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Cultivation of aerobic granules for simultaneous nitrification and denitrification by seeding different inoculated sludge

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Abstract: Cultivation of aerobic granules for simultaneous nitrification and denitrification in two sequencing batch airlift bioreactors was studied. Conventional activated floc and anaerobic granules served as main two inoculated sludge in the systems. Morphological variations of sludge in the reactors were observed. It was found that the cultivation of aerobic granules was closely associated with the kind of inoculated sludge. Round and regular aerobic granules were prevailed in both reactors, and the physical characteristics of the aerobic granules in terms of settling ability, specific gravity, and ratio of water containing were distinct when the inoculate sludge was different. Aerobic granules formed by seeding activated floc are more excellent in simultaneous nitrification and denitrification than that by aerobic granules formed from anaerobic granules. It was concluded that inoculated sludge plays a crucial role in the cultivation of aerobic granules for simultaneous nitrification and denitrification.

Keywords: aerobic granulation; inoculated sludge; nitrification; denitrification; inner structure; sludge morphology

Introduction

Granulation is a self-immobilization process of cell-to-cell, or cell-to-carrier surface attachment that involves biological, physical and chemical action (Liu, 2002). As compared to conventional floc-like sludge, granular sludge has the advantages of regular and strong microbial structure, good settling ability, high biomass retention, ability to withstand high organic loading rate (Beun, 1999; Peng, 1999) and capability of simultaneous nitrification and denitrification (SND; Ruan, 2003).

Previous research showed (Tay, 2001; Moy, 2002) that granulation and performances of aerobic sludge are affected by many environmental and operational parameters, but the granulation process of aerobic sludge has not been well investigated and the mechanism of sludge granulation is still a subject of discussion. Since there are many factors influencing the formation and characteristics of aerobic granules, it is supposed that inoculated sludge might be an effective factor influencing the formation and properties of the cell-immobilization community. Aerobic granules can be formed by (1) granulating activated flocs sludge into aerobic granules under favorable operations (Beun, 2002); (2) converting anaerobic granules into aerobic granules under aeration (Hu, 2004). It was thought that aerobic granules formed by inoculating anaerobic granules would mature easily and quickly. So the purpose of this study was focused on the influence of different inoculated sludge on aerobic granulation, physical characteristics and the simultaneous nitrification and denitrification of aerobic granules. However, it should be pointed out that little information is currently available on the essential role of inoculated sludge in the formation of aerobic granules. This work is expected to be useful for a better understanding of the mechanisms responsible for aerobic granulation.

1 Materials and methods

1.1 Reactor set-up and operation

Experimental set-up was two internal circulation sequencing batch airlift reactors (IC-SBAR) and each with the same geometrical configuration. The internal circulation reactor had an effective volume of 3.5 L (Fig. 1). The diameter of the down-comer was 80 mm. The riser was 900 mm in height, had an internal diameter of 40 mm, and was positioned at a distance of 20 mm from the bottom of the down-comer. Air was introduced by a fine bubble sparger aerator at the bottom of the reactor at an aeration volume of 0.06 m^3/h.

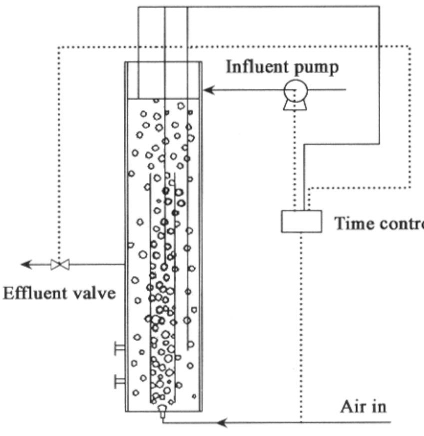

Fig. 1 IC SBAR technology process

Both reactors were operated sequentially: 20 min of influent filling, 92 min of aeration, 5 min of settling and 3 min of effluent withdrawal. Effluent was withdrawn at 500 mm height from the bottom of the reactors. The experiments were conducted in a temperature-controlled room at 22 ± 2℃. The pH was maintained about 7.0 using 1 mol/L NaOH and 1 mol/L HCl. In the experiments, reactors 1—2(R1, R2) were respectively inoculated with activated floc taken from a conventional nutrient (N and P) removing wastewater treatment plant and anaerobic granular sludge taken from a full-scale UASB reactor treating wastewater of brew house. The characteristics of inoculates are summarized in Table 1. Each reactor was seeded with 300 ml of inoculates.

