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Full Length Research Paper

A potential explanation for the effect of carbon source on the characteristics of acetate-fed and glucose-fed aerobic granules

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This paper proposes a new theory to account for the effect of carbon source on the characteristics of acetate-fed and glucose-fed aerobic granules. It is well known that reactor pH can vary in response to the oxidation of glucose or sodium acetate. As such, the effects associated with the carbon sources may be explained by the changed pH. The proposal was explored by experiments. Aerobic granules were cultivated in three identical sequencing batch reactors (SBRs, R1, R2 and R3), fed with sodium acetate, glucose, glucose and maintained pH at 4.5 - 5.5 (the variation of reactor pH in the oxidation of glucose), 4.5 - 5.5 and 7.5 - 8.5 (the variation of reactor pH in the oxidation of sodium acetate), respectively, and the effects of carbon source and reactor pH on the characteristics of aerobic granules were assessed. The results showed that the characteristics of aerobic granules, including microbial structure, mixed liquor suspended solids (MLSS), sludge volume index (SVI) and nitrification-denitrification, were strongly affected by reactor pH, but were independent with the carbon source supplied. These results fully supported the validity of the new theory. The theory suggests that the cultivation of aerobic granules with glucose or sodium acetate should take more attention to reactor pH rather than carbon source itself. The implications of this theory are discussed with regards to the other common carbon sources as well as better understanding of the mechanisms of aerobic granulation.

Key words: Acetate-fed granules, glucose-fed granules, reactor pH, carbon source, characteristics.

INTRODUCTION

Aerobic granulation is a recently developed biotechnology for wastewater treatment (Liu et al., 2005; Qin and Liu, 2006; Adav et al., 2008). Compared with conventional bioflocs, aerobic granules have a number of advantages, such as denser and stronger microbial structure, better settling ability, more effective sludge-effluent separation, greater biomass retention and enrichment, much improved capability to withstand shock loadings, simultaneous nitrification and denitrification and more resistance to inhibitory and toxic compounds (Qin and Liu, 2006; Li et al., 2007; Adav et al., 2008; Wang et al., 2008). From engineering and economic points of view, aerobic granulation is a promising process that has the potential to lead the next generation of biological wastewater treatment technologies.

Previous research reveals that aerobic granules can be successfully achieved with a number of substrates, including organic/inorganic carbon sources, toxic wastewater and real municipal and industrial wastewaters, and the characteristics of aerobic granules formed are highly affected by the carbon source supplied (Arrojo et al., 2004; Qin and Liu, 2006; Liu et al., 2008; Zhu et al., 2008; Ni et al., 2009). For the specific carbon source, it is easy

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Abbreviations: MLSS, Mixed liquor suspended solids; **SVI,** sludge volume index; **SBRs,** sequencing batch reactors.

to explain the strong selectivity of carbon source to microorganisms. From the selectivity theory, the microorganisms in activated sludge, which can take advantage of the carbon source, are remained in the reactor and further formed aerobic granules, and the microorganisms, which can not utilize the carbon source at all, would leave the reactor with effluents. But for the common carbon source, such as glucose and acetate sodium, although they do not have any selectivity to the microorganisms in activated sludge, but the characteristics of aerobic granules fed by them are also quite different from each other (Tay et al., 2002). It is unlikely to explain the strange phenomenon by the selectivity theory.

Under aerobic conditions, acetate and glucose can be oxidized as follows (O'Neill, 1998; Liu, 2008):

$$CH_3COO^- + 2O_2 + H^+ \rightarrow 2CO_2 + H_2O, \quad (1)$$

$$C_6H_{12}O_6 + 6O_2 \rightarrow 2C_3H_4O_3 + 2H_2O + 5O_2 \rightarrow 6CO_2 + 6H_2O \quad (2)$$

During the oxidation of acetate, hydrogen ion is consumed, which results in an increase in solution pH, whereas the oxidation of glucose generates pyruvic acid and carbon dioxide, which can acidify the solution. It can be inferred that in the degradation of glucose or acetate, solution pH can be changed to acid or alkaline. Can the changed pH resulting from the degradations of the carbon sources affect the characteristics of glucose-fed and acetate-fed granules, but not the carbon sources themselves? A new theory emerges and according to the theory, the characteristics of aerobic granules fed with glucose and acetate must be related to reactor pH, but not carbon source.

