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Influence of different substrates on the formation and characteristics of aerobic granules in sequencing batch reactors

SUN Fei-yun[1], YANG Cheng-yong[1,*], LI Jiu-yi[1], YANG Ya-jing[2]

(1. School of Civil and Architecture Engineering, Beijing Jiaotong University, Beijing 100044, China. E-mail: c.y.yang@263.net; 2. School of Environmental Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, China)

Abstract: The effects of different substrates on the aerobic granulation process were studied using laboratory-scale sequencing batch reactors (SBRs). Four parallel granules sequencing batch reactors (GSBR): R_1, R_2, R_3, and R_4 were fed with acetate, glucose, peptone and fecula, respectively. Stable aerobic granules were successfully cultivated in R_1, R_2, R_4, and smaller granules less than 500 μm were formed in R_3. Morphology and the physic-chemical characteristics of aerobic granules fed with different carbon substrates were investigated by the four reactors operated under the same pressure. The aerobic granules in the four reactors were observed and found that peptone was the most stable one due to its good settleability even after a sludge age as short as 10 d. A strong correlation was testified between the characteristics of aerobic granules and the properties of carbon substrates. The stability of aerobic granules was affected by extracellular polymer substances (EPS) derived from microorganism growth during feast time fed with different carbon substrates, and the influence of the property of storage substance was greater than that of its quantity. Optimal carbon substrates, which are helpful in the cultivation and retention of well-settling granules and in the enhancement of the overall ability of the aerobic granules reactors, were found.

Keywords: aerobic granules; different carbon substrates; extracellular polymers; GSBR

Introduction

As a key role in ensuring a success of a treatment process, effective separation of biomass from treated effluent has always been a focus to wastewater researchers and engineers. Suspended granules and attached biofilms are two well-known forms of microbial immobilization, in which microorganisms are separated from the effluent water and a large of biomass concentration is maintained in the treatment process. Microbial granules represent a more recent form of cell immobilization that has been probably best recognized in the up-flow anaerobic sludge blanket (UASB) reactor. In the UASB reactor, anaerobic microorganisms are self-immobilized under carefully controlled operating conditions into compact granules (Lettinga *et al.*, 1980; Pereboom, 1994). Anaerobic granulation is a process of dynamic selection in which environmental and operational pressures, both biologically and physically, favor the cultivation of bacteria that can form aggregates. These factors include microbial production of extracellular polymers and introduction of inorganic nuclei to initiate the formation of granules in an anaerobic reactor. In addition, the filamentous microorganisms also play an important role during the granulation in a binding system.

More recently, the sequencing batch reactor has been used to study granulation under aerobic conditions (Morgenroth *et al.*, 1997; Beun *et al.*, 1999, 2002; Peng *et al.*, 1999; Etterer and Wilderer, 2001). These aerobic granules are of characteristics that make them ideal bioagents for biological wastewater treatment. Compared to activated sludge, these granules have better settleability, higher density, stronger microbial structure, higher biomass retention and better nutrient removing capability. Some studies have shown that the aerobic granules sludge can be cultivated easily in sequence batch bioreactor (SBR) and sequencing batch airlift reactor (SBAR). Aerobic granular sludge can be cultivated in SBR, in which synthetic wastewater containing sodium acetate as an organic substrate was fed, and dissolved oxygen (DO) was controlled at a low concentration (0.7—1.0 mg/L) (Peng *et al.*, 1999). Investigation of aerobic granule technology tells that a short hydraulic retention time (HRT) (Beun *et al.*, 1999), a short selective settlement time (Qin *et al.*, 2004), a high capacity loading (Tay *et al.*, 2004) and a right shearing force (Tay *et al.*, 2001) posed by aeration are helpful in the formation and maintenance of aerobic granules in SBR and SBAR. Furthermore, the concentration of feeding condition (Mc Swain *et al.*, 2004) and the sludge age have also effects on the characteristics of aerobic granulation. But till the present, there has been no detailed investigation on the process and influence of the carbon substrates on the granulation in SBR reactors. Liu *et al.* (2004a) examined the structure of aerobic granules operated at different substrate N/COD ratios ranging from 5/100 to 30/100, and they believe controlling substrate N/COD ratio would be a useful strategy for improving the stability of aerobic granules because the observed growth rate and mean size of mature aerobic granules were found to decrease as the substrate N/COD ratio was increased. So far, the study on the characteristics of aerobic granules fed with different single carbon substrates is waiting to be improved. This paper focused on the effects of different carbon substrates on aerobic granulation and the change of granule morphology and structure. The

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results presented in this study can be used to evaluate the influence of cultivation conditions on aerobic granulation process.