1.2 Media

Both reactors were started up initially by feeding sucrose substrate in a batch mode. The compositions of synthetic wastewater used for this study were mainly consisted of sucrose as sole carbon source and other necessary elements. Details of the composition were as follows: sucrose, 2000 mg/L; NH_4Cl, 380 mg/L; KH_2PO_4, 88 mg/L; $CaCl_2 \cdot 2H_2O$, 80 mg/L; $MgSO_4 \cdot 7H_2O$, 20 mg/L; $FeCl_3 \cdot 6H_2O$, 20 mg/L, and 1 ml/L trace elements. The trace elements solution contained (g/L): EDTA, 15; H_3BO_4, 0.05; $ZnSO_4 \cdot 7H_2O$, 0.43; $CuSO_4 \cdot 5H_2O$, 0.25; $MnCl_2 \cdot 4H_2O$, 0.99; $NaMoO_4 \cdot 2H_2O$, 0.22; $Na_2SeO_3 \cdot 5H_2O$, 0.21; $AlCl_3$, 0.05; $CoCl_2$, 0.05; and $NiCl_2$, 0.199.

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Table 1 Characteristics of inoculates sludge

	Activated sludge	Anaerobic granular sludge
MLVSS, mg/L	4394	4678
SVI, ml/g	126.12	12.8
ρ, g/L	1.0018	1.0351
Water ratio, %	99	92
Size, mm	Flocs-like	1—3
Color	Brown	Black

1.3 Analytical procedures

The influents and effluents from both reactors were analyzed for NH_3-N, NO_2^--N, NO_3^--N and the sludge for mixed liquor suspended solids(MLSS), mixed liquor volatile suspended solids(MLVSS), sludge volume index (*SVI*), specific gravity and ratio of water containing according to standard methods(APHA, 1998).

In order to establish the size distribution of mature granules, representative granules were taken from the reactors and size classification was achieved by wet sieving with different sieves(0.5 mm, 1.0 mm, 1.5 mm, 2.0 mm) after the granules were washed several times with phosphate buffer.

Sludge morphology observation was conducted using optical microscopy. Granules harvested from the reactors were fixed in a 3 : 1 ratio with 4% paraformmaldehyde solution for 1 h at 4℃ to fixation, then the granules were rinsed with phosphate buffer and frozen overnight at − 30℃. The frozen granules were subjected to sectioning in two parts with a razor blade and the inner microbial morphology of the granules was examined in more detail with a scanning electron microscope.

2 Results and discussion

The experiments were conducted by inoculating different inoculated sludge in the reactors: conventional activated sludge in R1 and anaerobic granules in R2.

2.1 Effect of inoculated sludge on the morphology of aerobic granules

The activated flocs seeded in R1 were floc-like sludge while the anaerobic granules seeded in R2 had a diameter of 1—3 mm. The evolution of sludge morphology during operation was observed with an optical microscopy. Microscopy photography for both reactors on the day 8 and the day 15 are shown in Fig. 2.

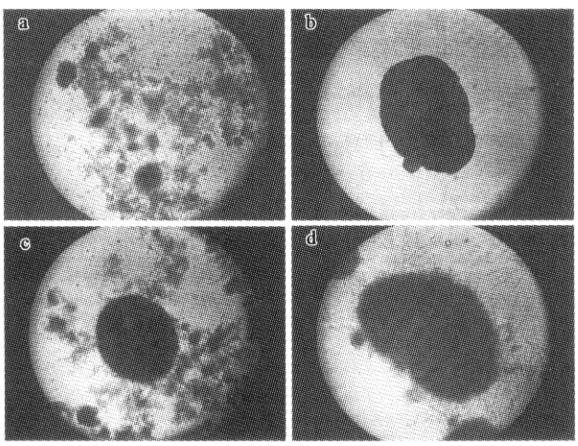

Fig. 2 Image analysis photographs of granules by microscopy(× 150)
a. the day 8 in R1; b. the day 8 in R2; c. the day 15 in R1; d. the day 15 in R2

On the day 8, sludge in both reactors had little change compared with inoculated sludge: only irregular, loose structured bioflocs were observed in R1(Fig. 2a) and black granular sludge in R2(Fig. 2b). On the day 15, tiny granules with a round shape appeared in R1(Fig. 2c), but the color of black inoculated granules in R2 turned to gray and many mildew grew on the surface of the granules(Fig. 2d).

The detailed microbial structures of aerobic granules taken from both reactors were further examined using SEM, which are shown in Fig. 3.

