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Aerobic Granulation under Low Selection Pressure

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Abstract. As inoculum sludge from activated sludge wastewater treatment plant was used, aerobic granular sludge was cultured under low selection pressure, which was operated with settling times of 20 and 10 minutes separately in the two SBRs fed with sodium acetate as carbon substrate. After 80 days operation, SVI values were up to 53 ml/g and 43 ml/g, the average particle diameter reached 0.4 mm and 1.0 mm, respectively. Although granules could be found on the 7th day, full granulation need longer time than higher selection pressure with shorter settling time. Compared with the results of settling time 20 minutes and 10 minutes, we found that granules in SBR with settling time of 20 minutes occurred later than that of 10 minutes, and were also relatively looser and smaller size. SEM images showed there were no filamentous bacteria in the granular sludge.

Keywords: aerobic granular sludge, low selection pressure, granulation, filamentous bacteria.

1. Introduction

As a self-immobilized biotechnology for wastewater treatment, aerobic granular sludge is characterized by outstanding settling rate, high biomass concentration and various species of bacteria just in a single granule [1-3], while the mechanisms behind aerobic granulation in SBR are not fully understood yet [4-5].

The physical settling-washing out action was a pure screening step without a demand for the microbes to respond to or to make changes upon the settling time carryout, hence having different intended meaning by the biological evolution theory. In SBR operation, only particles that settle within a given time frame could be retained in the reactor, while those with poor settle-ability were washed out from the system. According to biological evolution theory, this physical screening step was considered to provide a "selection pressure" to the biomass in the reactor, and only those which adapted to this challenge (to become big and dense enough to settle fast) would survive and be retained in the reactor. Tay et al. [6] studied nitrifying bacterial granulation at different selection pressures and concluded the need of high selection forces for granulation. The required selection pressures had been created by keeping the constant column height and varying the discharge port height. The low selection pressure in this study was to use a longer settling time to cultivate aerobic granular sludge.

In recent years, many researchers [7-10] have focus on reducing the settling time required for activated sludge flocs by forming dense flocs. The settling time acts as a major hydraulic selection pressure on microbial community. A short settling time preferentially selects for the growth of fast settling bacteria and the sludge with a poor settleability is washed out. Wang Q. et al. [11] concluded that aerobic granular sludge was cultivated by means of decreasing sedimentation time to wash out poor-settling biomass and increasing OLR to ensure sufficient new biomass growth, granules started to appeared on the 67th day. Qin et al. [12] reported that aerobic granules were successfully cultivated and became dominant only in the SBR operated at a settling time of 5 minutes. Mixtures of aerobic granules and suspended sludges were observed in the SBRs

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run at settling times of 20, 15 and 10 minutes. Therefore, choice of an optimal settling time was very important in aerobic granulation. Short settling time caused poorly settled suspended biomass washout and retained only well settled granules. Studies have indicated that short settling time could enhance aerobic granulation. McSwain et al. [13] demonstrated that after 2 minutes of settling time, the mature granules were well.

The main objectives of this study were to investigate the effect of low selection pressure on the formation of aerobic granulation and further to analyse the characteristics of aerobic granulation. Two SBRs were operated with sodium acetate as carbon substrate and two different settling times, each of which was inoculated with aerobic sludge from a conventional activated sludge wastewater treatment plant.

2. Materials and methods

2.1. Reactor and operation

Two cylindrical column type reactors with a working volume of 4 litres, a total height of 100 cm and an internal diameter of 9 cm were used for cultivating aerobic granules. R1-R2 were operated at a respective settling times of 20 and 10 minutes, while the other operation conditions were kept the same (3 h of cycle time, 10 min of influent filling, 2 h of aeration time, and 10 min of effluent withdrawal). Two reactors were supplied with an air flow rate of 0.4 m^3/h, equivalent to a superficial upflow air velocity of 1.75 cm/s. A substrate loading rate of 4.0 kgCOD/m^3 day was applied to R1-R2. Effluent was discharged at the middle point of the column. The only variation in operating strategy was the settling and idle times.

2.2. Wastewater composition

The synthetic wastewater [14] used in the study mainly consisted of: sodium acetate (COD≈1000 mg/l)，NH_4Cl (NH_4^+-N≈35 mg/l), KH_2PO_4 (PO_4^{3-}-P≈10 mg/l) and other necessary nutrients.

2.3. Seed sludge

Seed sludge was taken from an aeration tank of the Si-bao sewage treatment plant in Hangzhou city, P.R. China. The seeding sludge had a fluffy, irregular and loose-structure, and was brown in color. The SVI values were up to 160 ml/g.