1 Materials and methods

1.1 Experimental setting

The experimental work was carried out in four plexiglass cylinder sequencing batch reactors, marked by R_1, R_2, R_3 and R_4, separately. Fig.1 shows a diagram of the experiment system. The influent fed into each of the reactors contains different carbon substrates. The reactor has a work cubage of 3.1 L (7 cm in inner-diameter and 10 cm in height). Three electronic timer controlled SBR operation: feeding, aeration, settling and draining; the feeding and drain quantity were controlled by peristaltic pumps. Lasting times were set as: 10 min of feeding, 225 min of aeration, 3 min of settlement, and 2 min of draining, which makes up of the whole cycle time of 4 h in total. The effluence was drained from the mid of the reactor, so that the volumetric exchange ratio (VER) was constant at 0.5 throughout the experimental period and the hydraulic retention time was 8 h while the organic loading rate (OLR) was kept at (4.0±0.2) kg COD/(m^3·d). Compressed air was introduced to each SBR by means of sintered stone diffusers. The airflow was controlled with the aid of pre-calibrated rotameters to ensure a constant level of mixing, resulting in an airflow velocity of 0.4 cm/s. DO levels in the unit were similar and always greater than 3 mg/L. The whole reactor was kept in a constant temperature instrument at a maintenance temperature of 25℃. The reactor was inoculated using 1.5 L fresh activated sludge (its initial mixed-liquor suspended solid being 3.0 g/L) taken from an aeration tank of a local municipal wastewater treatment plant. The wall of the reactor was cleaned and the biofilm growth was discarded once every three days.

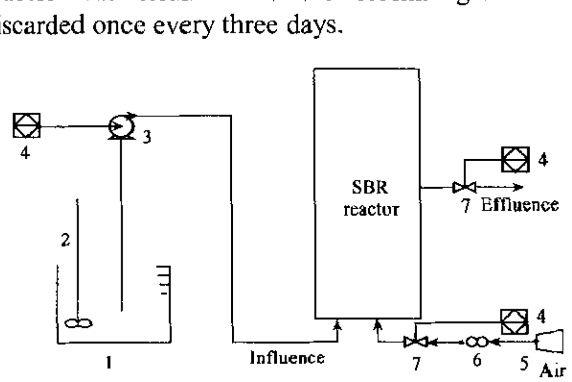


Fig. 1 Schematic representation of the SBR system
1. substrate feed container; 2. whipper; 3. metric pump; 4. electronic timer; 5. aerator; 6. pre-calibrated rotameters; 7. electromagnetic valve

1.2 Media

The compositions of the concentrated media were as follows. Media A is so-called carbon substrate; R_1 was fed with NaAc of 1.23 g/L; while R_2 with glucose of 1.25 g/L; R_3 with peptone of 1.25 g/L, and R_4 with fecula of 1.25 g/L. Media B is composed of NH_4Cl of 0.2 g/L, KH_2PO_4 of 0.2 g/L, $MgSO_4$ of 0.06 g/L and $CaCl_2·2H_2O$ of 0.05 g/L. It was fed to every reactor with the same concentration and quantity. The composition of trace element solution was taken according to Tay *et al.* (2003).

1.3 Analytical procedures

Regular sampling at the influence, effluence and mixed-liquor, as well as the analysis of the mixed-liquor suspended solids (MLSS), soluble chemical oxygen demand (SCOD), suspended solids (SS), specific oxygen utilization rate (SOUR), sludge volume index (SVI) and specific gravity as specified by "Standard Methods" (APHA, 1980), were performed so as to assess the performance of the system. SVI was determined in 100 ml graduated cylinders at 30 min of sludge settlement. For the determination of oxygen utilization rate, the granules were carefully washed with tap water and placed in a biological oxygen demand bottle (Orinon 862A) containing pre-aerated substrate and nutrient solution. In order to avoid breaking the granules, the bottle was gently mixed with a magnetic mixer at 50 r/min. The temperature was kept at 25℃. The decrease in dissolved oxygen concentration was recorded at a time interval of 30 s. SOUR was then calculated according to the change in dissolved oxygen concentration over time and the MLVSS in the bottle. The size and granularity of sludge in each reactor were determined by a laser particle size analysis system (Mastersizer 2000, Malvern Instrument, UK) and microscopic observation, and the zeta potential of sludge surface was measured by zeta potential analysis system (Zetasizer 2000, Malvern, UK). Periodically, well-mixed samples of aerobic granules were taken from each reactor and analyzed to determine the morphological and chemical characteristics of the aerobic granules such as size and specific gravity etc. The morphological and structural characteristics of aerobic granules were observed and measured qualitatively with a scanning electronic microscope (FEI Quanta 200, USA). Granule samples were first washed with distilled water and dewatered with a graded ethanol series (30%, 50%, 70%, 85%, 95% and 100%). The dewatered samples were then dried with a critical point dryer (CPD030, BAL-TEC AG, Balzers, Liechtenstein) and were held intact or bisected for observation of the external and interior structures. The samples were further sputter-coated with gold (SCD005, Bal-Tec AG, Balzers, Liechtenstein) for SEM observation.

