Effect of the food-to-microorganism (F/M) ratio on the formation and size of aerobic sludge granules

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ABSTRACT

Laboratory experiments were carried out to investigate the effect of the sludge loading, or the food-to-microorganism (F/M) ratio, on the rate of aerobic granulation and the size of the granules in biological wastewater treatment. Four column batch reactors were used with a similar sludge suspended solids (SS) concentration of around 2000 mg/L. The reactors were fed with a glucose-based wastewater at different chemical oxygen demand (COD) concentrations, resulting in F/M ratios from 0.3 to 1.1 g COD/g SS-d. A higher F/M ratio appeared to promote faster formation of larger granules and a lower F/M ratio led to slower formation of smaller granules. Upon complete granulation, the granules became rather stable in size, and the mean diameter of the granules in different reactors increased from 1.2 to 4.5 mm linearly with the F/M ratio applied. Molecular analysis of the sludge did not show the domination of any particular bacterial species during the granulation process. It is apparent that applying different F/M ratios in different granulation stages, e.g., a higher F/M in the early stage and a reduced F/M in the later stage, can be an effective start-up strategy to facilitate rapid granule formation and sustain small and healthy granules in bioreactors.

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1. Introduction

Aerobic sludge granulation is a new microbial immobilization technique that has the potential to fundamentally advance the biological wastewater treatment technology [1–3]. Compared to conventional activated sludge, the dense granule structure confers on granular sludge an excellent settling ability that allows for rapid sludge-effluent separation, a high level of biomass concentration, and a greater organic treatment loading capability [3–5]. Aerobic granules are considered as a special form of bacterial biofilm growth in suspension [5,6]. It has been demonstrated that granulation can be achieved by means of selective discharge of small and slow-settling sludge flocs [7]. However, the influences of process conditions on the quality and property of granules are still issues of investigation. For instance, the size of aerobic granules has a profound impact on the stability and treatment performance of granular sludge, and larger granules may become less stable in wastewater treatment. However, effective measures

for controlling the size and improving the stability of aerobic granules in a bioreactor remain to be developed.

During the start-up of aerobic granulation, a short settling period is commonly adopted to force the discharge of small and loose sludge flocs from the reactors and hence to retain denser sludge [3,7]. Such an early washout of small and slow-settling sludge from the suspension leads to a loss of biomass, resulting in an increase in sludge loading rate [1,3]. A high food-to-microorganism (F/M) ratio would enhance microbial growth [8] and hence facilitate the aerobic granulation process [9,10]. In connection to biomass growth, the sludge loading rate, or the F/M ratio, in a bioreactor could be an essential parameter that regulates the size of granules. However, the correlations between the F/M ratio and the rate of aerobic granulation and the size of granules have not been well established.

F/M ratio is a process variable that can be easily adjusted in operating bioreactors. A suitable F/M can be favorable for both the progress of granulation and the size control of granules. In this study, laboratory experiments were conducted with batch reactors to investigate the effect of the F/M ratio on the formation, size, and stability of granules. The rates of sludge granulation and the stable size of granules formed under different F/M conditions were determined. The findings are essential to the development of an effective start-up strategy for the formation and maintenance

of small and healthy granules in long-term biological wastewater treatment operation.

2. Materials and methods

2.1. Experimental set-up and operation

Four 0.4-L graduate cylinders (H 22 cm $\times$ D 5.2 cm) were used as column batch reactors for the experimental study on aerobic granulation. Activated sludge from a full-scale sewage treatment plant (Stanley Sewage Treatment Works, Hong Kong) was used as the seed sludge after one month of laboratory acclimation with a glucose-based synthetic wastewater. The seed sludge was well mixed before loading into the four reactors to have the same initial mixed liquor suspended solids (MLSS) concentration of 2000 mg/L. The reactors were fed once a day with a synthetic wastewater consisting of glucose, NH_4Cl, KH_2PO_4 and $NaHPO_4 \cdot 6H_2O$, and other nutrients that was prepared according to the formula given by Tay et al. [10].

