Aerobic granulation in sequential sludge blanket reactor

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Abstract Aerobic granulation was studied in a column-type of sequential sludge blanket reactor. Reactor was operated 4 hours per cycle under a chemical oxygen demand (COD) loading rate of 6.0 kg/m^3/d by using acetate as substrate. Results showed that aerobic granules with a mean diameter of 0.35 mm were observed at cycle 42. With granulation proceeding, the sludge volume index (SVI) value gradually decreased, and to an average value of 50 mL/g at stable granulation period. Observation of granules' microstructure by scanning electron microscopy (SEM) showed that rod bacteria were dominant in granules with lots of cavities presented. An increase in cell hydrophobicity was observed after the appearance of aerobic granules. The cell hydrophobicity of sludge was found to be about 50% higher after granulation. It appears that high hydrophobicity could induce cell attachment and further strengthen cell–cell interaction; cell hydrophobicity might therefore play a major role in the formation of aerobic granules.

Keywords Aerobic granulation; hydrophobicity; sequencing reactor; SVI

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Introduction

The performance of a biological system for wastewater treatment depends significantly on the active biomass concentration, the overall biodegradation rates, the reactor configuration and the feeding rates of the pollutants and dissolved oxygen. For the conventional activated sludge process, separation efficiency of biosolids from liquid phase has a significant implication for the design of biological wastewater treatment systems. It is well known that the separation efficiency of biosolids is inversely related to the biomass concentration in the aeration tank (Kiely, 1997). Research on anaerobic granulation showed that microbial granulation would be an ideal engineering way to solve technical problems encountered in conventional suspended culture. In fact, anaerobic granules cultivated in upflow anaerobic sludge blanket (UASB) reactors exhibit excellent settling ability leading to good solid–liquid separation (Lettinga *et al*., 1984). As compared to conventional activated sludge flocs, the advantages of granular sludge are known as: regular, denser and stronger microbial structure, good settling ability, high biomass retention, and ability to withstand high organic loading rate.

A very comprehensive literature has been documented for anaerobic granular sludge, both in microbiology and in engineering. Granulation by methanogens (Lettinga *et al*., 1984), acidifying bacteria (Vanderhaegen *et al*., 1992), nitrifying bacteria (De Beer *et al*., 1993; Van Benthum *et al*., 1996) and denitrifying bacteria (Van der Hoek, 1988) have also been reported. These previous works seem to indicate that granulation process would not be strictly restricted to some specific species. It should be pointed out that there is little information currently available for aerobic granulation. Therefore, the main objective of this research is to study granule formation under aerobic conditions. Meanwhile, the microstructure of granules was also investigated by scanning electron microscopy (SEM). It is expected that the research results would be useful for better understanding of the mechanism responsible for aerobic granulation.

Materials and methods

Experimental set-up

One column with a working volume of 2.3 L was used as sequential aerobic sludge blanket reactor during the study. Reactor has a height of 80 cm with a diameter of 60 mm. It was operated sequentially as 5 minutes of influent filling, 225 minutes of aeration, 5 minutes of settling and 5 minutes of effluent withdrawal. Effluent was discharged at the middle port of the column. An air velocity of 4.0 L/min was supplied to the reactor, equivalent to a superficial upflow of 2.4 cm/s. Reactor was applied with a substrate loading rate of 6.0 kg COD/m^3/d, corresponding to the influent COD concentration of 2000 mg/L. The experiment was conducted in a temperature controlled room at 25°C.

Media

Reactor was started up by using 650 mL of sludge acclimatized for one week by acetate substrate in a batch mode. Original seeding sludge for acclimatization was taken from a local municipal wastewater treatment plant (average floc size 0.12 mm; sludge volume index, 205 ml/g). The composition of synthetic wastewater used for this study is as follows: sodium acetate 2.93 g/L, NH_4Cl 350 mg/L, K_2HPO_4 30 mg/L, KH_2PO_4 25 mg/L, $CaCl_2$ $2H_2O$ 30 mg/L, $MgSO_4$ $7H_2O$ 25 mg/L, $FeSO_4$ $7H_2O$ 20 mg/L, and microelement solution 1.0 mL/L. This gives a COD of 2000 mg/L. Microelement solution contained: H_3BO_3 0.05 g/L, $ZnCl_2$ 0.05 g/L, $CuCl_2$ 0.03 g/L, $MnSO_4$ H_2O 0.05 g/L, $(NH_4)_6$ Mo_7O_{24} $4H_2O$ 0.05 g/L, $AlCl_3$ 0.05 g/L, $CoCl_2$ $6H_2O$ 0.05 g/L, $NiCl_2$ 0.05 g/L (Tay *et al*., 2001).

