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Characteristics of aerobic granules grown on glucose a sequential batch shaking reactor

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Abstract: Aerobic heterotrophic granular sludge was cultivated in a sequencing batch shaking reactor(SBSR) in which a synthetic wastewater containing glucose as carbon source was fed. The characteristics of the aerobic granules were investigated. Compared with the conventional activated sludge flocs, the aerobic granules exhibit excellent physical characteristics in terms of settleability, size, shape, biomass density, and physical strength. Scanning electron micrographs revealed that in mature granules little filamentous bacteria could be found, rod-shaped and coccoid bacteria were the dominant microorganisms.

Keywords: aerobic granulation; sequential batch reactor; granule characteristics

Introduction

A high biomass concentration and bioactivity with good settlingability in biological wastewater treatment system increases the volumetric conversion capacity and the flexibility to the fluctuated loading rate. A conventional suspended culture system has some disadvantages such as a lower sludge concentration resulted in a relatively low volumetric conversion capacity(0.5—2 kgCOD/($m^3 \cdot d$)); a large settler required due to poor settling ability; a high surplus biomass production and the low flexibility with respect to loading rate and toxicants. Cell immobilization and aggregation have proved to be the ideal way to solve the problem. Compared with conventional activated sludge flocs, hereotrophic granular sludge has regular, dense and strong microbial structure, good settling ability, high biomass retention, and the ability to withstand shock loading rate. In fact, granulation is a phenomenon that the cells of microorganisms gather together to form a fairly stable, contiguous association under physiological conditions (Liu, 2002). It can be considered as a special case of biofilm growth. The technology has been successfully applied in anaerobic wastewater treatment system. The volumetric conversion capacity of upflow anaerobic sludge blanket (UASB) can be 45 kgCOD/($m^3 \cdot d$) (Kazuaki, 1998). Granulation is not restricted to special species. Granulations by methanogens, acidifying, nitrifying bacteria and denitrifying bacteria have been reported (Lettinga, 1984; Van, 1988; 1996). Recently, research has been focused on the development of heterotrophs aerobic granules (Beun, 1999; Peng, 1999; Zhu, 1999; Etterer, 2001). The mechanism of granulation, however, is still a subject of discussion. A discontinuous operation system has been proved to be advantageous to cultivate granular sludge. It is reported that aerobic granular sludge had been cultivated in aerobic sequencing batch reactor(SBR).

The aim of this research is to provide characteristics information of aerobic granules grown on glucose in sequencing batch shaking reactor(SBSR). It is expected that the characteristics information could be helpful for the completely study aerobic granule.

1 Materials and methods

1.1 Synthetic wastewater

A synthetic wastewater with the following composition was used: Glucose 2 g/L, NH_4Cl 0.382 g/L, K_2HPO_4 0.112 g/L, $CaCl_2 \cdot 2H_2O$ 0.2 g/L, $MgSO_4 \cdot 7H_2O$ 0.025 g/L, $FeSO_4 \cdot 7H_2O$ 0.02 g/L, EDTA 0.02 g/L, and trace solution 1 ml/L.

The composition of the trace solution was: H_3BO_3 0.05 g/L, $ZnCl_2$ 0.05 g/L, $CuSO_4 \cdot 5H_2O$ 0.05 g/L, $MnSO_4 \cdot H_2O$ 0.07 g/L , $COCl_2 \cdot 6H_2O$ 0.05 g/L, $(NH_4)_6MoO_{24} \cdot 4H_2O$ 0.03 g/L, KI 0.03 g/L, $AlCl_3 \cdot 5H_2O$ 0.05 g/L. The influent pH value was adjusted to 7.0 ± 0.5 by the addition of $NaHCO_3$ and H_2SO_4.

1.2 Sludge inoculum

The seed sludge used for the experimental startup was taken from the Minhang Sewage Treatment Plant, China. It had a biomass concentration of 2.42 mg/L and a sludge volume index(SVI) value of 183 ml/g.

1.3 Experimental set-up

The experimental triangular reactor had working volume of 0.5 L. The internal diameter of the reactor was 12.5 cm at the bottom and 3.5 cm at the top and with 22 cm in height. The reactor was vibrated at 200 r/min. Aeration occurred by sucking air through liquid surface during vibrating period. The temperature of the reactor was maintained at 25℃ by placing the reactor in a temperature controlled closet. The pH of the reactor was adjusted at 7.0 ± 0.5 using $NaHCO_3$ and H_2SO_4.

The reactor was operated as a sequencing batch reactor. Each successive cycle lasted for 12 h. The timing of a cycle is given in Table 1. Aeration is off during feeding, effluent withdrawal and settling. The reactor was totally operated for 57 d.

