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The formation and characteristics of aerobic granules in sequencing batch reactor (SBR) by seeding anaerobic granules

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Abstract

The cultivation of aerobic granules in SBR by seeding anaerobic granular sludge was investigated using acetate-based synthetic wastewater. Morphological variation of granular sludge in the reactor was observed and results revealed that the inoculated anaerobic granules experienced a process of disintegration—recombination—growing up. The disintegrated anaerobic sludge may play a role of nucleus for the granulation of aerobic sludge. The sludge concentration increased for the first 4 weeks, then decreased and reached a stable value of 5 g/l at 60 d with a SVI of 30–40 ml/g. Granular sludge dominated in the reactor and suspended sludge concentration was less than 0.5 g/l at the end of the process. In the inoculated anaerobic granular sludge, spherical bacteria were the main microorganisms, however, rod-shaped and filamentous microorganisms prevailed in the cultivated aerobic granules. This is ready way to cultivate aerobic granules by seeding anaerobic granular sludge.

Keywords: Aerobic granules; Granulation; Anaerobic granule; Microbial aggregates; SBR process

1. Introduction

Microbial populations exist in biological wastewater treatment systems as free or aggregated cells, such as flocs and granules. The self-immobilization of microorganisms into aggregates or granules is a process that has been exploited for the biological treatment of municipal and industrial wastewaters. The aerobic granules have several advantages over conventional activated sludge flocs, such as a strong and compact microbial structure, improved settling ability and higher biomass retention. Because of their ability to retain biomass, aerobic granules are capable of significantly higher organic loading rates compared to conventional activated sludge systems [1]. Granular growth can be considered as a special case of biofilm growth. Nevertheless, unlike biofilms or anaerobic granules, the aerobic granule is remains a newly acknowledged microbial structural organization and thus there is a lack of profound understanding about most of its characteristic changes.

Granulation is not only restricted to anaerobic sludge. Granulation by acidifying bacteria, nitrifying bacteria [2], denitrifying bacteria and aerobic heterotrophs [3,4] has been observed. All these observations have been done in a continuously operated system. For many applications a discontinuous operation is advantageous. In these sequencing batch reactors (SBR) aerobic granules can also be formed [5].

Although granulation of anaerobic sludge has been well documented, very little information on the formation of granular sludge under aerobic conditions is available. In recent years more and more attentions have been paid to the cultivation of aerobic granules in SBR [1,5–10]. Most of these successfully cultured the aerobic granules of heterotrophic microorganisms using short HRT and a relative high shear. They also investigated different operational conditions on granular sludge formation. However, all these studies used aerobic sludge as seed to cultivate the aerobic granules and the conditions for cultivating aerobic granules are uncertain.

The purpose of this study was to investigate the possibility of cultivating aerobic granules in SBR by seeding anaerobic granular sludge, because the cultivation of granules under anaerobic conditions is well established and anaerobic

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granules are available in large amounts. The work could be helpful in the development of aerobic granule-based systems for full-scale application.

2. Materials and methods

2.1. Experimental set-up

An aerobic sequencing batch reactor was used for the experiments. The cylindrical reactor had an inner diameter of 17.6 cm and a height of 50 cm. The working volume was 5 l. Four air diffuser were placed at the bottom of the reactor symmetrically, and the air flux was controlled by flow meter. Temperature was controlled at $25 \pm 2^{\circ}$. The schematic diagram of experimental set-up was shown in Fig. 1.

One operation cycle had 6 h totally, including 3 min for filling, 330 min for aeration, 10 min for sedimentation (decreasing to 5 min from the 35th day) and 17 min for draw and idle. 4 l effluent was discharged at the end of each cycle. The procedures of the reactor operation, including feeding, aerating, setting and discharging, were controlled by timers automatically.