2 Results and discussion

2.1 Experimental performance

Every reactor was inoculated with activate sludge taken from a local municipal wastewater treatment plant without any acclimation. No oxygen control was made, which means that the oxygen concentration (DO) during aeration was almost saturated. The pH was kept between 6.8 and 7.6 throughout the experiment by adding proper dosage of diluted HCl and sodium bicarbonate. Every reactor was aerobic during all the feeding period. According to the change of sludge morphology and the sludge age controlled by draining mixed liquor from every reactor, 80 d of experiment can be divided into three phases: Phase I (start-up phase), Phase II (maturation phase), and Phase III (sludge retention time (SRT) controlling phase). Due to the distinct performance of reactors, the start-up phase lasted for different periods of time. It took 19 d for R_1, 13 d for R_2, 10 d for R_3, and 18 d for R_4, respectively. The maturation phase run from the end of start-up phase till the day 35. The Phase III went till the end of the experiment.

With the growth of MLSS (Fig.2a) and the size of the granules in every reactor during start-up phase, a large amount of floc sludge with bad settleability appeared and needed to be washed out under the selective settlement time from the reactor when the VER went down to 0.5. Owning to the difference in adaptation capacity and the conversion rate of different carbon source of the biomass, the decrease rate of MLSS and washed-out quantity of the floc varied from one reactor to another. In R_1 and R_2, MLSS decreased from 1.6 to 0.3 g/L on the day 6. In R_2, the average diameter of yellow granules grew higher than 600 μm on the day 7. In R_4, the decrease of MLSS is slower than in other reactors. MLSS decreased to 0.34 g/L on the day 12, but the size of granules is similar to that of R_2 fed with glucose. In R_3, the size of the granules stayed larger than 120 $\pm$20 μm almost all the time during the experimental period, and the MLSS in this reactor did not see significant decrease over the strictly selected settling time. This phenomenon is beyond our expectation and the reason might be due to the unexpected TN/COD ratio which was 20/100—30/100 in peptone and the high nitrogen concentration led to the accumulation of inorganic matter and protein which prevented the aggregation of microorganisms.

Maturation phase conducted from the end of

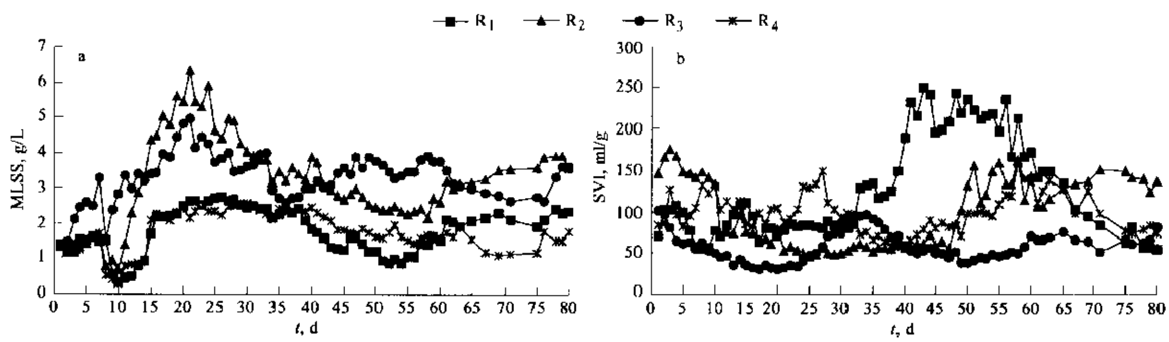


Fig.2 Evolution of MLSS (a) and SVI (b) in every reactor

start-up phase till the day 35. This phase is characterized by high organic loading rate, well COD removal rate and good sludge settleability. Most of the floc biomass was washed out and visible granules appeared increasingly in the following days. Large filamentous granules became predominant in R_1, R_2 and R_4 reactors while the floc biomass went almost disappeared. The granules in every reactor came out to be obviously different in size. In R_1, the size of granules is around 1.5 to 3 mm, and the MLSS falls in between 3.5 and 4.4 g/L. In R_2, the texture of granules is much denser than in the others, although the size of granules is similar to R_1 and R_4, causing the MLSS in R_2 to be as high as 6.5 g/L. As to reactor 3 fed with peptone, the size of sludge distributed in the whole cylinder remained unchanged.