2.4. Analytical methods

The mixed liquor suspended solids (MLSS), Sludge volume index (SVI), NH_4^+-N and COD_{Cr} were measured according to Standard Methods, P.R. China. The images of flocs and granules were digitally captured under a kind of Motic electron microscope (EMS) and analyzed by a computerized image analysis system with the software “Image pro-plus”. Scanning electron microscopy (SEM) photos consisted of a series of procedure. The samples of aerobic granule sludge were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4; 0.1 M) for 4h at 4℃, washed in phosphate buffer and post-fixed in 1% osmium tetroxide at 0℃ for 1.5h. The fixed material was then dehydrated using a graded ethanol series and infiltrated. For SEM, fixed samples were dehydrated and subjected to critical-point drying using liquid CO_2. They were then sputter-coated with gold and examined by means of a kind of XL-30ESEM scanning electron microscope at an accelerating voltage of 25 kV.

3. Results and discussion

3.1. The morphology changes of sludge

The morphology of aerobic granular sludge in two reactors was shown in Fig. 1.

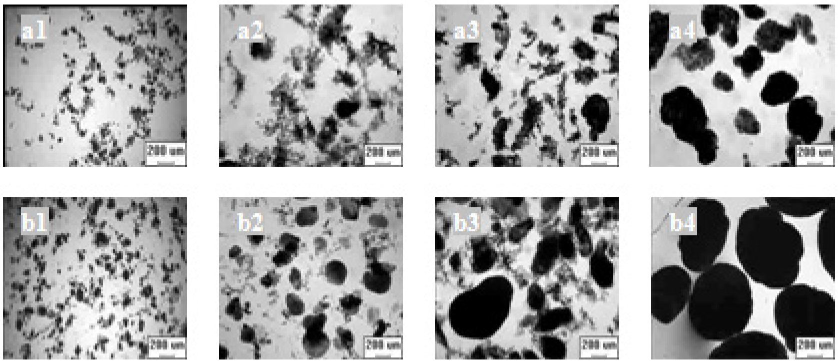

Fig. 1: Morphology of aerobic granules developed in two reactors. (a1, a2, a3, a4. Morphology of granules developed on day 7, 32, 53 and 80 from R1 reactor, respectively. b1, b2, b3, b4. Morphology of granules developed on day 7, 32, 53 and 80 from R2 reactor, respectively. Bar=200μm)

As illustrated in Fig. 1, tiny granules first appeared in two reactors after 7 days running, and granules in R2 reactor had advantage on the size and quantities. On 32th day, flocs still became dominant and granules started to disappear in R1 reactor. Meanwhile, Small particles were dominant in R2 reactor. The number of granules was decreased because of a slight viscous expansion. The floc-like sludge gradually changed to granular sludge with time, granular sludge began to appear again whereas flocs still remained dominant in R1 reactor. The granule sludges had a layer of fluffy materials at the edges of the granules, and were approximately 160 μm in diameter, most of which were light-yellow in color. Granules in R2 reactor had a compact structure with a clear outer shape and smooth surface, The mean diameter was up to 310μm. It can be seen from the test that the initial time granule sludge formed was similar to some researches using high selection pressure (1-5 min). Granules were both appeared after 6-7 days of operation, while the time granule sludge formed completely was much longer. Due to relatively short settling time and high selection pressure, granules in R2 reactor were larger in size and had a higher quantities than the R1 reactor.

Fig. 2 showed SEM images of mature granules in two reactors after 70 days. It can be seen that granules formed in R1 reactor (Fig. 2 a, c) had distinct gaps between granules inside and outside. It had a rough profile in which cells were banded together with the structure of bulk without apparent nuclear area in the central of the granule. Granules in R2 reactor (Fig. 2 b, d) had a relatively smooth surface and a very compact microstructure in which cells were tightly linked together. The rod-like bacteria were predominant in two reactors, no filamentous bacteria had been discovered.

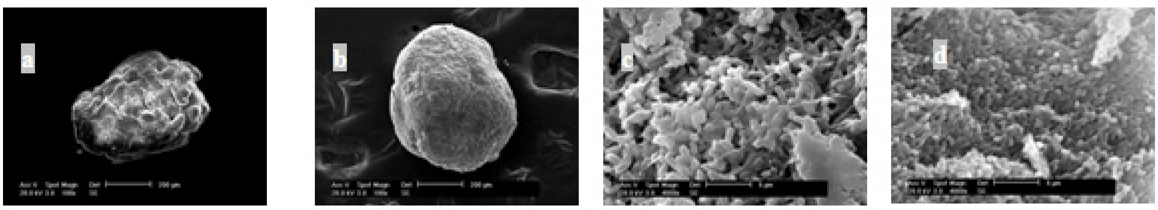

Fig. 2: SEM images of mature granules in reactors. (a. granule surface from R1 reactor(Bar=200 μm); b. granule surface from R2 reactor(Bar=200 μm); c. granule interior from R1 reactor(Bar=5 μm); d. granule interior from R2 reactor(Bar=5 μm).)