2.2. Influent and seeding sludge

Synthetic wastewater was used in this experiment, with sodium acetate as organic source. The influent contained NH_4Cl, KH_2PO_4, $NaHCO_3$, Mg^{2+}, Ca^{2+} and other trace elements. The COD concentration was about 500 mg/l and NH_4^+–N about 50 mg/l. The influent pH was adjusted to 7.0 by adding $NaHCO_3$. All chemicals were obtained as analytical grades.

A anaerobic granular sludge from a UASB treating brewery wastewater was used as seeding sludge. The average diameter of seeder was about 1.1 mm, it was black, ball-shaped or oval-shaped. The sludge concentration in the reactor after inoculation was about 3.3 g/l.

2.3. Analytical methods

The samples were periodically collected and analyzed for COD, pH, sludge volume index (SVI), suspended solids (SS), volatile suspended solids (VSS), and dissolved oxygen (DO) according to standard methods [11]. The settling velocity was measured by recording the time taken for individual granules to fall from a certain height in a measuring cylinder.

The granular sludge was drained off and harvested at the steady state. Bulk volumes for the total granules in the whole reactor were measured. Assuming the granule was spherical in shape, the number of total granules (n) in every category that resided in the reactor could be estimated, using the average mean diameter (d, in millimeters) and bulk volume of granules. The granules were washed several times with phosphate buffer before sorting the granules according to size, employing the wet-sieving method [12]. All analyses of the samples were done at least in duplicate, unless stated otherwise.

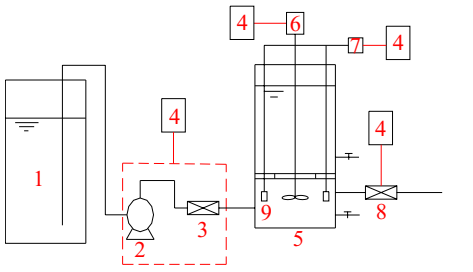

Fig. 1. Schematic diagram of the experimental set-up.

3. Results and discussion

3.1. General observations

The reactor was started-up by seeding anaerobic granular sludge from a UASB, which was operated at a brewery wastewater treatment plant. During the start-up period, the settling time was kept short. After inoculation of the reactor, highly filamentous granules were formed over several days. From observations both in the reactor and under the microscope it could be concluded that the granules in this first stage were formed by filamentous bacteria. These granules were not stable at all and broke up into pieces after a few days. Subsequently a large part of the biomass was washed out and a new granulation occurred. The granules formed in this second stage hardly contained any filaments and consisted dominantly of bacteria. The variation of anaerobic granules and the granulation of aerobic sludge were observed by light microscopy and the photographs are shown in Fig. 2.

Fig. 2 shows the process of morphological change of granules in the reactor. The seeding anaerobic granules were regular in shape, and black in colour, the average diameter was around 1.1 mm (Fig. 2(A)). After 1 week the anaerobic granules began to shrink (Fig. 2(B)) and then began to disintegrate due to the aerobic conditions (Fig. 2(C)). Two weeks later, the outside colour of the granules became yellow, indicating that the anaerobic microorganisms gradually phased out and the aerobic microorganisms became dominant in the reactor, the suspended solids increased and grew fast. Under aerobic conditions the suspended solids began to recombine (Fig. 2(D) and (E)). From 35 day, the settling time decreased to 5 min, most of suspended solid were washed out. The granulation occurred in the reactor, small granules were present after 5 weeks of operation, the photograph showed many filaments around the surface of the granules. (Fig. 2(F)). On day 50 the granules had a more or less “steady state” average diameter of about