Maturation phase began when MLSS became constant and the SVI_{30} got to be well. The MLSS of R_3 increased from 2.1 in the beginning to 5.0 g/L along with the growth of biomass. The dense structure and small size of the sludge resulted in a well settleability represented by SVI_{30}. Owing to a high TN/COD ratio, SVI_{30} run always below 45 ml/g, which partly assumed that the absorption and deposition of carbon substrates are restrained intracellular during aeration feeding period. The uptake of substrates in this biomass was different from traditional aerobic granules cultivated by easy-to-biodegraded substrates. NH_4^+, which reduced along with the peptone degradation process, was also believed to be responsible for this unexpected phenomenon. High concentration of NH_4^+ can affect the efficiency of substrates transportation from wastewater to the inside of cells, which helps decrease the growth rate of biomass and then hinder the aggregation of microorganisms. In R_4 reactor, the size of the granules increased from 1.2 to 2 mm, and the MLSS developed the same as in R_1 and R_2. The maximum MLSS reached 3.7 g/L.

To investigate the stability of granules in every reactor fed with different carbon substrates, phase III was conducted over a fixed retention time being 10 d. During phase II, the high OLR and the selective pressure helped the growth and proliferation of larger and denser aerobic granules so that the biomass was eventually dominated by the microorganism aggregation in R_1, R_2 and R_4. Meanwhile, the R_3 reactor enjoyed better performance characteristics such as settleability and removal efficient owning to a large amount of denser biomass being consisted of smaller granules of (120 ± 20) μm in diameter. With respect to the stability of aerobic granules which were studied in the evolution process under short SRT pressure, 0.31 L mixed liquor was drained from every reactor everyday during the aeration period, bringing about that the SRT in the reactor was able to maintain for 10 d. In R_1, which was cultivated by acetate, the MLSS began to decrease smoothly and lasted for 15 d. Then the MLSS reached another constant level at 2.7 g/L, but the SVI_{30} increased rapidly for almost 30 d during the beginning time period. The maximum of SVI_{30} reached 250 ml/g, although the size of granules was just around 2 mm in diameter. Holes could be seen inside of the granules and a loose structure is the main reason for the decrease of MLSS and increase of SVI in the beginning of the drainage of mixed liquor. The loose and light granules are assumed to be caused by the increasing growth rate of biomass over a short SRT. van Loodrecht (1995) thought that a lower growth rate of the microorganisms could favor the stability of biofilm, because the shear force caused by aeration and cycling of hydrology can break up the newly grown biomass that could not attach to the former biofilm. This conclusion perhaps explains the fast growth of biomass under the short SRT that results in the bad performance of granules. Therefore, except R_3 reactor, the other three reactors have an obvious trend of deterioration at the beginning of SRT control period. However, this trend would be alleviated and a new balance between biomass and environment could be reached after 60 d, while the MLSS and SVI came to a stable level. The detailed data are shown in Figs.2a and 2b.

Fig.3 shows the soluble chemical oxygen demand (SCOD) removal efficiency throughout the experiment in every reactor. The OLR was kept around 4.0 ± 0.2 kgCOD/(m^3·d), and the HRT was 8 h. Even after the three phases, the four reactors had soluble COD removal efficiency as high as more than 90%. In R_2 fed with glucose source, its SCOD removal percentage reached as high as 95%. Although the floc biomass was washed out in the start-up phase and the mixed liquor was manually drained, the COD removal efficiency was yet not low. In R_3 fed with peptone, its SCOD removal efficiency reached 91% in the beginning phase and 87% on the day 50. A relatively lower substrate removal rate goes beyond our expectation because almost all aerobic granules cultivated in SBR have high carbon source removal capacity (Beun *et al.*, 1999; Tay *et al.*, 2001, 2004). Furthermore, the MLSS and the SOUR of these small granules were excellent. Then a high concentration of free ammonia ion in influent is believed responsible for this occurrence. Yang *et al.* (2003) reported that free ammonia could result in a significant decrease of cell hydrophobicity, and also block the production of cell polysaccharides. The TN/COD in R_3 was 20/100—30/100, the free ammonia concentration can therefore be worked out being equal to 9.8—18.0 mgN/L, according to the equation proposed by Anthonisen *et al.* (1976). This FA (free ammonia) concentration is much higher than the inhibition threshold suggested by Yang *et al.* and resulted in a reduced production of cell polysaccharides. In addition, a higher FA concentration also inhibited the catabolic activity of microorganism that favors the decrease of cell polymer. Meanwhile, in reactor