Analytical procedures

Effluent sample was analysed for COD and sludge sample for mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI), specific oxygen utilization rate (SOUR) and specific gravity by following the standard methods (APHA, 1995). Granule size was measured by laser particle size analysis system (Malvern MasterSizer Series 2600), or image analysis system (Image-Pro Plus, V4.0, Media Cybernetics) with an Olympus SZX9 microscope. Microbial observation was conducted by using either microscopy or image analysis (IA). Cell hydrophobicity was determined with the method described by Rosenberg *et al*. (1980). Hexadecane is used as the hydrophobic phase. Hydrophobicity is expressed as the percentage of cells adhering to the hexadecane after 15 minutes of partitioning. Microbial structure was observed with a scanning electron microscopy (SEM) (Stereosan 420, Leica Cambridge Instruments). The granule samples were gently washed with phosphate buffer solution and allowed to settle naturally. Granules were then fixed with 4% paraformaldehyde and left for 4 hours. The fixed granules were dehydrated by successive passages through 20, 40, 60, 80, 100% ethanol. Then they were dried either by Freeze Dryer (Edwards, England) or Critical Points Dryer (E3000, VG Microtech, England) and finally observed by SEM.

Results

Observation of aerobic granule formation

Seeding sludge used for the experimental startup had a mean floc size of 0.12 mm. It shows a fluffy, irregular and loose-structured morphology, as see in Figure 1A. The evolution of sludge morphology with the operation was monitored by using the IA technique. Size variation with the operation time was monitored by mastersizer analysis system. Floc size gradually increased since startup as seen in Figure 2. At cycle 42, it increased to 0.35 mm. Tiny granules were observed at this time. Granules with a mean size around 0.4 mm prevailed in the reactor during later operations (Figure 2). Figure 3 shows a comparison of size distribution by volume between seeding sludge and granular sludge at cycle 120. Most of

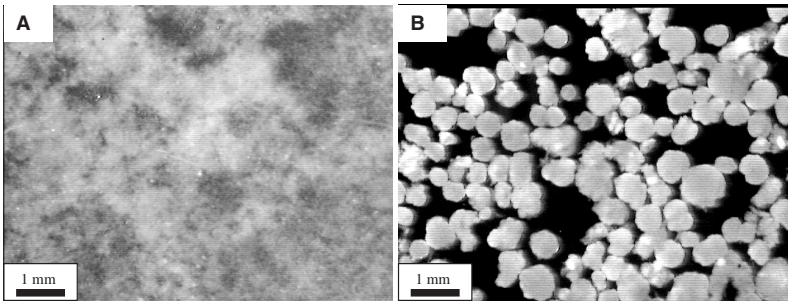

Figure 1 Comparison of seeding sludge (A) and aerobic granules (B) by image analysis