Table 1 Timing of a 12-h cycle

Successive phases	12-h cycle
Effluent withdrawal, min	5
Influent feeding, min	2
Aeration, min	711
Settling, min	2
Total cycle length, min	720

1.4 Analytical procedures

The dissolved oxygen(DO) concentration in the reactor was measured online with a DO-electrode(Haitch, German). The effluent of chemical oxygen demand(COD), suspended solid(SS), and sludge sample for mixed liquor suspended solids(MLSS), sludge volume index (SVI) were monitored daily or once every 2—3 d according to the Standard Methods (APHA, 1995).

For estimating the size distribution of the granules, the sludge samples taken from the reactor were classified into six fractions using laboratory sieves with various openings (0.2, 0.6, 1.0, 2.0, 3.0 mm). The sludge particles were first placed in the biggest opening (3.0 mm). During gentle intermittent swirling the granules settled through the sieve plates according to their size. The procedure was repeated until the five sieves were used.

The structure and microbial composition of the granular sludge were examined with scanning electron microscopy. The samples were gently washed with phosphate buffer solution then fixed with a 2.5% (w/v) glutaraldehyde solution (pH 7.0) for more than 3 h and dehydrated using a graded series of ethanol (10%, 25%, 75%, 90%, 100%). The dehydrate granules were critical dried then sputter-coated with gold for SEM examination with the Hitachi SEM (model S-450) in the Department of Cell Science, The Chinese Academy of Sciences in Shanghai.

The settling velocity of the granules was calculated from the experimentally determined settling time in a 1.6 m water column as described by Pereboom (Pereboom, 1994). Biomass density was measured by following the method used by Beun *et al*. (Beun, 2002). The physical strength of the granules was determined by following the method of Ghangrekar *et al*. (Ghangrekar, 1996). The physical strength was expressed in terms of integrity coefficient (%), which is defined as the ratio of residual granules to the total weight of the granular sludge after 5 min of shaking at 200 r/min on a platform shaker.

2 Results and discussion

The mature aerobic granules were obtained in the sequencing batch shaking reactor (SBSR) after 57 d operation. The shape of the granule was nearly spherical with a clear outline as shown in Fig. 1. The relevant reactor parameters are listed in Table 2.

Table 2 Process and granules characteristics of the SBSR in steady state

Parameters	SBSR
Reactor temperature, ℃	25
Influent COD, g/L	4
Effluent COD, g/L	0.25
HRT, h	24
COD removal efficiency, %	94
Biomass concentration in reactor, g/L	6.53
Biomass density, g/L	45.2
SVI, ml/g	65
Granule average diameter, mm	1.2
Settling velocity, m/h	52
Physical strength, %	99

2.1 Morphology of granules

Sludge granulation was first observed on day 9. After 31 d operation, the reactor reached its steady state. During the granulation particles grew steadily. Fig. 2 illustrates the distribution of the granular size on day 9 and day 31. On day 9, the first day that granules were visible, most of the granules were smaller than 0.2 mm in diameter. While on day 31, representative for the steady state situation of the reactor, there was a normal distribution with some smaller granules of about 0.2 mm. The average granule diameter was around 1.2 mm.

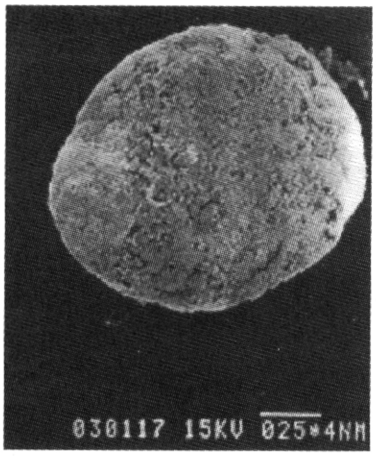

Fig. 1 SEM micrograph of granule(× 60)

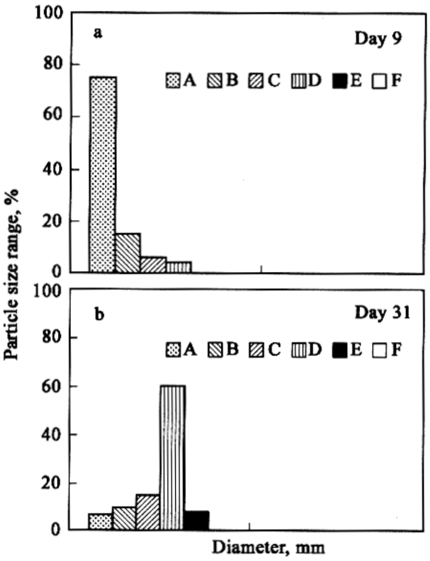

Fig. 2 Granular size distribution in the reactor at day 9(a) and day 31(b)
A. d < 0.2 mm; B. 0.2 < d < 0.6 mm; C. 0.6 < d < 1 mm; D. 1 < d < 2 mm; E. 2 < d < 3 mm; F. d > 3.0 mm

2.2 Microbiology of granular sludge

Electron micrographs from the SEM examination are shown in Fig. 3. In initial granules, there were many filamentous bacteria in the granules served as framework with rod-shape or coccoid bacteria entrapped in. The pores were formed mainly by filamentous intercrossed. Matured granules were mainly constituted of rod-shaped and coccoid bacteria with many fine pores. No filamentous bacteria were observed.