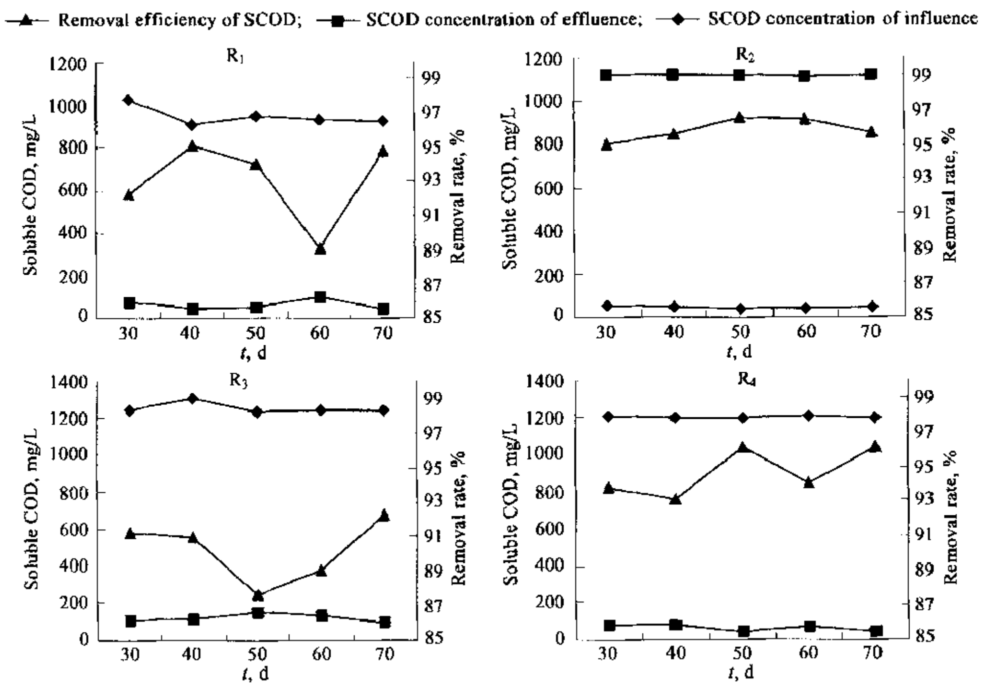


Fig.3 SCOD removal efficiency of every reactor

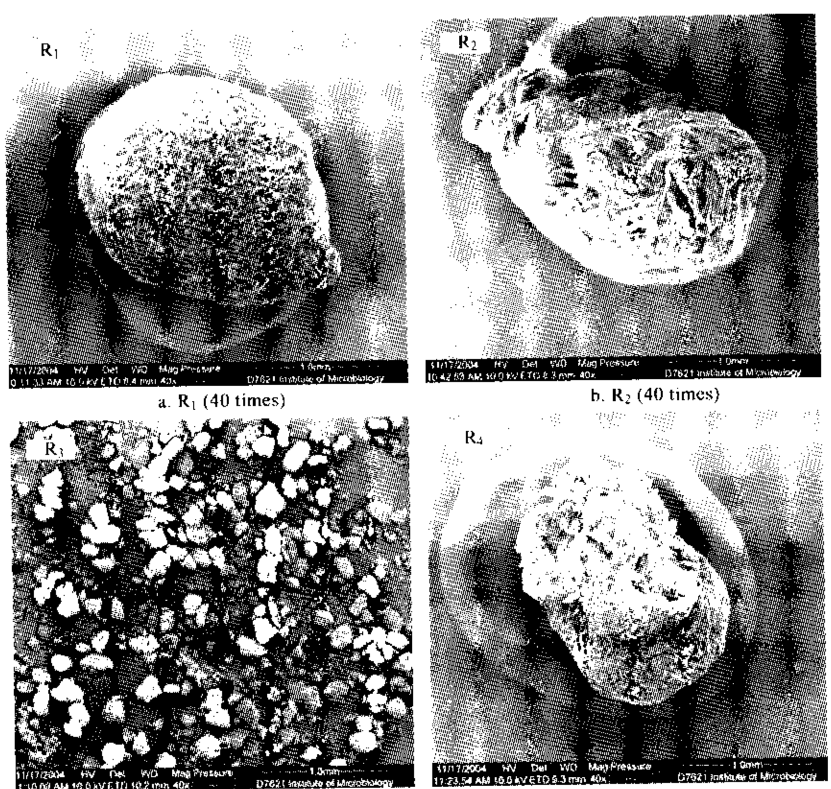
systems that were pulse fed, more than 80% of the substrate was used for synthesis of extracellular polymers while the rest was used for growth and maintenance process (Martins *et al.*, 2003). So a decrease in the catabolic activity of these small granules is often expressed by a low substrate removal rate even at a sufficient biomass concentration. Since the mechanism why and how the free ammonia influences the physicochemical and biological properties of cell surface is still unclear, further study on this topic is needed.

2.2 Morphological characteristics of aerobic granules

SEM observation had been applied to analyze the characteristics of aerobic granules under different carbon sources fed with. The inoculated sludge was taken from an aeration tank of a local wastewater treatment plant (MLSS: 2.5 g/L; SVI: 154 ml/g).