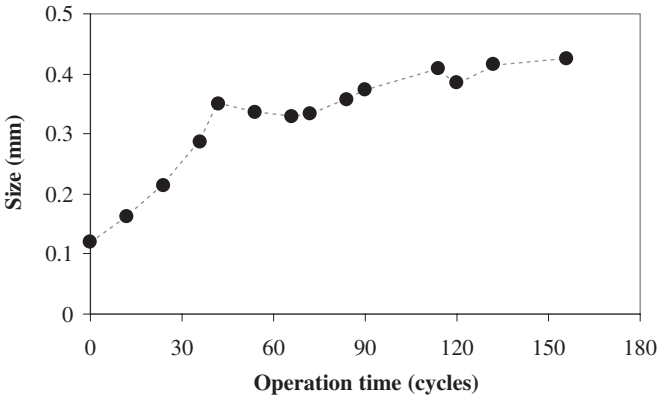

Figure 2 Sludge size versus the operation cycles

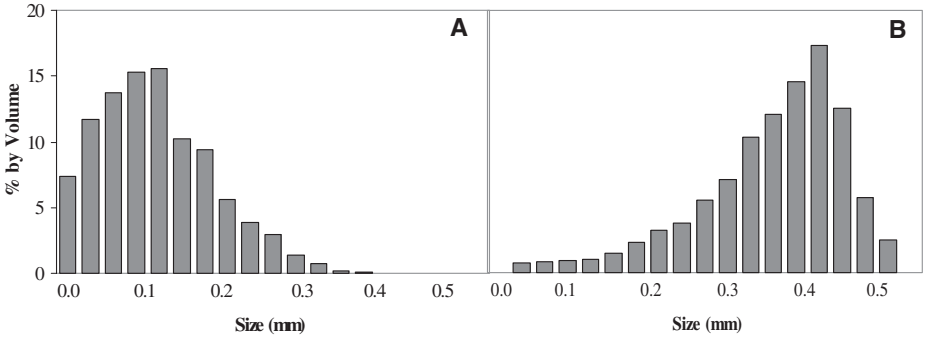

Figure 3 Comparison of size distribution between seeding sludge (A) and granular sludge (B) at cycle 120

the biopartible size of seeding sludge concentrates around 0.1 mm (Figure 3A). But at cycle 120, the concentration was shifted to 0.4 mm. It can be seen that small sized particles co-existed with large sized granules (Figure 3B). The morphology of aerobic granules by IA is shown in Figure 1B. Compared with seeding sludge, it can be seen that aerobic granules have a regular round shape and a clear outer surface. An SEM view of the granule and its surface at high magnification shown in Figure 4. As seen in Figure 4B, rod bacteria were predominant in granules, and lots of cavities were present. These cavities can enhance substrate transfer from the bulk to granules and intermediate or by-product transfer from inside granules to the bulk.

Sludge settling property

Seeding sludge had a SVI value of 205 mL/g. It gradually decreased along with the formation of aerobic granules as seen in Figure 5. After the formation of aerobic granules, it

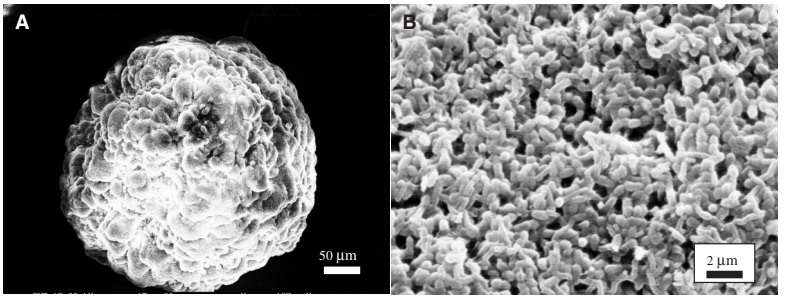

Figure 4 Photographs of granule (A) and its surface view at high magnification (B) by SEM

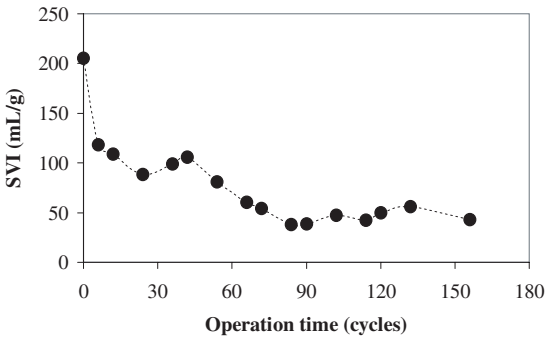

Figure 5 SVI versus the operation cycles

decreased to an average value of 50 mL/g. Obviously, the settling ability of sludge was improved significantly after aerobic granule formation.

The specific gravity of granular sludge also increased after granulation. It was 1.0008 kg/L at the beginning of the experiment, and increased to an average value of 1.0069 kg/L during the granulation period. The specific gravity of sludge reflects the compactness of a microbial community. The significant improvement of specific gravity for granular sludge indicated its highly compact structure.

Cell surface hydrophobicity

A significant difference in cell hydrophobicity was observed before and after the formation of aerobic granules. It increased from a value of 50.6% in the period abefore granulation to 75.1% after granulation, i.e. about 50% higher for granular sludge. It appears that the formation of aerobic granules is coupled to an increase in thecell hydrophobicity. Hydrophobicity of cell surface has generally been considered to play an important role in the self-immobilization and attachment of cells to a surface.