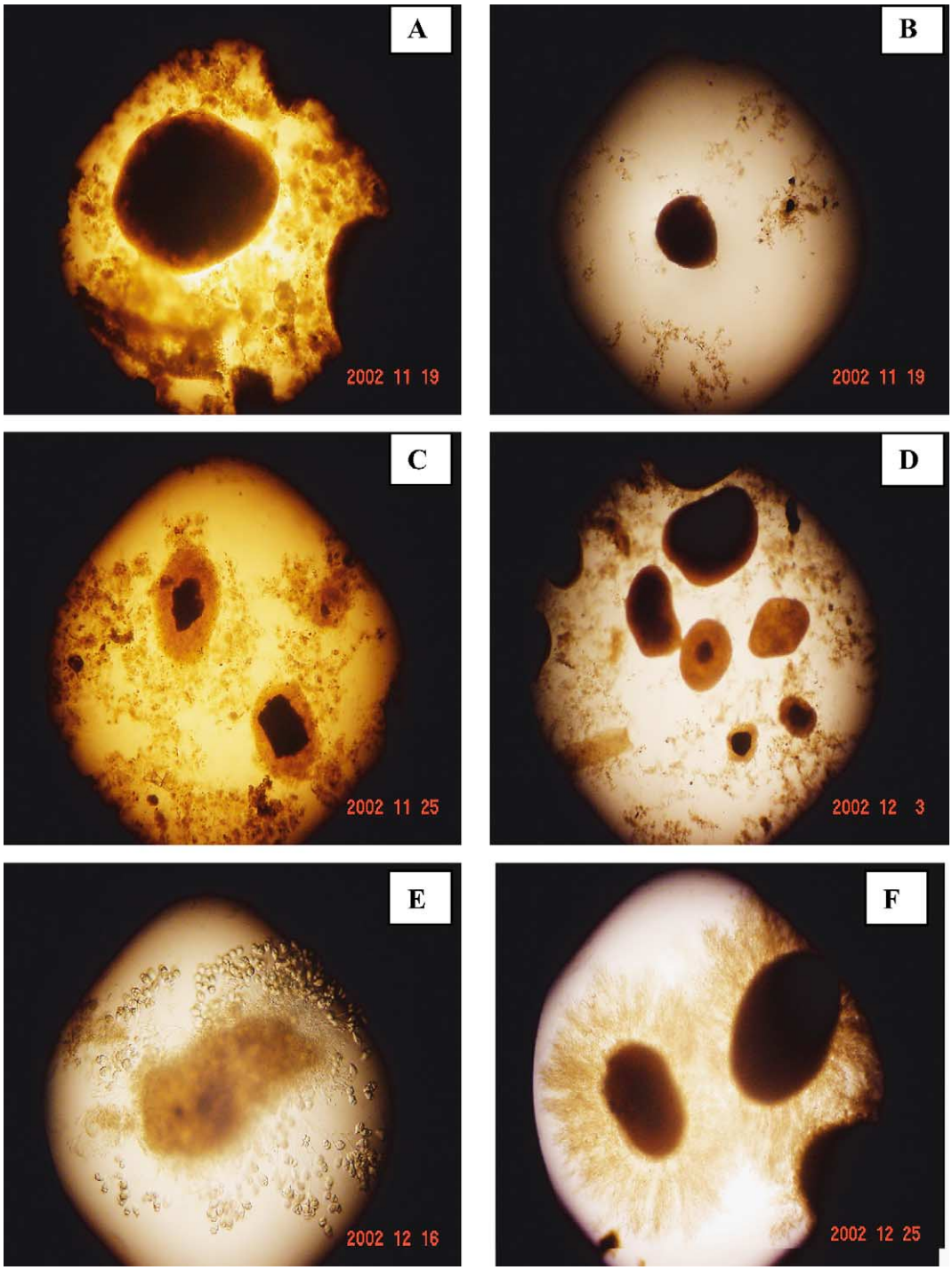

Fig. 2. The morphological variation of granular sludge during the experimental period (Magnification = 40×). (A) Anaerobic granule used as seeder; (B) after 1 week; (C) after 2 weeks; (D) after 3 weeks; (E) after 5 weeks and (F) after 5 weeks.

1.2 mm. Thereafter no significant change of the granules occurred.