In order to test the theory, the variations of reactor pH in the degradations of glucose and acetate, 4.5 - 5.5 and 7.5 - 8.5, were achieved in preliminary experiments, and in the current study, three sequencing batch reactors (SBRs, R1, R2 and R3) were operated with sodium acetate, glucose and glucose as substrates and were held pH at 4.5 - 5.5 (the variation of reactor pH in the oxidation of glucose), 4.5 - 5.5 and 7.5 - 8.5 (the variation of reactor pH in the oxidation of sodium acetate), respectively, to study the effects of carbon source and reactor pH on the characteristics, including microbial structure, mixed liquor suspended solids (MLSS) and sludge volume index (SVI) as well as nitrification-denitrification.

MATERIALS AND METHODS

Experimental setup and operation

Experiments were performed in three identical sequencing batch reactors. Each reactor had a working volume of 1.0 L and an internal diameter of 5 cm, giving a working H/D of about 10. Fine air bubbles for aeration were supplied through an air sparger at the bottom of the reactor at an airflow rate of 3 l/min, equivalent to a superficial upflow air velocity of 2.5 cm/s. All of the reactors were operated in a sequential mode for a 4-h cycle consisting of 5 min of feeding, 225 min of aeration, 5 min of settling and 5 min of effluent withdrawal from the middle ports of the reactors. The reactors were operated at room temperature, and water temperature was 20 - 25°C. R1 and R2 were maintained at pH 4.5 - 5.5 and R3 at 7.5 - 8.5 through addition of 1 M HCl or 1 M NaOH.

Activated sludge taken from a local municipal wastewater treatment plant (Shanghai Minhang district water purification plant) was used as seed sludge for inoculation, with initial MLSS of 2.87 g/l and SVI of 174 ml/g.

The composition of the synthetic wastewater used in this study was as follows: carbon source (1025 mg/l sodium acetate for R1 or 750 mg/l glucose for R2 and R3), 320 mg/l NH_4Cl, 58 mg/l K_2HPO_4, 23 mg/l KH_2PO_4, 90 mg/l $MgSO_4$, 53 mg/l $CaCl_2$, 20mg/l ethylenediamine tetraacetic acid (EDTA) and 1.0 ml/l microelement solution. This provided a chemical oxygen demand (COD) of 800 mg/l for all the reactors. The microelement solution contained 0.05 g/l H_3BO_3, 0.05 g/l $ZnCl_2$, 0.03 g/l $CuCl_2$, 0.05 g/l $MnSO_4 \cdot H_2O$, 0.05 g/l $(NH_4)_6MoO_{24} \cdot 4H_2O$, 0.05 g/l $AlCl_3$, 0.05 g/l $CoCl_2 \cdot 6H_2O$ and 0.05 g/l $NiCl_2$ (Ren et al., 2008).

Analytical methods

The measurements of $NH4^+$-N, MLSS and SVI were conducted according to the standard methods (APHA, 2005). Total nitrogen (TN) was analyzed with a total organic carbon/total nitrogen (TOC/TN) analyzer [N/C3000 (ChD), Analytik Jena AG, Germany]. A pH meter was used for pH measurements. The average particle size was measured by a laser particle size and shape analyzer (CIS-100, Ankersmid Co., Netherlands) with a range of 0.002 - 6000 µm.

Microbes were observed using an optical microscope equipped with a charge coupled device (CCD) camera (Nikon E400, Japan). The morphology and surface structure of the granules were studied with a scanning electron microscope (SEM; JSM-5610 LV JEOL, Tokyo, Japan).

RESULTS AND DISCUSSION

Microbial structure of aerobic granules

Granulation of aerobic sludge was satisfactorily achieved in reactors R1, R2 and R3. The microbial structures of mature granules in R1, R2 and R3 are shown in Figures 1, 2 and 3, respectively. All granules were round in shape with a clear outline, but there were obvious differences in microbial composition and microstructure. SEM images revealed that the granules in R1 and R2 were mainly composed of fungi, while the granules in R3 consisted predominantly of bacteria. In R1, the long filamentous cells tangled with each other and protruded from the surface, forming a fluffy and loose structure. In R2, the long filamentous cells likewise tangled with each other, forming a fluffy and loose structure. In contrast, the granules in R3 were composed of bacterial aggregates with a rather strongly compact structure.