Retainable biomass in reactor and COD removal

Biomass concentration in terms of MLVSS was 0.81 mg/L at the beginning of the experiment (Figure 6). It was increased to 2.5 mg/L at cycle 6, and then fluctuated at this level until the appearance of aerobic granules at cycle 42. Biomass concentration then gradually increased, and reached a relatively stable level of about 6.0 mg/L after cycle 90. It is obvious that granulation significantly increased the biomass concentration in the reactor. COD removal showed a very stable efficiency of 95% during the granulation period, as seen in Figure 6. Granulation could lead to more biomass being kept in the reactor because of the good settling property of granular sludge. This in turn would be favourable for reactor performance and stability.

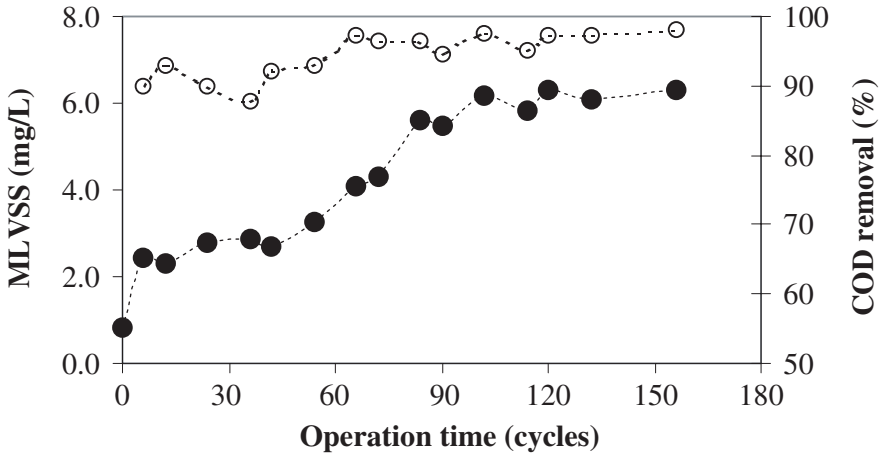

Figure 6 Biomass retained in reactor (•) and COD removal (o) versus the operation cycles

Discussion

Granules were observed after 42 cycles of operation. They show a smooth, compact and round shape, compared with seeding sludge which has a fluffy, loose, and irregular structure, as seen in Figure 1. Granulation is a gradual process from a bioparticle size of 0.1 mm to final stable granules with sizes around 0.4 mm. With the proceeding of granulation, the SVI value gradually decreased, to a stable level of about 50 mL/g at granulation period. There is a significant improvement, compared with the seeding sludge of 205 mL/g. Granulation greatly improved sludge's settling property. The specific gravity of granular sludge increased to 1.0069 kg/L from the seeding level of 1.0008 kg/L. Because of the compact and dense granules with good settling property, a higher biomass concentration of 6.0 g/L (MLVSS) was retained in the reactor.

Formation of granules was correlated with an increase in cell surface hydrophobicity. It is around 50% higher for granular sludge than seeding sludge. Hydrophobic binding has a prime importance for self-immobilization and cell-to-cell attachment (Marshall and Gruickshank, 1973; Pringle and Fletcher, 1983). The high cell hydrophobicity might induce cell attachment, and initiate the aerobic granulation, and finally keep the cell together. Previous research also indicated that the hydrophobicity of microorganisms would play a crucial role in the formation of anaerobic granules (Mahoney *et al*., 1987; Tay *et al*., 2000). Mahoney *et al*. (1987) reported that the biosolids washed out from the UASB reactors were more hydrophilic than the reactor sludge. In a thermodynamic sense, increasing the hydrophobicity of cell surfaces causes a decrease in the excess Gibbs energy of the surface, which is in favor of solid (cells)–liquid phase separation, i.e. the formation of microbial aggregate. Consequently, a higher hydrophobicity of the cell surface would result in a more strengthened cell-to-cell interaction and further a dense and stable structure. The present research shows that hydrophobicity increased after granulation. It is reasonable to consider that cell hydrophobicity might play an important role for the aerobic granulation process.

Conclusions

Aerobic granules, which mainly consist of rod bacteria, were successfully cultivated in a sequential sludge blanket reactor. Granules have a smooth, round and compact structure compared with loose, fluffy and irregular seeding sludge. Settling property of the granular sludge improved significantly in terms of the SVI value and specific gravity. Results show that hydrophobicity of cells increased by about 50% after granulation. It is reasonable

to consider that cell hydrophobicity might induce cell attachment and further strengthen cell-to-cell interaction. Cell hydrophobicity might play an important role for the aerobic granulation process.

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