Cambridge, UK). For determination of the particle size distributions (PSD), the sludge matter
in a sample was separated by a screen with an opening of 1,000 μm. The smaller particles and flocs left in the sludge sample were measured for their size distribution by a laser diffraction particle counter (LS13 320, Beckman Coulter, CA, USA). The large particles and granules collected by the screen were placed on the stereomicroscope (Figure 1(b)) and sized by a computer-based image analysis system (analySIS 3.1, Olympus Soft Imaging Solutions, Germany). The size of an individual particle or floc was determined according to its projected area, \( A \), and expressed by its equivalent diameter according to
\[
d = \sqrt{\frac{4A}{\pi}} \quad (Li \ et \ al. \ 2003).
\]

RESULTS AND DISCUSSION

Simulation of the aerobic granulation process

The seed sludge was assumed to consist of five biomass classes with different structural features: looser flocs \( X_1 \), loose flocs \( X_2 \), dense flocs \( X_3 \), denser flocs \( X_4 \) and compact granule or granule precursors \( X_5 \). The total initial sludge MLSS concentration \( X \) used for the bioreactors was 2,000 mg/L. According to the particle size distribution measurement (LS 13 320, Beckman Coulter), the floc size of the seed sludge ranged from 1 to 1,500 μm, with a peak size of around 80 μm. The five sludge groups were classified initially for the seed AS based on the particle sizes, i.e. \( X_1 < 60 \mu m \), \( X_2: 60–150 \mu m \), \( X_3: 150–250 \mu m \), \( X_4: 250–400 \mu m \) and \( X_5 > 400 \mu m \). In R1 seeded with only the AS, estimated from the flocs size distribution, the initial MLSS concentrations for the five sludge groups were 800, 600, 300, 200 and 100 mg/L. Accordingly, in R2, R3 and R4 seeded with the mixture of AS and GS, the initial MLSS concentrations in the five sludge classes were 400, 300, 200, 100 and 1,000 mg/L. In connection to the experimental condition, the influent substrate concentration was 1,000 mg COD/L, the hydraulic retention time (HRT) was 24 hrs and the organic removal efficiency was around 95%. A typical \( Y_{obs} \) value of 0.3 mg SS/mg COD was assumed for the bioreactors. Loose flocs are believed to have a better substrate uptake capability than dense flocs and granules (Yang et al. 2004; Sears et al. 2006). Thus, the substrate uptake factors for the five sludge classes were assumed to have a ratio \( p_1:p_2:p_3:p_4:p_5 = 1.2:1.05:1.01:0.95:0.9/0.6 \) (0.9 for granule precursors and 0.6 for mature granules). These relative ratios were largely validated by the results of the organic uptake tests reported in the section below. Loose flocs normally have slower settling velocities than denser flocs and granules. Thus, a sludge discharge factor ratio \( q_1:q_2:q_3:q_4:q_5 = 2.0:1.5:1.0:0.5:0.1 \) was assumed for the method of selective sludge discharge from the top of the reactor column during the settling phase. In contrast, a discharge factor ratio \( q_1:q_2:q_3:q_4:q_5 = 0.1:0.5:1:1.5:2 \) was assumed for the method of selective sludge discharge from the bottom during the settling phase. When a mixed sludge discharge was used, \( q_1–q_5 \) would be equal to each other.

The biomass dynamics in relation to the granulation process can be well simulated. The changes in sludge concentrations over time in the five classes were calculated using the section-based model (Equation (2)) for the four reactors with different seed sludge mixtures and different sludge discharge methods. In R3 with unselective sludge discharge at an overall ratio of 15%, the loose sludge flocs \( X_1 \) and \( X_2 \) became more dominant and there was no increase in dense flocs \( X_4 \) and granules \( X_5 \) (Figure 2).
With the selective discharge of small and loose sludge flocs, the concentrations of $X_1$ and $X_2$ became lower and the granule precursors or granules accumulated in R1 and R4 (Figure 2). Granulation could also be achieved completely after running 40 days in R1 that had no seed of granules. It took only 10 days to complete granulation in R4 with both AS and GS as seed sludge. The result suggests that the selective discharge of loose sludge flocs is the crucial operating measure to achieve sludge granulation. If mature granules and granule precursors were included in seed sludge, the granulation process could be considerably accelerated (Figure 2). In contrast, with the selective discharge of dense flocs and granules from the bottom in R2, the concentrations of $X_1$ and $X_2$ increased rapidly and dense flocs and granules $X_4$ and $X_5$ disappeared after about 10 days (Figure 2). Changing the sludge removal method to selective discharge of small and loose sludge flocs from the top led to re-growth of the granules in R2. Comparison between the simulation results for different reactors indicates that sludge granulation cannot be achieved in a conventional activated sludge process which has the settled sludge discharged. The selective discharge of loose sludge flocs is the crucial operating factor for the formation and accumulation of aerobic granules in bioreactors.

**Experimental tests on the granulation process in the batch reactors**

The four laboratory batch reactors were operated in a comparable manner in terms of HRT, sludge discharge ratio, sludge retention time (SRT), biomass concentration and F/M ratio (Figure 3(a, b)). As a result, the treatment performance of the four BCRs was rather similar. For the same loading of 1.0 kg COD/m$^3$ d, all reactors performed well in organic removal (Figure 3(c)). The TOC removal efficiency was maintained at around 90% for treating the wastewater with a TOC of 380 mg/L.