It could be clearly seen from Fig. 3, the granules in R1 had a very compact bacterial structure, in which cells were tightly linked together and a rod-like species are predominant. Compared with R1, the microstructure of bacteria was loose in R2 and the amount of microorganisms was by far less than that in R1. The reason of this phenomenon is probably that the anaerobic bacteria in the initial inoculated anaerobic granules were dead and decayed under the condition of aeration, so that most interspaces of the granules center become void and loose.

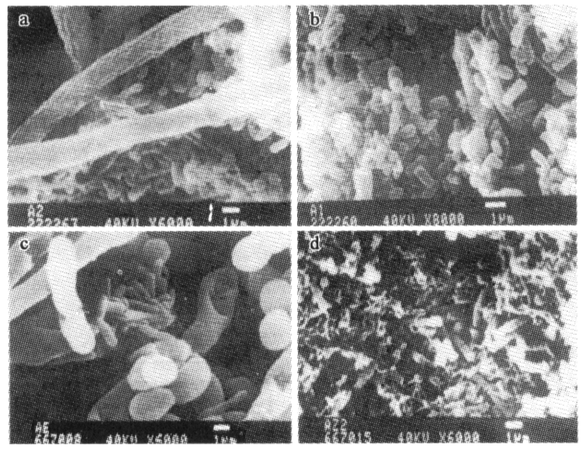

Fig. 3 The microbial structures of aerobic granules by using SEM
a. surface of aerobic granules in R1(× 6000); b. inner profile of aerobic granular sludge in R1(× 8000); c. surface of aerobic granular sludge in R2 (× 6000); d. inner profile of aerobic granular sludge in R2(× 6000)

2.2 Effect of inoculated sludge on retainable biomass in the reactors

After five weeks of operation, both reactors reached a steady state, indicated by stable granular sludge content and constant granules size. The biomass concentration in the reactors is shown in Fig. 4. A stable biomass concentration of 5.4 g MLVSS/L in R1 was achieved and the average granular diameter was around 1.0—1.5 mm. But, the biomass concentration was only 3.2 g MLVSS/L and the average granular diameter was 2 mm in R2. It was suggested that the formation of aerobic granules by seeding activated flocs lead to a high biomass concentration, although the diameter of the granules was smaller than that of granules by seeding anaerobic granules.

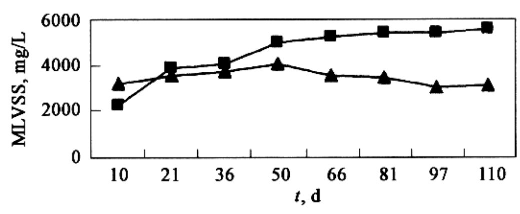

Fig. 4 The MLVSS in the rectors(■ R1, ▲ R2)

2.3 Effect of inoculated sludge on settling ability and specific gravity

The *SVI* changes of sludge in the reactors are clearly indicated in Fig. 5. The inoculated sludge used in R1 had a *SVI* value of 126.12 ml/g, and it distinctly decreased to a stable value (28.16—33.486 ml/g) after the formation of granular sludge. Compared with R1, a *SVI* value of 89—100 ml/g was obtained in R2 after the maturity of aerobic granules, but the *SVI* of inoculating anaerobic granules was only 12.8 ml/g. Since *SVI* was a representative parameter to settling ability of granular sludge, it was made clear that the granulation of aerobic sludge formed by seeding activated flocs could significantly improve the settling ability of sludge, but the aerobic granules formed by acclimating anaerobic granules had a worse settling ability compared with anaerobic granules. The reason may be that many mildew grown on the aerobic granules caused a bad settling ability of granules.

It was also indicated in Fig. 5 that it was different distinctly between the specific gravities of aerobic granules in the reactors. As

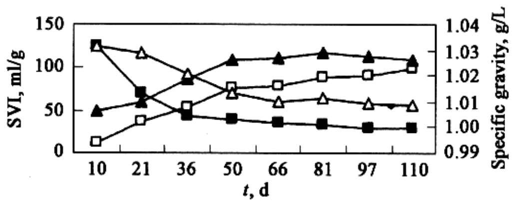

Fig. 5 Changes of sludge volume index(■ R1; □ R2) and specific gravity (▲ R1; △ R2) in the rectors

pointed out, the aerobic granules prevailed in R1 had a specific gravity of 1.025, but the specific gravity of aerobic granules matured in R2 was only 1.0087. The result is owing to that the inner section of granules in R2 has less biomass than that of granules in R1, which led to a low specific gravity.