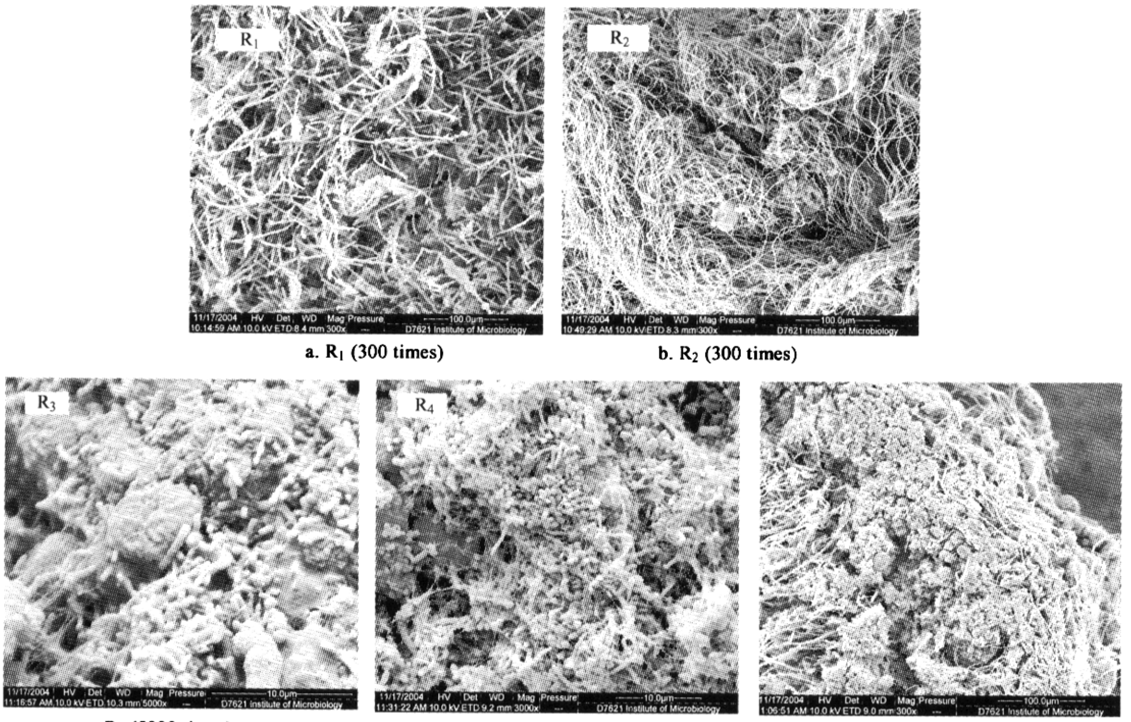
In R_1 fed with acetate, yellow small granules appeared after 7 d operation during start-up phase, and 2 mm diameter granules became dominant on the day 13. The SEM observation is plotted in Fig.4a and Fig. 5a, which indicates that the filaments built up the framework of granules and the fungi and cocci like bacteria were entrapped in the granules. An intercross of filaments comprises the main body of the outermost layer of the granules. Contacting granules were retained in the reactor and the substrate penetration was carried out through the holes on the surface of the granules. In the subsequent days the reactor became full of granules, and only a little visible floc biomass could be seen in it. The color of the granules changed to dark yellow and their structure became much denser, which were caused under selective conditions. The aerobic granules had a significant shift into a loose density and a light color in SRT control phase. Simultaneously, the concentration and the performance of granules went down, and the filamentous bacteria became dominant to adapt to the high growth rate. When the granules slowly got stable on the day 70, the structure of the granules turned comparatively looser and the SVI_{30} went as high as 145 ml/g, which is similar to traditional aerobic sludge in wastewater treatment plant. At this situation, the advantages of aerobic granules will disappear.

Fig.4b and Fig.5b show the morphology and structure of the granules generated in R_2. The granules are composed of filaments and some large sporules growing from fungi and other bacteria. The surface of granules is compact because of the intertwist of filaments and the junction of large sporules. The mycelium, which makes up of the outer layer of the aggregation, is smaller than the filaments in granules of R_1. The change of the form and the structure of aerobic granules cultivated with glucose was the least one among these four reactors, and the sludge characteristics and the removal efficiency were almost not influenced by the mixed liquor drainage operation, although bad filaments were also visible on the surface of the granules. Comparatively, R_3 reactor is somewhat special, there being no large granules above 0.5 mm in diameter, and the color of these small



a. R_1 (40 times) b. R_2 (40 times) c. R_3 (40 times) d. R_4 (40 times)

Fig.4 Comparison of appearance forms of aerobic granules fed with different carbon substrates



a. R_1 (300 times) b. R_2 (300 times)

c. R_3 (5000 times) d. R_4 (3000 times) e. The cross sections of aerobic granules (40 times)

Fig.5 SEM photos of aerobic granules cultivated with different carbon substrates

pellets is dark brown. Fig.4c and Fig.4c illustrate the size and the structure of these small granules. The compact surface is hard and the dominant micro-organism is bacilli. There were no obvious extracellular polymers found on the granules. All these characteristics were correlated with the effect of NH_4^+ reduced from peptone that has smaller C/N ratio. This inhibition of substrates absorption would lead to the decrease of growth rate of fungi and filaments that are key factors for the stability of microorganism aggregations. From Fig.4d and Fig.5d, a higher concentration of extracellular polymers can be seen and the glutinosity of this composition is likely to be caused by highly biodegradable fecula. The cocci and short bacilli are very common to grow on the surface of this kind of granules. During phase III, the MLSS and the SVI_{30} were relatively stable, although the size of granules decreased to 1 mm due to the increase of growth rate of microorganisms. This behavior of granules can also be attributed to the difficulty of degradation of fecula.