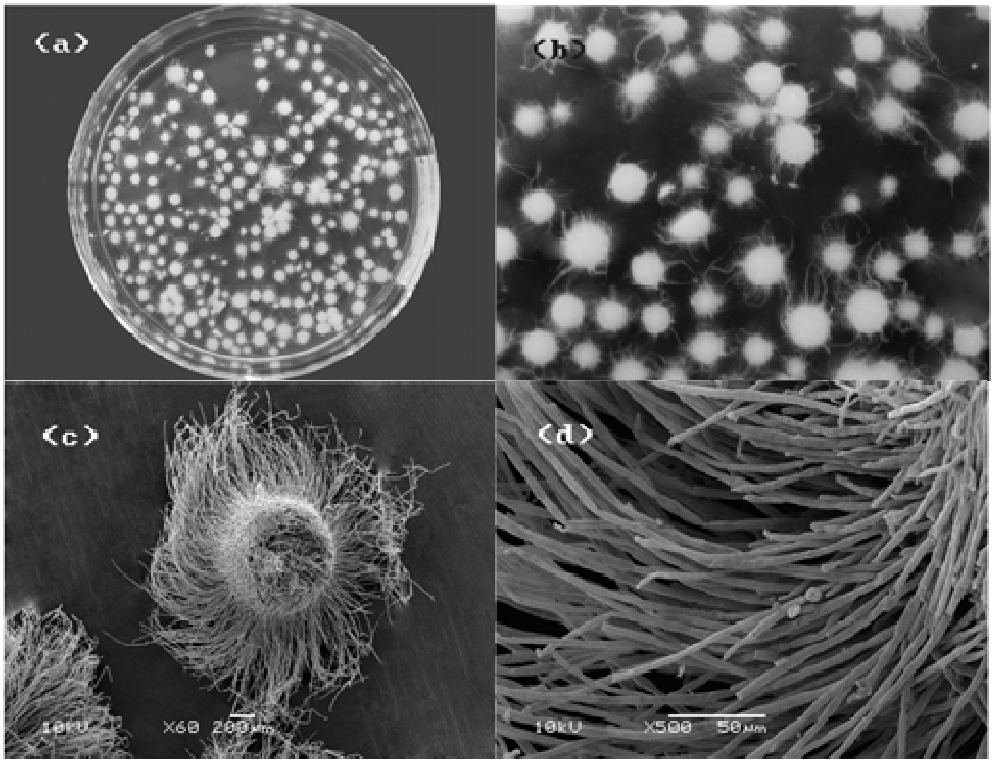

Figure 1. Microbial structure of aerobic granules in R1 (acetate-fed; maintained in acid pH).