The operating condition was the same for the four reactors, R1, R2, R3, and R4, except the feed substrate concentration. Different influent organic concentrations in terms of the chemical oxygen demand (COD) – 600, 1400, 2200, and 2200 mg/L – were used for the four reactors to have F/M ratios of 0.3, 0.7, 1.1, and 1.1 g COD/g SS-d for R1, R2, R3, and R4, respectively. For R4, however, the influent COD concentration changed from 2200 to 600 mg/L in the third week after its start-up, which reduced the F/M ratio from 1.1 to 0.3 g COD/g SS-d. The experiments were carried out at room temperature, and the water temperature was 20–22 °C. $NaHCO_3$ was dosed into the feed solution to maintain the pH of the reactors in the neutral range between 7.0 and 7.5. Aeration was conducted from the bottom of the reactors at an air flow rate of around 1.0 L/min during the aeration phase, and the dissolved oxygen (DO) concentration in the sludge suspension was in a range of 2–5 mg/L.

At the end of each 24-h cycle, the sludge was allowed to settle in the column without aeration. During the early settling phase, a certain amount of the sludge suspension was withdrawn by siphon below the water surface. The slow-settling sludge flocs in the suspension were therefore removed from the reactors. The sludge concentration in each reactor and the amount of daily sludge discharge from the reactor were measured. Accordingly, the rate of the daily sludge discharge was then adjusted to maintain the MLSS concentration at about 2000 mg/L in the reactors. The purpose of this operation was to selectively remove the small and slow-settling sludge from the sludge mixture while keeping the sludge concentration and F/M ratio at the predetermined levels in each reactor [7]. After another 30 min of sludge sedimentation, the supernatant was withdrawn from the reactors, and the wastewater influent was added into each reactor to restore its original water volume of 0.4 L.

2.2. Determination of the organic uptake capability and settling behavior of the granules

After the completion of aerobic granulation, the granular sludge was characterized for its organic uptake rate and settling velocity. For each sludge sample from a reactor, the organic uptake test was performed in a 250-mL glass beaker, and the sludge and glucose concentrations were 1000 mg MLSS/L and 300 mg/L, respectively. The sludge mixture was sampled at various time intervals and the glucose concentration in the liquid phase of the sludge was measured. The mass-balance equation for organic in the reactor may be written as $(dS/dt)V = QS_0 - QS + rV$, where S is the substrate concentration and S_0 is its initial concentration, t is time and r the rate of the substrate removal. Since $Q = 0$ for a batch reactor and a first-order correlation $r = -(kX)S$ may be assumed for the early phase of substrate uptake, where k is a specific rate coefficient and X the biomass concentration in the batch reactor, the rate of organic removal may be approximated by $dS/dt = -kXS$. By linear regression of $\ln(S_0/S)$ versus Xt, the apparent specific organic uptake rate coefficient of the sludge can be estimated.

The settling experiments were conducted for individual mature granules following the procedure described by Xiao et al. [11] in water column. The acrylic settling column was 90 cm in height and 8.1 cm in diameter with a corn bottom and a valve. For each setting test, a granule was placed at the top of the water column, and the settling velocity of the granule through the lower 60 cm was measured. The granule reaching the bottom was then released and retrieved. The granule was placed on a stereomicroscope (S8 APO, Leica, Germany) equipped with a digital camera (EC3, Leica, Germany), and the granule was sized according to its projected area, A, and expressed by the equivalent diameter of $d = \sqrt{4A/\pi}$ [12].

2.3. Water and sludge analysis

The COD and SS concentrations and the sludge volume index after 5 min (SVI_5) were measured following the Standard Methods [13]. The total organic carbon (TOC) concentration was measured using a TOC analyzer (IL550, HACH-Lachat, USA). The glucose concentration was determined by a UV/VIS spectrophotometer (Lambda 25, Perkin Elmer, USA) according to the phenol–sulphuric acid method [14]. The morphology of the aerobic granules was observed under a stereomicroscope (S8 APO, Leica, Germany). The particle size distributions (PSD) of the sludge samples (<2000 μm) were measured using a laser diffraction particle counter (LS13 320, Beckman Coulter, USA). When granules grew larger, photographs of the granules

in a sludge sample were taken by a digital camera with the stereomicroscope. The photo images of the granules were analyzed by an image analysis system (analySIS 3.1) for PSD of the granular sludge.