However, some researchers suggested that a cavity may appear at the center of the granules where bad substrates penetration efficiency exists. In this experiment, the absence of this kind of cavities may be because of short operation time and the comparative smaller size of granules cultivated. Therefore, the gradients of oxygen and substrate transfer rate are large enough for biomass growth. Fig.5e shows that along the cross sections of the granules, obvious layers exist. On the outermost layer of this section, the filaments and the fungi that have better activity at the three-phase interface where high diversity of concentration took place during the feast time got dominant, and this kind of filaments can successfully compete for substrates with other organisms. Filaments that have low μ_{max} occupied the inner side of granules. The middle junction of these two layers is assumed to be the lower degradable polymer such as poly-β-hydroxy-butyrote(PHB), and the glucose accumulating bacteria would be responsible for this substance, which can exert positive effect on lower growth rate microorganisms. Those substance and the lower growth rate microorganisms can contribute to the stabilization of aerobic granules.

2.3 Physic-chemical characteristics of aerobic granules

Settlement time and shear force, which are key operation conditions, were controlled in the same way during the experiments. The difference among the experiments was assumed to be induced by different organic substrates. A representative index is used to express this different characteristic of aerobic granules caused by cultivated substrates. Fig.6 gives the Zeta potentials on the surface of aerobic granules during start-up phase. From Fig.6 we know that, in the low area of Zeta potential, the aggregation of micro-organisms in R_3 is less intense than in other reactors, while the variation of this index is similar among the other reactors. It can be concluded that granules fed with single carbon substrate have no direct relation to Zeta potential of sludge surface and that the change of Zeta potential of sludge surface during start-up phase is not a necessary condition for aerobic granules generation.

Tables 1 and 2 list the specific gravity and the

SOUR of aerobic granules in each reactor on the day 37, separately. The specific gravity of traditional activated sludge varies from 1.002 to 1.006 g/cm^3(Zhu and Liu, 1999), and the aerobic granule is larger than the average value in this study. The greater the molecular weight of carbon substrates is, the larger the specific gravity of aerobic granules will be. As a criteria for the ability of a reactor to degrade organic substance, the SOUR can be determined in batch experiment under 20℃. In comparison with the value of SOUR of traditional activated sludge, the aerobic granules of R_1, R_2 and R_3 have stronger activity and can bear higher organic loading rate, which helps save the space of a wastewater treatment plant. Therefore, it becomes a popular topic of research nowadays. The SOUR of R_4 is comparatively low and the reason may be ascribed to the low degradability of carbon substrates. This outcome would affect the availability of granules in the sequencing batch reactor in treating factory wastewater that bears high fibre and fecula concentrations.

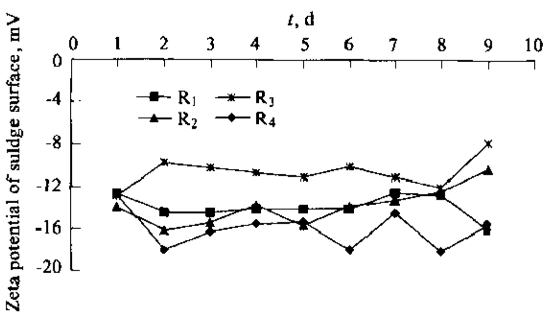


Fig.6 Zeta potentials of sludge surface during start-up phase

Table 1 Calculation on the specific gravity of aerobic granules

Reactor	Weight of bottle and granules, g	Weight of water and bottle, g	Weight of bottle, g	Correction on temperature, F	Specific gravity of aerobic granules, g/cm^3
R_1	80.4352	80.2035	30.2895	0.998	1.0065
R_2	79.6443	79.4727	29.5157	0.996	1.0074
R_3	75.5147	75.4650	25.817	0.993	1.0081
R_4	75.4021	75.3083	25.819	0.994	1.0079