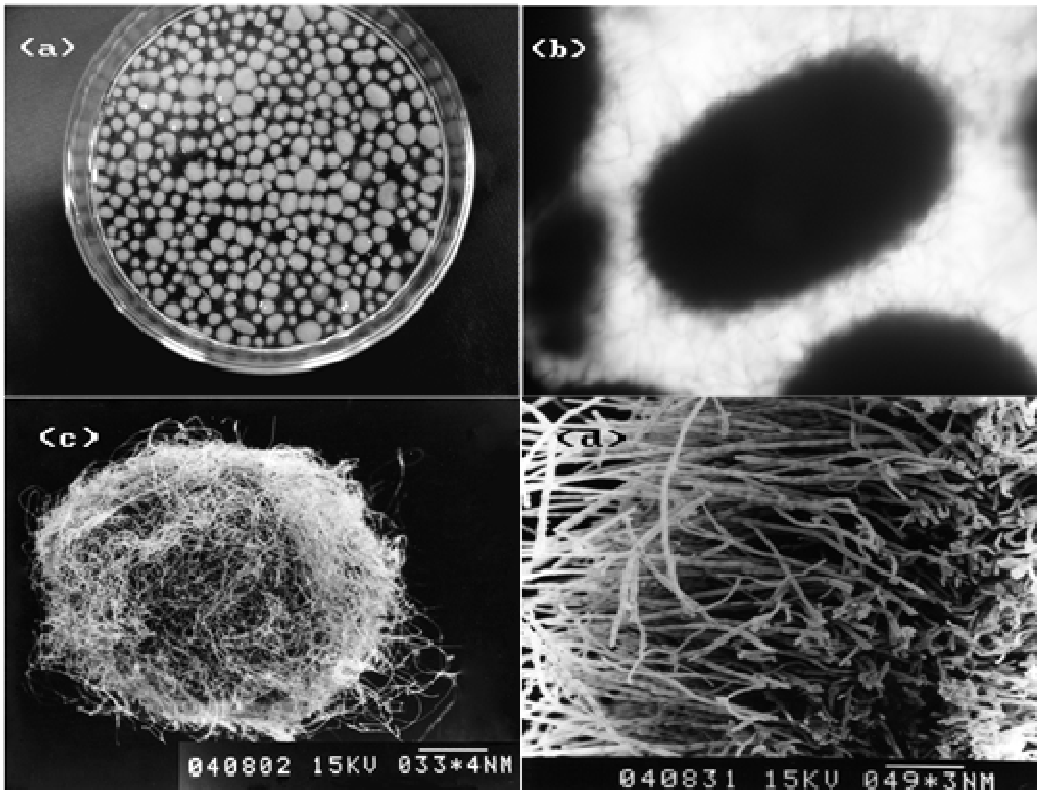

Figure 2. Microbial structure of aerobic granules in R2 (glucose-fed; maintained in acid pH).

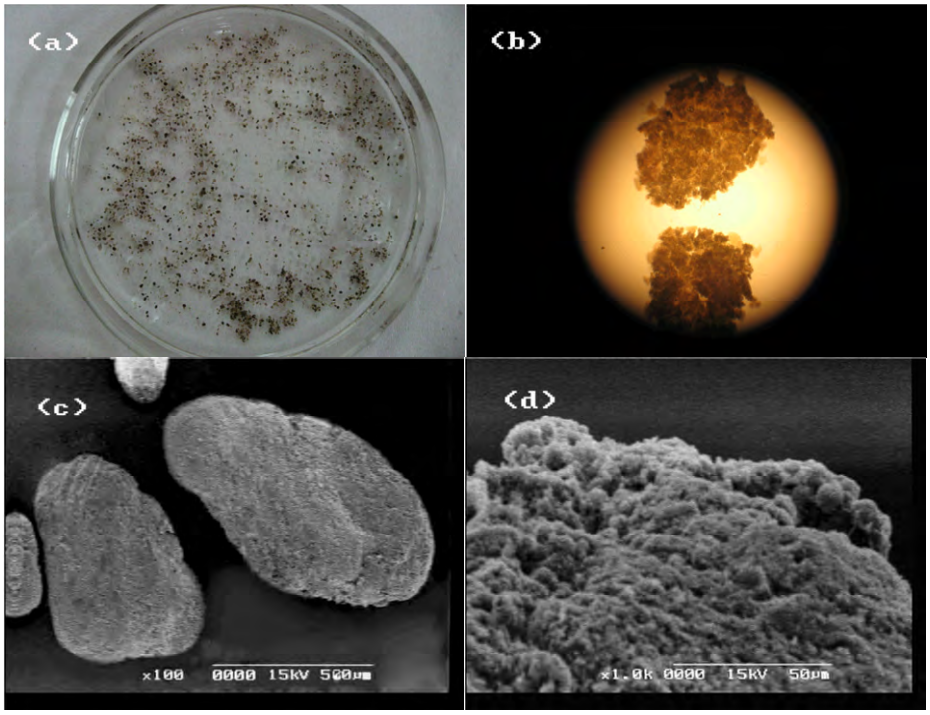

Figure 3. Microbial structure of aerobic granules in R3 (glucose-fed; maintained in alkaline pH).