2.3 Sludge settling properties

Sludge volume index (SVI) value of matured granules was 65 ml/g which was little lower than the values of 80—100 ml/g (Peng, 1999) cultured in an upflow sequencing batch reactor, while conventional activated sludge is 183 ml/g. The average settling velocity of granules was 52 m/h. Such settling velocity of aerobic granules is comparable with those of anaerobic granules cultivated in UASB, and at least three times higher than those of activated sludge flocs having a settling velocity of less than 10 m/h. It is obvious that the settleability of sludge improved in a very significant way after the formation of aerobic granules in the reactors. The settling ability of aerobic granules is a key factor that

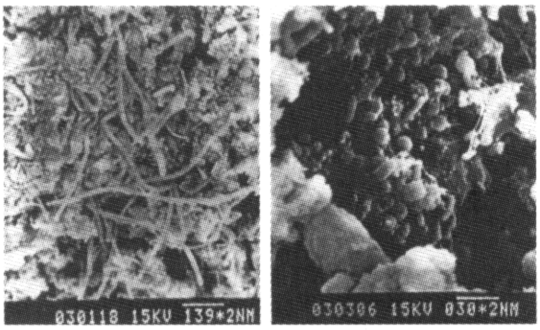

Fig. 3 Scanning electron photomicrographs of granules from sequencing batch shaking reactor(SBSR)
a. initial granule(× 500); b. mature granule(× 5000)

determines the efficiency of solid-liquid separation, which is an essential for the well-functioning of biological wastewater treatment systems.

2.4 Biomass in reactor

After the formation of aerobic granules, a high concentration of 6.53 g MLSS /L (MLSS = mixture liquor suspended solids) in the sequencing batch shaking reactor was achieved. The result further showed that the density of the biomass granules obtained in the reactor was 45.2 g/L which is comparable with that of SBAR (sequencing batch airlift reactor) system, and at least two times higher than that in SBBC (sequencing batch bubble column) system. Higher biomass density of aerobic granules showed that a denser microbial structure. In a biomass system, the higher biomass density, the more biomass retains in the reactor, and the higher biomass in consistent with better settling ability. Therefore the system would faster and more efficient remove the organic pollutants, and has high flexibility with respect to loading rate and toxicants.

2.5 Physical strength

The physical strength of the granules, expressed as integrity coefficient after shaking at 200 r/min on a platform shaker, is 99% for the glucose-fed granules. The higher the integrity coefficient the higher is the strength of granules. High strength showed that the aerobic granules are able to withstand high abrasion and shear. It is because during the operation, the biomass in the reactor underwent a relatively homogenous circular flow that produced a pressure. The pressure hastened microbial to aggregate for adapting to the pressured environment(O'Flaherty, 1997; Bossier, 1996). In this research, the SBSR formed a horizontal circular shear velocity, while a SBAR provided vertical circular shear velocity. The biomass aggregates grew to dense, strong granules by constantly subject to circular hydraulic attrition. The higher strength of aerobic granules would be very important for industrial steady operation.

2.6 Reactor efficiency and stability

The system was operated in successive 57 d. A high COD removal efficiency of more than 90% was observed during the whole operation period of 57 d. When volumetric loading rate increased to 5 kg COD/(m^3 · d) which was equivalent to an influent COD concentration of 4000 mg/L, the COD removal efficiency was more than 93% and the effluent COD concentration about 250 mg/L(Fig. 4). The effluent COD keeps stable after the formation of mature granular sludge. Granular sludge in the reactor systems with high biomass concentration and physical strength would be favor of the stable operation.

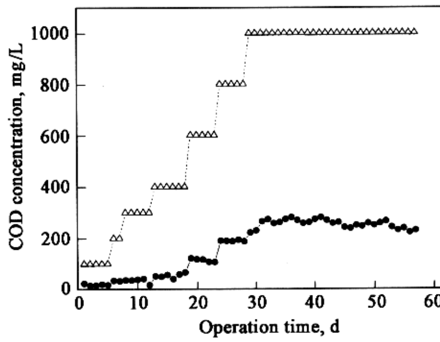

Fig. 4 Changing of COD during the experiment period
△influent(× 4) ●effluent

3 Conclusions

Formation of heterotrophic aerobic granular sludge was possible in the sequencing batch shaking reactor(SBSR) fed with glucose as carbon source. The granular sludge has a nearly spherical shape. In mature granules little filamentous bacteria can be found, rod-shaped and coccoid bacteria are the dominant morphology. Granules possess of good physical characteristics on settleability, biomass density, and physical strength. Compared with conventional activated sludge flocs, aerobic granular sludge exhibit excellent characteristics that would favor of industrial application.

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