2.4 Effect of inoculated sludge on its contained water

During the studies, a very significant difference in ratio of water containing was observed on the sludge in the reactors. In R1, ratio of water containing decreased from a value of 99% to 96% after the granulation of sludge. On the contrary, ratio of water containing increased from 95% to 98% after the maturity of aerobic granules in R2. Further more, it was found that the ratio of water containing of granules in R1 was somehow lower than that of granules in R2(Fig. 6). The results indicated that, even under the same operation condition, the sludge water content in reactors was slightly different because seeding different inoculated sludge. It was probably that the microorganisms in reactors were different when inoculating different inoculated sludge, for zoogloea, bacteria were predominant in R1 while mildew was predominant in R2, which caused different water content in granules.

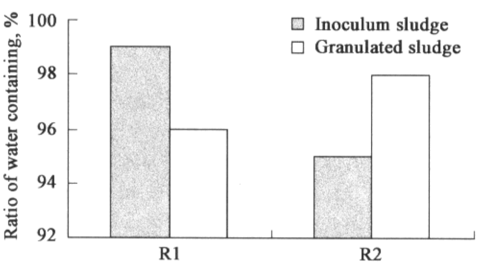

Fig 6 Ratio of water containing in the reactors

2.5 Nitrification and denitrification of aerobic granules in the reactors

It has been demonstrated that aerobic granule was a microbial community having the capability of simultaneous nitrification and denitrification. In the experiments, microbial activity and biodegradability of aerobic granules to ammonia was analyzed in the reactors. The concentrations of NH_3-N, NO_2^--N and NO_3^--N in the effluent of the reactors are shown in Fig. 7. It was obvious that aerobic granules formed in R1 were superior to granules in R2 in terms of the capability of performing simultaneous nitrification and denitrification.

As mildew was predominant on the surface of granules in R2, it led to characteristics of granules inferior to that of granules in R1, including the ability of nitrification and denitrification of granules.

3 Conclusions

This study investigated the influence of inoculated sludge on aerobic granulation and physical characteristics of aerobic granules. Further investigation on the performances of aerobic granules for simultaneous nitrification and denitrification was studied. From the results of investigation, the conclusions could be drawn as follows:

(1) Different inoculated sludge leads to different granulation and inner structures of aerobic granules. The granule formed by granulating activated floc has a very compact bacterial structure and smooth surface while the granule formed by acclimating anaerobic granules has a loose structure and fluffy shape because of heavy growth of mildew.

(2) The physical characteristics of aerobic granules, namely

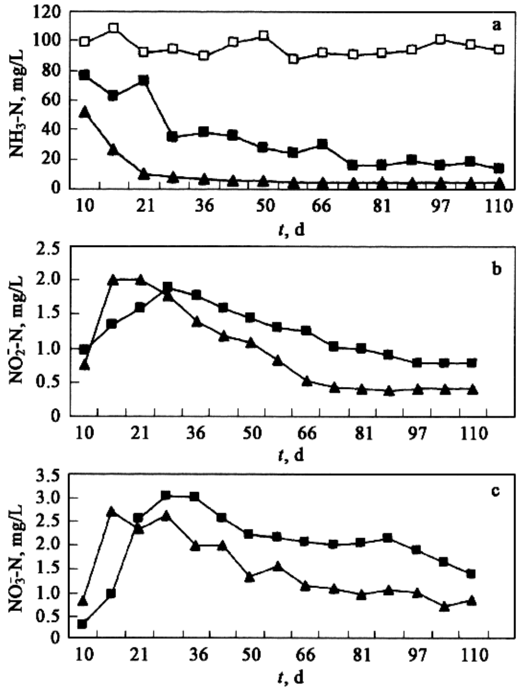

Fig. 7 The concentrations of NH_3-N (a), NO_2^--N (b) and NO_3^--N (c) in the reactors

□ influent; ▲ effluent of R1, ■ effluent of R2

biomass content, sludge volume index, specific gravity and ratio of water containing, are closely related to inoculated sludge. The granules formed by granulating activated flocs have the favorable physical characteristics superior to the granules formed by anaerobic granules.

(3) The aerobic granules formed by granulating activated sludge have a high potentiality of simultaneous nitrification and denitrification than that of granules formed by anaerobic granules.

(4) It seems that different inoculated sludge leads to different microorganisms in the reactors, which is the main reason for different characteristics of aerobic granules in the reactors. And further studies at molecular or genetic level are required for a more profound understanding of the aerobic granulation mechanisms.

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