As described previously, the methods of daily sludge discharge were different for the four column reactors. The different sludge discharge methods did lead to completely different sludge transformations in the reactors. With the selective discharge of small and slow-settling sludge flocs from the top, aerobic granulation was well achieved in reactors R1 and R4. It took a longer time of about 30 days to achieve granulation in R1 in which all seed sludge was AS flocs, whilst it took a much shorter time of 10 days or less to complete granulation in R4 in which half of the seed sludge was granules. For reactor R3 with mixed sludge discharge, the granules in the seed sludge disappeared gradually and the reactor turned into a typical activated sludge reactor. In R2 seeded with both mature granules and AS flocs, the granules disappeared rapidly when the method of selective discharge of settled sludge from the bottom was used. After the method was changed to selective discharge of small and slow-settling sludge flocs from the top during the settling phase, granule formation and accumulation was observed, and granulation was achieved eventually in the reactor. Therefore, the selective discharge of small and loose sludge flocs was shown as the controlling factor for aerobic granulation.

Particle size distributions of the sludge in different reactors were measured throughout the experimental study.

![Figure 3](https://iwaponline.com/wst/article-pdf/62/10/2442/446147/2442.pdf)
In a PSD of the sludge mixture, the biomass was classified in terms of size as follows: the sludge particles smaller than 60 \( \mu \)m were considered as small and loose flocs \( X_1 \), the sludge particles sizing between 60 and 400 \( \mu \)m were grouped as larger and denser flocs \( X_2 + X_3 + X_4 \), and the particles larger than 400 \( \mu \)m were granules or granule precursors \( X_5 \). The results of PSD measurements during the laboratory experiments are presented in Figure 4 and compared with the results of model simulations. Due to the selective sludge discharge in R1, small granules became visible about 10 days after the start-up of the experiment, and \( X_5 \) became dominant in sludge after about 30 days. Granulation nearly completed in R1 after 40 days, which is in agreement with the simulation result (Figure 4(a–e)). In contrast, in the early phase of R2 with selective discharge of granules and denser flocs from the bottom, granules \( X_5 \) disappeared after 20 days and flocs became dominant under the same loading conditions (Figure 4(f)). From day 40 onward, changing the sludge removal method to selective discharge of loose flocs from the top led to the accumulation of granular sludge \( X_5 \) again. This experimental result also compares fairly well with the model predictions for the biomass growth dynamics (Figure 4(b)).

In R3, which was subject to mixed sludge discharge, granulation did not appear to take place even when mature granules were seeded in the reactor during start-up. The sludge in the form of flocs \( X_1 - X_4 \) became dominant after 60 days of the reactor operation. The experiment result is consistent with the modeling result (Figure 4(c–g)). The comparison between the results of R1, R2 and R3 proves that the selective discharge of small and slow-settling sludge flocs is the key condition for the formation and growth of aerobic granules. Mature granules in the attached-growth mode would not lead to complete sludge granulation if loose flocs were not discharged from the sludge mixture. However, seeding with granular sludge, together with selective discharge of loose flocs, could accelerate the granulation process, as observed in R4 (Figure 4(h)). Due to the increased portion of granules in the seed sludge, granular sludge \( X_5 \) would take up more substrate to grow and became dominant rapidly in the reactor. The granulation dynamics were predicted well by the numerical simulations (Figure 4(d)). In addition, the comparison between R1 and R4 demonstrates the benefit of adding granules in the seed sludge for aerobic granulation. Granulation was achieved in less than 20 days in R4, which was considerably faster than that in R1 under the same loading and operating conditions (Figure 4(e–h)).

**Comparison in substrate uptake capability between the biomass in different batch reactors**

The substrate uptake rates by the biomass sludge in the four reactors were tested at different phases of the
experimental study. Based on the TOC reduction after the substrate feeding into the reactors, the specific organic uptake rates were estimated for the biomass in sludge suspension (Figure 5). The organic uptake rates show a certain degree of fluctuations with time. Nonetheless, the comparison of the substrate uptake rates among the four reactors indicates that the sludge with a high portion of granules had a lower substrate uptake rate. For example, the sludge flocs in R3 had an organic uptake coefficient more than two times higher than that of the granular sludge in R4 (Figure 5). Loose sludge flocs had a clear advantage over dense sludge flocs and granules in the uptake of substrates and nutrients. Compared with tightly-packed large flocs and granules, small and loose flocs apparently can obtain substrates more easily from the suspension, which allows them to grow faster (Yang et al. 2004; Li & Li 2009). In other words, loose activated sludge flocs can readily out-compete small granules for substrates. In a mixture of sludge flocs and granules, there is less substrate available for uptake by dense flocs and granules due to the competition from loose sludge flocs. Without selective sludge discharge, it is impossible for granules to grow and become dominant, as observed in R2 and R3. In contrast, selective discharge of small and slow-settling flocs eliminates these competitors from the system and allows more granule growth, resulting in granulation, as observed in R1 and R4. In future work, more effective strategies are to be developed to control the rate of aerobic granulation and the size of granules in order to improve the stability of the granular sludge system for wastewater treatment.

CONCLUSIONS

- A section-based mathematical model was used to describe the biomass dynamics during aerobic granulation in a bioreactor. The simulation results compare well with the experimental results obtained from four batch reactors using different seed sludge and sludge discharge methods.
- The results of both the model simulation and the laboratory experiment indicate that the selective discharge of relatively small and loose sludge flocs is the crucial operating factor for a batch reactor to achieve aerobic granulation. Addition of granules in the seed sludge can accelerate the granulation process.
- The main mechanism of the selective sludge discharge method for granulation is that discharge of loose sludge flocs removes these competitors in the suspended-growth mode and makes the substrates more available for uptake by the attached-growth biomass. This would allow more growth and accumulation of granules in a bioreactor, resulting in complete granulation.

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