A heat extraction method was modified to extract extracellular polymeric substances (EPS) from activated sludge and granules [15]. The sludge was first washed three times and dewatered by centrifugation (5810R, Eppendorf, Germany) in a 25-mL tube at 4000 $\times$ g for 5 min. The sludge pellet in the tube was then homogenized into 2.5 mL of 0.05% NaCl solution by a beadbeater (Mini-beadbeater™, Biospec, USA) without beads. The sludge mixture was then diluted with the NaCl solution to its original volume of 25 mL. The sludge suspension was heated to 60 °C in a water bath for 30 min, and the sludge mixture was then centrifuged at 4000 $\times$ g for 15 min. The supernatant collected was regarded as the EPS extract of the sludge, which was analyzed for TOC, polysaccharides (PS), proteins, and humic-like substances (HS). The PS content was determined using the phenol–sulphuric acid method [14] with glucose as the standard. Proteins and HS were analyzed by a UV/VIS spectrophotometer (Lambda 25, Perkin Elmer, USA) following the modified Lowry method [16] using bovine serum albumin (Sigma) and humic acid (Fluka) as the standards, respectively.

2.4. DNA extraction and denaturing gradient gel electrophoresis (DGGE) analysis of the sludge

DGGE band profiles were used to reveal the most abundant DNA types among the microbial species in a sludge sample [17]. The genomic DNA of the biomass was extracted following the protocol described by Zhuang et al. [18] using a beadbeater (Mini-beadbeater™, Biospec, USA) and a microcentrifuge (MiniSpin plus®, Eppendorf, Germany). Subsequently, the variable V3 region of the bacterial 16S rDNA gene sequence was amplified by polymerase chain reaction (PCR) [19] with a DNA Engine® Peltier Thermal Cycler (PTC-200, MJ Research, USA). A touchdown thermal profile technique was used for the PCR procedure [20]. As described by Li et al. [21], the PCR-amplified DNA products were then separated by DGGE, and the DGGE gel images were acquired using the ChemiDoc (Bio-Rad, USA) gel documentation system. The DGGE band patterns were then used to calculate the Shannon–Weaver index for the species diversity of different sludge samples [21].

3. Results and discussion

3.1. Formation and physical properties of the aerobic granules

Aerobic sludge granulation was well achieved in all four batch reactors operated at different F/M ratios (Figs. 1 and 2). The sludge MLSS concentrations were kept largely comparable between the four reactors during the experimental study. Within the range tested, the F/M ratio, or the biomass loading rate, did not appear to be the crucial factor for granule formation. As indicated previously [7], selective discharge of small and slow-settling sludge flocs from the sludge suspension was the determining factor for aerobic granulation. However, the F/M ratio displayed a profound effect on the rate of granulation and the morphological property of the granules. In general, a higher F/M ratio brought about faster formation of larger granules, and a lower F/M ratio led to slower formation of smaller granules (Figs. 2 and 3). In R1 at a low F/M of 0.3 g COD/g SS-d, small granules were observed after 25 d and granulation was fully achieved throughout the column reactor after 40 d. In R2 at a medium F/M of 0.7 g COD/g SS-d, granules appeared after 16 d and granulation was completed after 20 d. In R3 and R4 at a high F/M of 1.1 g COD/g SS-d, granules were observed after only 7 d and granulation was completed in about 14 d. A comparison between the different column reactors suggests that faster biomass growth under a higher F/M condition would facilitate a rapid granule formation and growth. Nonetheless, a high biomass loading, e.g., F/M > 0.5 g COD/g SS-d [22], may not be a necessity for complete sludge granulation.

As described previously, upon complete granulation, the F/M ratio for R4 was reduced from 1.1 to 0.3 g COD/g SS-d after 20 d of the start-up. With the decrease in F/M, breakage of large granules occurred, resulting in losses of the biomass. However, the aerobic granules in R4 became stabilized eventually at smaller sizes after about 10 d, and the granules appeared to be comparable to those formed in R1 at the low F/M of 0.3 g COD/g SS-d (Figs. 2 and 3). Based on the rate of sludge discharge from the four reactors, the sludge retention time (SRT) was kept at 15, 8, and 5 d for R1, R2, and R3,