The results revealed clearly that it is possible to cultivate aerobic granules in SBR by seeding anaerobic granular sludge. The settling time is a crucial factor for the formation of granules, mainly because it determined the amount of sludge accumulation in the reactor. Too long a settling time will result in the formation of flocculated biomass. A too short settling time does not lead to the accumulation of sufficient granules. By applying a appropriate settling time, i.e. selecting for biomass particles with a high settling velocity, it was possible to obtain a granular sludge in the reactor.

Many researchers [2,4,13] clearly showed that sludge granules can be formed by a wide variety of organisms, not

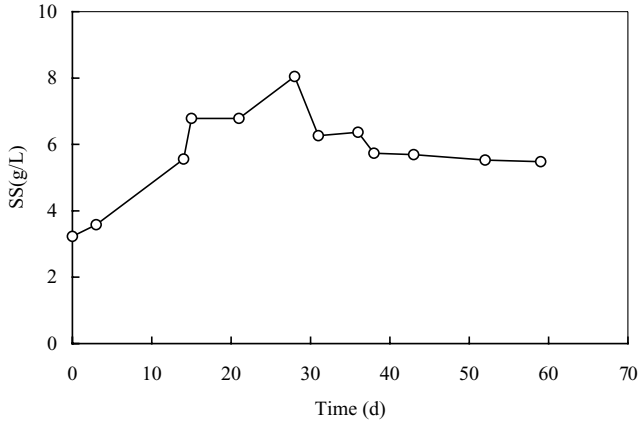

Fig. 3. Variation of SS in the reactor.

restricted to certain microbiological groups, but related to the way reactors are operated. The pulse-wise addition of substrate may partly attribute to the granulation of aerobic sludge. The formation of aerobic granules in general leads to a better sludge settling. Due to the diffusion limitation of substrate, the granular biomass will grow slower than suspended cells. By preventing the accumulation of suspended cells (by adjusting the HRT) or flocs (by the settling velocity), proper granules will be formed.

3.2. Variation of sludge concentration in the reactor

The concentration of suspended solids (SS) in the reactor after inoculation was about 3.3 g/l (Fig. 3). During the start-up, the anaerobic granules gradually disintegrated and the some debris with poor settling ability was washed out, so the anaerobic biomass decreased. The aerobic microorganisms began to grow at the aerobic conditions, the total suspended solids increased and reached maximal value of 8 g/l. After that, due to decreasing the sedimentation time, the smaller flocs and granules subsequently washed out, only the microbes attached to the granules can be retained in the reactor, so the MLSS gradually decreased and then reached a stable state, the average concentration of aerobic granules in the reactor was around 5 g/l, the suspended sludge in the reactor was less than 0.5 g/l. The dominan microorganisms were filamentous twisted around the granules and bacillus attached to granules. During 40–60 day, along with reaching the stable suspended solids concentration in the reactor, the SVI value became stable at 30–40 mg/l.

3.3. SEM photographs of granular sludge

The morphology and inner structure of the granules was observed in more detail using scanning electron microscopy, the section of the aerobic granules was observed after cutting the granular sludge in two parts with a razor blade. Fig. 4(A) and (B) demonstrated the anaerobic granules used as seed. The inoculated anaerobic granules were more or less spherical particles with a smooth surface (Fig. 4(A)), and consisted of mainly the spherical bacteria. Fig. 4(C) revealed the section of the aerobic granule cultivated in the SBR. In general two layers could be observed in the granule—(1) the center of the granule, which was about 0.7–1.2 mm in diameter, and (2) the outer layer with a thickness of about 0.4 mm. The outer layer had a rather fluffy and loose structure and was more transparent compared to the center of the granule. The center of the granule had a dense structure. The reason may that due to the limitations in diffusion and mass transport the nutrient availability in the inner region of the granule was scarce, whereby certain starved bacterial cells started to die and were consumed by outer bacteria, the inorganic components accumulating in the center of granules. No big empty holes resulting from complete lysis of the interior biomass were observed in the center of the granule. Fig. 4(D) indicated that the dominant microorganisms in inner aerobic granules are rod-shaped and filamentous bacteria.