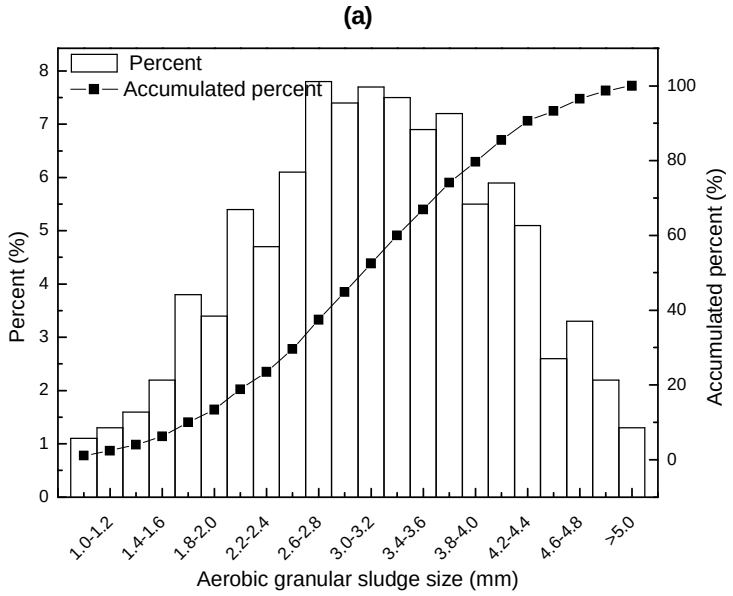

Figure 4a. Particle size distribution of aerobic granules in R1 (acetate-fed; maintained in acid pH).

The particle size distributions of aerobic granules in R1, R2 and R3 are shown in Figure 4. The granules in R1 and R2 had a wide range, from 1.0 to 5.0 mm, while the granules in R3 were mainly distributed around the range of 0.40 - 1.60 mm. From SEM images and particle size distributions, it was clear that the microbial structures of aerobic granules in R1 and R2 were almost the same, but they were quite different from those in R3.

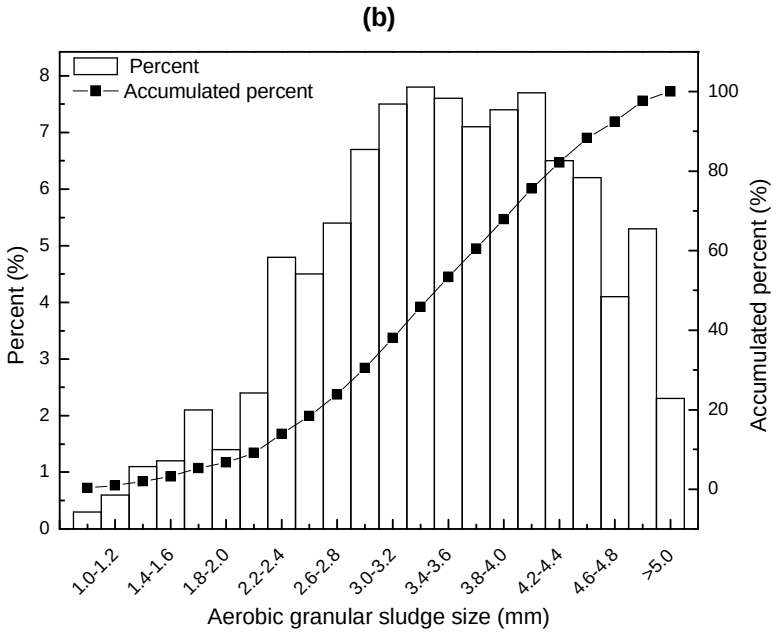

Figure 4b. Particle size distribution of aerobic granules in R2 (glucose-fed; maintained in acid pH).