Table 2 SOUR of aerobic granules

	R_1	R_2	R_3	R_4
SOUR, mgO_2/(gVSS·h)	150.6	381.6	308.4	59.28

2.4 Stability of aerobic granules

Aerobic granulation is known as a microbial self-immobilization process that should be similar to the growth of biofilm (Li and Liu, 2005; Beun *et al*., 2002). In the study of biofilms, there is evidence that the strength of biofilms is negatively related to the growth rate of microorganism. Then a suitable index to denote the stability of microorganism aggregation such as granules or biofilm is the actual growth rate of the microorganisms inside the granules (van Loodrecht, 1995). Some researchers linked the stability of bio-aggregation to the diffusion of the substrates in the biomass, using the gradients of substrates inside the aggregation to explain the correlation between the stability of aggregation and the diffusion rate of substrates (de Kreuk and van Loosdrecht, 2004). When the substrates gradient is large inside the aerobic granules, the heterogeneity or floc-like bacteria will develop and the stability will be bad. On the contrary, when this gradient is small, the microorganisms with lower growth rate would dominate and the regular granules can be formed. Heterotrophic organisms decrease the growth rate when they grow on the hard-to-biodegraded storage polymer PHB or glycogen, comparing to the growth on the easily biodegradable substrates such as acetate and glucose. The previous study of aerobic granules has proved this conclusion and obtained stable granules. In addition, similar to this research on the activated sludge floc, the extracellular polymers, which are a major factor for the absorption and removal of organic source in activated sludge, also lead to the unstable aerobic granules. Liu *et al*. (2004b) studied the effect of different organic substrates on the formation of the extracellular polymeric substance and the experimental results demonstrate that the sludge fed with acetate has a high polysaccharide-to-protein ratio. In the sludge fed with glucose, the formation of extracellular polymer substances (EPS) is more rapid. The sludge fed with peptone contains only a little polysaccharide. The size of EPS is different in this sludge too. This research's outcome is in accord with our results of aerobic granules, and the amount of EPS and the properties of the polymers have significant effect on the granules' morphology and structure. Tay *et al*. (2004) conclude that when the polysaccharide- to-protein ratio is less than 2, aerobic granules are unable to develop. Low polysaccharide-to-protein ratio in R_3 fed with peptone leads to a low capability of biomass aggregation and the difficultly biode- gradable protein contributes to the low growth rate of microorganisms that is important to the stability of a GSBR system.

Liu *et al*. (2004a) reported that, in order to improve stability of aerobic granules, selecting slow-growing nitrifying bacteria will be an effective way. A higher N/COD ratio results in a lower specific growth rate of aerobic granules and in a higher cell hydrophobicity that contributes to a compact structure of aerobic granules. In addition, higher TN/COD ratio of R_3 system conduces to the selection of nitrifying bacteria in small granules. The nitrifying population was mainly located at a depth of 70—100 μm from the surface of a granule (Tay *et al.* 2003). Since nitrifying bacteria are slow-growing species, the stable structure would be resulted from the demand of nitrifying population on nutrients, and small size granules in turn guarantees that nitrifying population in aerobic granules can maximize access to nutrients. Furthermore, through increasing the protein level in the EPS, a better settleability (low SVI) of activated sludge and a higher negatively charged floc surface could be achieved, which helps increase the stability of aerobic granules (Sponza, 2002). So, a high TN/COD ratio is the main reason for the good stability and settleability of small granules in R_3, even if the size of granules in R_3 is the smallest among the four reactors. Meanwhile, the bad stability of R_1, R_2 and R_4 is also relevant to the high growth rate of biomass and the physicochemical properties of EPS of their aerobic granules. Polysaccharide and protein content are recognized as important factors that could affect the granulation process. High polysaccharide-to-protein ratio exerts a positive influence on the granulation process, but has low anti-impulsion ability to the change of sludge retention time; low polysaccharide-to-protein ratio would hinder the microorganism aggregation but is helpful in maintaining the structural integrity of biomass.

3 Conclusions

Four SBRs, reactors R_1, R_2, R_3, and R_4, were fed with acetate, glucose, peptone and fecula respectively. Three phases of biomass development were observed. In the acclimation phase, the seed sludge developed into large flocs. Coming up next is the maturation phase, which is characterized by a significant increase in MLVSS concentration in the reactors and well characteristics of granules. Large aerobic granules were not detected in the reactor fed with peptone. Aerobic granules first appeared on day 12 in R_2 cultivated with glucose. In R_3, the flocs in the acclimation phase are mostly consisted of small and dense granules of (120 ± 20) μm. The young granules in the maturation phase contain a variety of cell morphotypes, including filamentous microorganisms, rods, and cocci that are embedded in an extracellular polymeric matrix. In the cases of R_1 and R_4 which were fed with acetate and facula, aerobic granules were initially observed on the day 19 and 18, respectively. However, these granules were unstable and went loose in the following days. In SRT control phase, R_3 made no difference and the SVI got as low as 45 ml/g. Under certain operation conditions such as DO, shear force and selective time, the characteristics of aerobic granules such as morphology, structure, specific gravity, size, settleability and stability are strongly dependent on the properties of carbon substrates. Compared to flocs, granules are more compact, with a greater specific gravity, and higher strength or resistance to deformation. The relative amounts of SS are higher in the effluent biomass than in the reactor biomass in R_1, R_2, and R_4, and this is due to the washing-out of poorly settling flocs.

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