Fig. 4(E) shows that extracellular polymers (ECP) bridge the bacterial cells together. Fig. 4(F) shows the morphology of aerobic granule, it was clear that the aerobic granules had an uneven surface, which was more filamentous than anaerobic granules.

3.4. Physical characteristics of granules

3.4.1. Granule size

Granule size was a direct parameter to show the growth and aging process in the microbial organizations [14–16]. It also played a significant role in the limitation of mass transport and diffusion [17–19], due to the porosity in the granular structures, which diminished with the increase in size and age [15]. The particle size was important for the nutrient accessibility and product releasing, which also had great impact on microbial viability, microenvironment and the microstructure of the microbial organization. Hence, granule size is an eminent factor in molding the physical performance and characteristics of aerobic granules.

The average diameter of inoculated anaerobic granules was about 1.1 mm. The micro-observation of aerobic granules showed that the aerobic granules were more filamentous than anaerobic granules, therefore the boundary of aerobic granules was difficult to determine, which caused difficulty in measuring the particle size. The cultivated aerobic granules were sorted using metal sieves, the results indicated that the aerobic granules had a wide range of sizes, approximately 0.5–4.0 mm in diameter, the average granule diameter was around 1.2 mm. More than 70% granules displayed a range of diameter between 0.6–1.4 mm. The particle size distribution curve of day 50 was depicted in Fig. 5, which is representative for the steady state situation of the SBR.

3.4.2. Water content

The water content in inoculated anaerobic granules was about 92.7%, the water content in cultivated aerobic granules was about 94.3%.