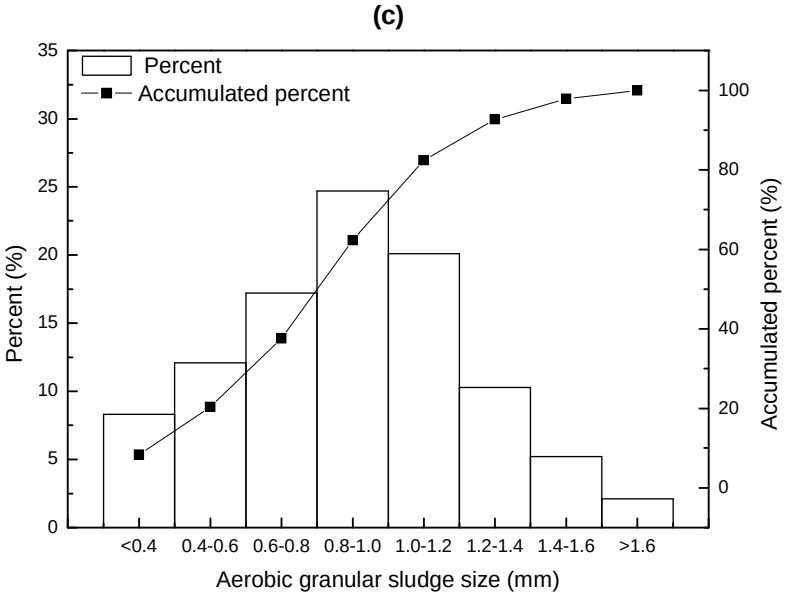

Figure 4c. Particle size distribution of aerobic granules in R3 (glucose-fed; maintained in alkaline pH).

R1 and R2 were maintained at acid pH (4.5-5.5), which encouraged the growth of most fungi and inhibited the growth of most bacteria (Rousk et al., 2009). With the continuous elimination of bacteria, fungi gradually became dominant and formed fungal granules. In contrast, maintaining R3 at an alkaline pH (7.5-8.5), which is optimum for the growth of most bacteria and unfavorable to fungi (Rousk et al., 2009), allowed bacteria to survive and aggregate to form the compact granules. So it could be seen that reactor pH played an important role in the microbial structure of the aerobic granules. Yang et al.