Fig.3 showed the size distribution of two reactors obtained after 80 days. Particle size distribution curves were made using the measured diameters. It showed that there were mainly small granules of around 0.4 mm in R1 reactor, while granules cultivated in R2 reactor had mainly diameter distribution ranged 0.8-1.0 mm. It can be seen that particle size was much smaller and it increased slowly relatively.

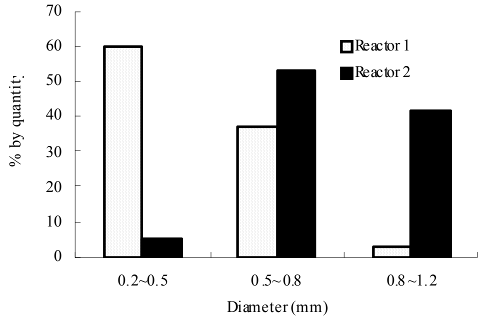

Fig. 3: The granule size distribution by number after 80 days of operation.

3.2. Changes of MLSS and SVI in reactors

Fig. 4 showed the MLSS and SVI in two reactors had similar changes.

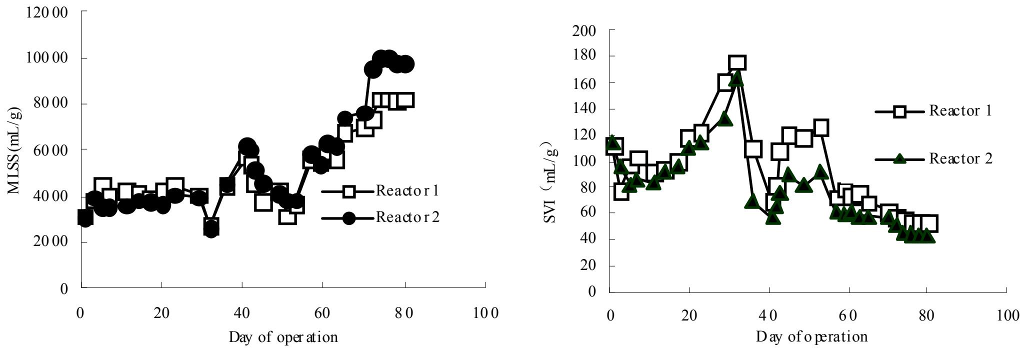

Fig. 4: MLSS and SVI over reactor operation.

There may be subjected to cohesive expands about on the 32th day, resulting in the rising of SVI and the loss of MLSS. With the development of incubation periods, SVI values gradually decreased with MLSS gradually raising, even were finally down to 53 and 43 ml/g, respectively. This seemed to indicate that SVI values can be decreased to about 50 ml/g under low selection pressure, while it did not favor the decreasing of SVI values and the growth of sludge concentration.

3.3. Variation of COD and NH_4^+-N during the typical periods

Fig. 5 showed the variation of COD and NH_4^+-N concentration during the typical periods. Two reactors had been the similar removal on COD and NH_4^+-N, basically arrived at full removal. It showed that bacteria were subjected to an aerobic starvation phase during the late stage of aeration, in accordance with the Feast-Famine regime.

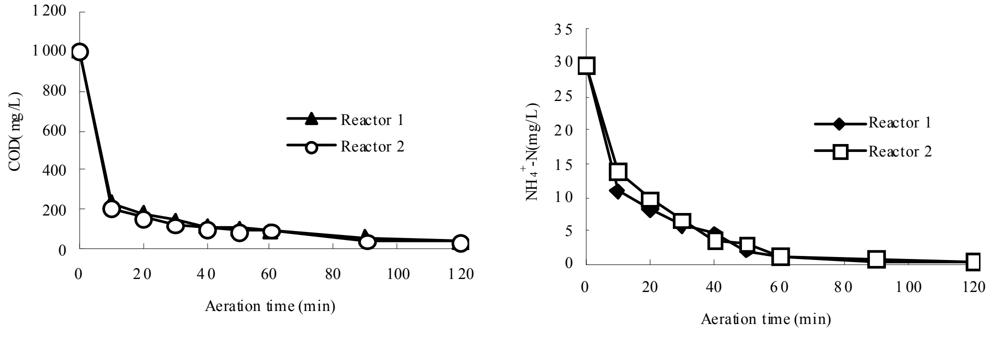

Fig. 5: NH_4^+-N and COD profiles on different settling times.

4. Conclusions

That was feasible for achieving aerobic granular sludge even under low selection pressure with longer settling time. According to the analysis of the time of formation on the granular sludge, diameter size, SVI and microscope inspection, a shorter settling time could accelerate aerobic granulation since poor settling sludge might be washed out from the reactors.

5. Acknowledgements

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