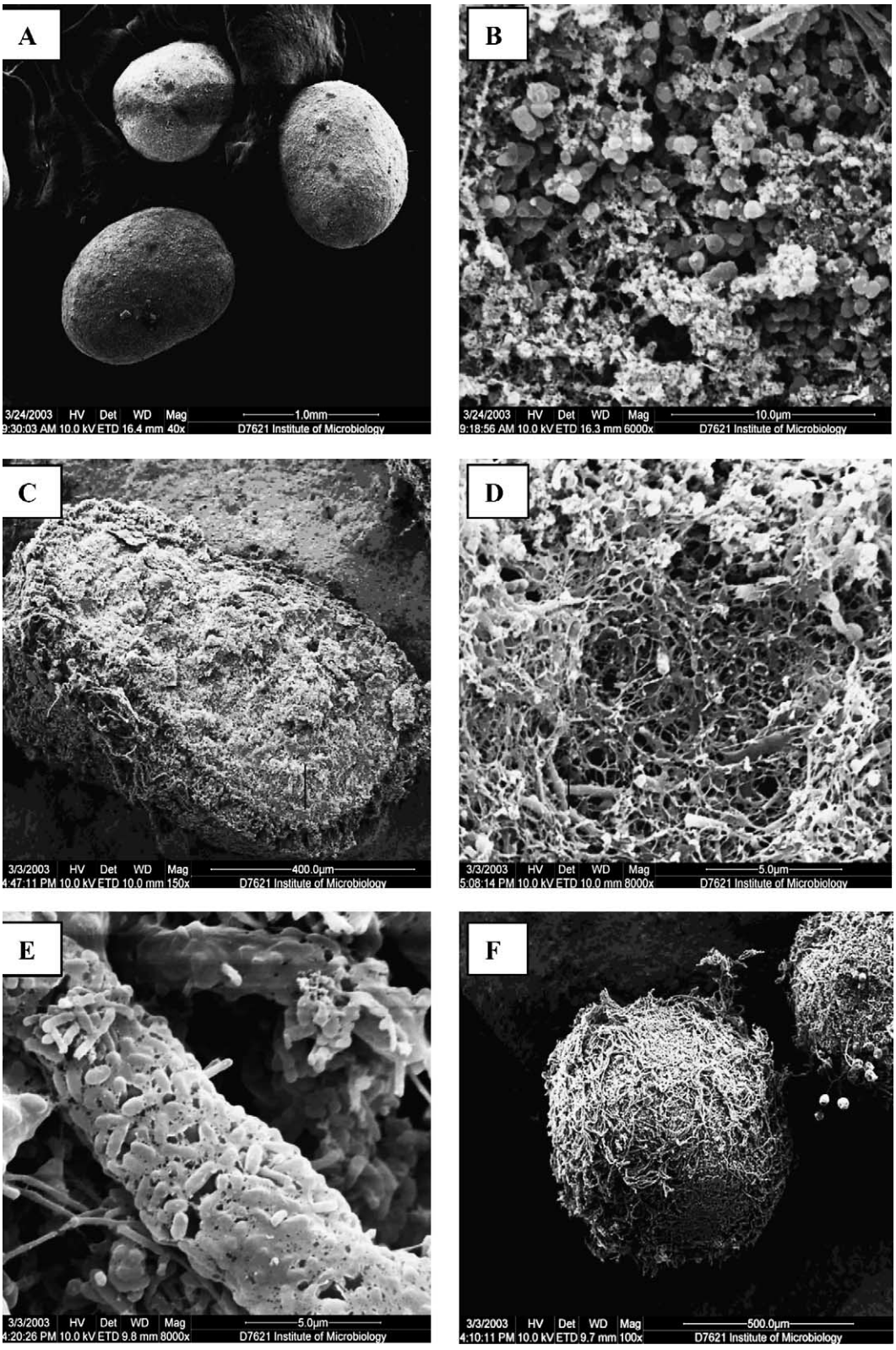

Fig. 4. Scanning electron micrographs of granular sludge, (A) morphology of anaerobic granules used as seeder (40× magnification); (B) inner structure of anaerobic granules (6000× magnification); (C) section of aerobic granule cultivated in the SBR (150× magnification); (D) inner structure of aerobic granule (8000× magnification); (E) inner structure of aerobic granule (8000× magnification) and (F) morphology of aerobic granule (100× magnification).

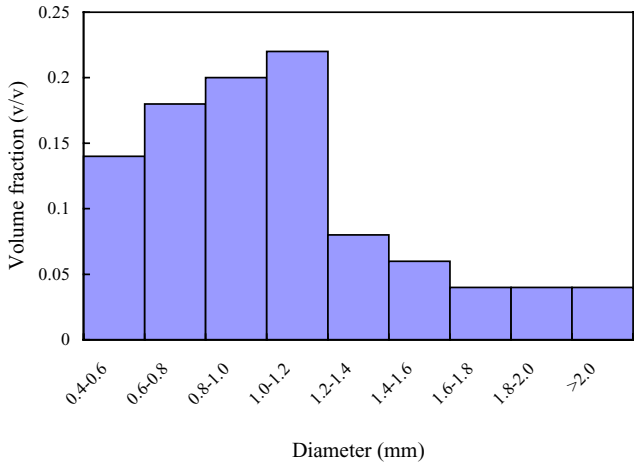

Fig. 5. Aerobic granular size distribution in the reactor.

3.4.3. Settling velocity

The settling velocity of cultivated aerobic granules was in the range of 22–60 m/h, the average settling velocity was 38.4 m/h, compared with 72 m/h for inoculated anaerobic granules, the settling velocity decreased, which was corresponding to the fact that the water content in the aerobic granules increased. Schmit and Ahring [20] reported that the settling velocity of anaerobic granular sludge ranged from 18–100 m/h, the settling velocity of the aerobic granules cultured in this study was in this range.

3.4.4. VSS/SS ratio

The initial ratio of inoculated anaerobic granules was about 0.57, showing that the relative biological components were low. Along with the reactor operation, the VSS/SS of aerobic granules gradually increased to 0.71. However it was lower than that of normal activated sludge (the VSS/SS of activated sludge cultured with the same influent was about 0.85).

3.5. Mechanism of aerobic granulation

From previous studies on microbial structural self-assembly, either in the form of flocs, biofilms or anaerobic granules, it was known that the structure consisted of biomass, extracellular polymer substances (ECP), inorganic precipitates, bound water and cavities [21–25], amongst which EPS was the main scaffold.

There are several mechanisms for the formation of granular sludge. In a UASB the up-flow velocity created a selective pressure to which the organisms have two responses: to be washed out or to bind together and form easily settable granules [26]. In anaerobic granules, the methanogenic microorganisms exhibit natural tendencies to aggregate and resulted in the formation of granular sludge [27,28]. Extracellular polymers bridge the bacterial cells together and hold granules together [29,30].

Based on the microscopic observations done during this research, a mechanism for the formation of aerobic granules in SBR by seeding anaerobic granules may be proposed as follows. Anaerobic granules underwent serial morphological and physical changes. Firstly, the anaerobic granular sludge disintegrated under aerobic conditions after inoculation, forming irregular and small flocs and debris; then the flocs and debris from the disintegrated granules recombined under aerobic conditions; and finally the granules grow up, resulting the formation of aerobic granular sludge. The disintegrated anaerobic sludge may play a role of nucleus for the granulation of aerobic sludge.

4. Conclusions

This study demonstrated for the first time the possibility of aerobic granulation in SBR by seeding anaerobic granules. The formation and characteristics of aerobic granules were investigated, and the following conclusions can be drawn:

1. Aerobic granular sludge can be cultivated in an aerobic SBR by seeding anaerobic granules. The settling time was a key parameter for granulation of aerobic sludge.
2. The sludge concentration could be maintained at about 5 g/l and the SVI at 30–40 ml/g, due to the formation of aerobic granules in SBR.
3. The anaerobic granules experienced a process of disintegration—recombination—growing up. The domina microbes in the anaerobic were spherical bacteria, but rod-shaped and filamentous microbes prevailed in the cultivated aerobic granules.
4. In comparison with inoculated anaerobic granules, the average diameter, the water content and the biological components of the aerobic granules increased, the settling velocity decreased.

Acknowledgements

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References

[1] Moy BYP, Tay JH, Toh SK, Liu Y, Tay STL. High organic loading influences the physical characteristics of aerobic granules. Lett Appl Microbiol 2002;34:407–12.
[2] De Beer D, van der Heuvel JC, Ottengraf SPP. Microelectrode measurements in n itrifying aggregates. Appl Environ Microbiol 1993;59:573–9.
[3] van Benthum WAJ, Garrido-FernaÂ ndez JM, Tijhuis L, van Loosdrecht M, Heijnen JJ. Formation and detachment of biofilms and granules in a nitrifying biofilm airlift suspension reactor. Biotechnol Prog 1996;12(6):764–72.

[4] Tijhuis L, van Loosdrecht M, Heijnen JJ. Formation and growth of heterotrophic aerobic biofilms on small suspended particles in airlift reactors. Biotechnol Bioeng 1994;44:595–608.
[5] Morgenroth E, Sherden T, van Loosdrecht M, Heijnen JJ, Wilderer PA. Aerobic granular sludge in a sequencing batch reactor. Water Res 1997;31(12):3191–4.
[6] Beun JJ, Hendriks A, van Loosdrecht M, Morgenroth E, Wilderer PA, Heijnen JJ. Aerobic granulation in a sequencing batch reactor. Water Res 1999;33:2283–90.
[7] Etterer T, Wilderer PA. Generation and properties of aerobic granular sludge. Water Sci Technol 2001;43:19–26.
[8] Tay JH, Liu QS, Liu Y. Microscopic observation of aerobic granulation in sequential aerobic sludge reactor. J Appl Microbiol 2001; 91:168–75.
[9] Tay JH, Liu QS, Liu Y. The effects of shear force on the formation, structure and metabolism of aerobic granules. Appl Microbiol Biotechnol 2001;57:227–33.
[10] Toh SK, Tay JH, Moy BYP, Ivanov V, Tay STL. Size-effect on the physical characteristics of the aerobic granule in a SBR. Appl Microbiol Biotechnol 2003;60:687–95.
[11] APHA, Standard Methods for the Examination of Water and Wastewater, 19th ed., New York: American Public Health Association, 1995.
[12] Pereboom JHF. Size distribution model for methanogenic granules from full scale UASB and IC reactors. Water Sci Technol 1994;30: 211–21.
[13] van der Hoek JP. Granulation of denitrifying sludge. In: Lettinga G, Zehnder AJB, Grotenhuis JTC, Hulsho PLW, editors. Granular aerobic sludge. Pudoc, The Netherlands: Wageningen, 1988. pp. 203–10.
[14] Grotenhuis JTC, Smit M, Plugge CM, Yuan SX, Lammeren AAM, van Stams AJM, Zehnder AJB. Bacterial composition and structure of granular sludge adapted to different substrates. Appl Environ Microbiol 1991;57:1942–9.
[15] Bishop PL, Zhang TC, Fu YC. Effects of biofilm structure, microbial distributions and mass transport on biodegradation processes. Water Sci Technol 1995;31:143–52.
[16] Beun JJ, Loosdrecht MCM, van Heijnen JJ. Aerobic granulation in a sequencing batch airlift reactor. Water Res 2002;36:702–12.
[17] Picioreanu C, van Loosdrecht M, Heijnen JJ. Effect of diffusive and convective substrate transport on biofilm structure formation: a two-dimensional modeling study. Biotechnol Bioeng 2000;69:504–15.
[18] Yu T, Bishop L. Stratification of microbial metabolic processes and redox potential change in an aerobic biofilm studied using microelectrodes. Water Sci Technol 1998;37:195–8.
[19] van Loosdrecht M, Tijhuis L, Wijdieks AMS, Heijnen JJ. Population distribution in aerobic biofilms on small suspended particles. Water Sci Technol 1995;31:163–71.
[20] Schmit E, Ahring BR. Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors. Biotechnol Bioeng 1996;49:229–46.
[21] Lawrence JR, Korber DR, Hoyle BD, Costerton JW, Caldwell DE. Optical sectioning of microbial biofilms. J Bacteriol 1991;173:6558–67.
[22] Massol-Deya AA, Whallon J, Hickey RF, Tiedje JM. Channel structures in aerobic biofilms of fixed-film reactors treating contaminated groundwater. Appl Environ Microbiol 1995;61:769–77.
[23] Keiding K, Wybrandt L, Nielsen PH. Remember the water—a comment on EPS colligative properties. Water Sci Technol 2001;43:17–23.
[24] Schmitt J, Flemming HC. Water binding in biofilms. Water Sci Technol 1999;39:77–82.
[25] Sponza DT. Extracellular polymer substances and physicochemical properties of flocs in steady- and unsteady-state activated sludge systems. Proc Biochem 2002;37:983–98.
[26] Guiot S, Pauss A, Costerton J. A structured model of the anaerobic granule consortium. Water Sci Technol 1992;25(7):1–10.
[27] Chui F, Li F. Microbial structure and activity of UASB granules treating different wastewaters. Water Sci Technol 1994;30(12):87–96.
[28] Kosaric N, Blasczyzk R. Microbial aggregates in anaerobic WWT. Adv Biochem Eng Biotechnol 1990;42:28–55.
[29] Ross W. The phenomenon of sludge pelletization in the anaerobic treatment of a maize processing plant. Water SA 1984;10(4):197–204.
[30] Shen C, Kosaric N, Blasczyzk R. The effect of selected heavy metals on anaerobic granules and their extracellular polymeric substance (EPS). Water Res 1993;27(1):25–33.