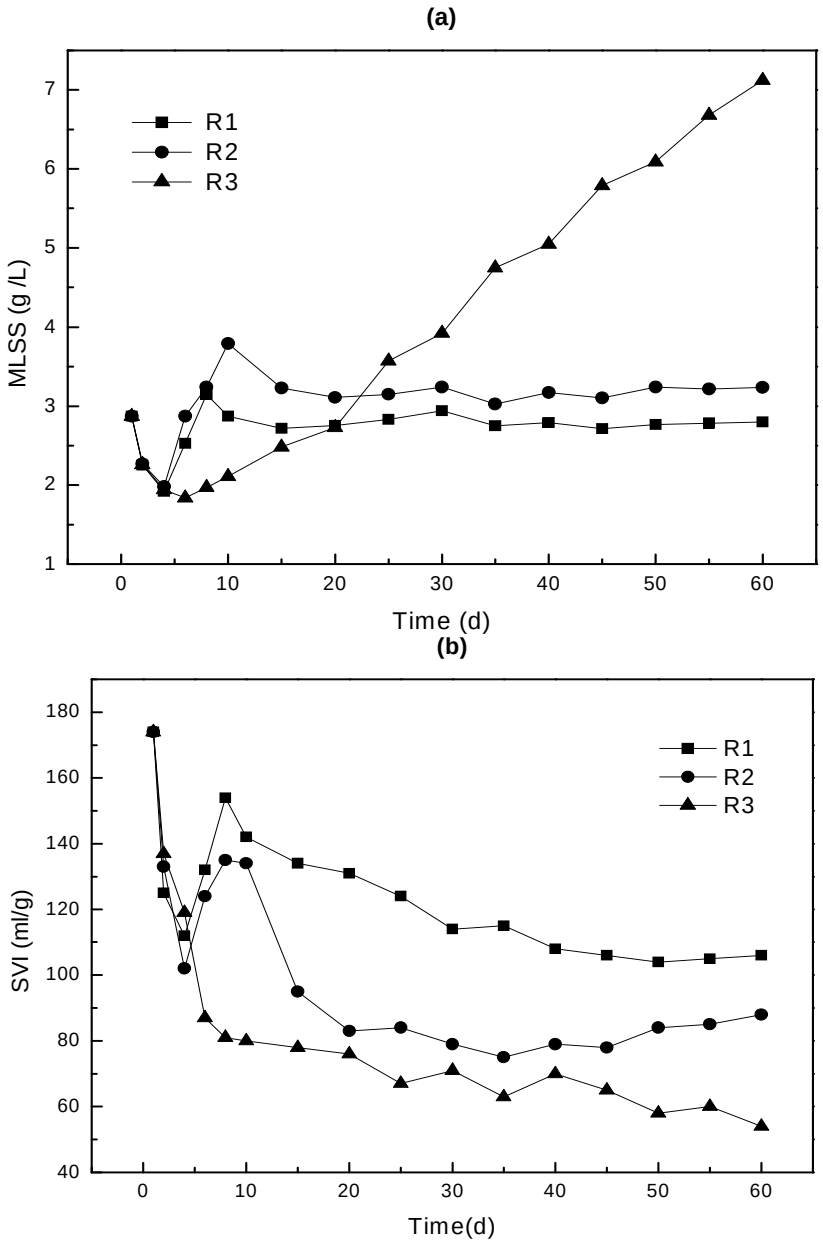

Figure 5. MLSS and SVI of aerobic granules: (a) mixed liquor suspended sludge (MLSS); (b) sludge volume index (SVI); R1: acetate-fed, maintained in acid pH; R2: glucose-fed, maintained in acid pH; R3: glucose-fed, maintained in alkaline pH.

(2008) has achieved fungal-dominant granules in acidic condition and bacterial-dominant granules in alkaline condition using the same carbon source. In contrast, R2 and R3 in this study were fed with the same carbon source (glucose), but they resulted in quite different microbial structures. Therefore, the microbial structure of the aerobic granules was significantly influenced by reactor pH, and was not related to carbon source.

MLSS and SVI of aerobic granules

The evolutions of MLSS and SVI in the reactors throughout the operation are shown in Figure 5. Due to poor settling ability (174 ml/g in SVI) and short settling time (5 min), seed sludge was washed out of the reactors during the initial days, leading to the lowest MLSS of 1.92 g/l in R1 and 1.98 g/l in R2 on the fourth day and 1.84 g/l in R3

on the sixth day. Then, with the retention of fast-settling biomass and granulation, MLSS in R3 increased continuously to a level of more than 7 g/l, and corresponding SVI gradually decreased to 54 ml/g. This variation is similar to previous findings reported for bacterial granules in SBRs (Tay et al., 2004). In contrast, MLSS in R1 and R2 increased to the maximum of 3.15 g/l after 8 days and 3.79 g/l after 10 days, respectively. Then MLSS slightly decreased and stabilized at 2.797 and 3.237 g/l finally, with corresponding SVIs of 106 and 88 ml/g, for R1 and R2, respectively. For the granules in the three reactors, MLSS and SVI in R1 and R2 were quite similar, and MLSS of fungal-dominant granules (R1 and R2) was much lower than that of bacterial-dominant granules (R3), and SVI was slightly higher. The same results were reported by Yang et al. (2008).

SVI denotes the degree of looseness of aerobic granules, which is closely related to microbial structure of aerobic granules (Dick and Vesilind, 1969). The granules in R1 and R2 were fluffy and loose, so SVI was high. The granules in R3 were tight and compact, so SVI was low. A high SVI means that if the granules have good settling ability, MLSS must be low, so MLSS in R1 and R2 was low. A low SVI means that if MLSS is high, the settling ability of the granules must be good, so the granules in R3 not only had a high MLSS but also a low SVI. Thus, MLSS and SVI of aerobic granules determined by microbial structure were indirectly influenced by reactor pH, and had no relation with carbon source.

Nitrification-denitrification of SBRs

The removal efficiencies of NH_4^+-N and TN are shown in Figure 6. R1 and R2 were considerably different in nitrogen removal efficiency with R3. R1 and R2 showed consistently low NH_4^+-N and TN removal efficiencies (less than 25 and 20%) throughout the operation, which indicates poor nitrification-denitrification by fungal-dominated granules. In R3, NH_4^+-N removal efficiency gradually increased and reached 88.5% on the 60th day; TN removal efficiency and slightly increased in the first days, then increased rapidly after the appearance of granules before finally stabilizing at about 40%. R3 had good nitrification-denitrification capacity after 40 days.

Nitrifying and denitrifying bacteria grow best at pH 7.5 - 8.2 and 8.0 - 9.0, respectively, so they would grow poorly in the acidic condition of R1 and R2 but would grow well in the alkaline condition of R3. For the fungal-dominant granules in R1 and R2, the fluffy and loose structure allows oxygen to diffuse into the entire aerobic granules, making the whole granules to be in aerobic condition. Li et al. (2005) have reported that dissolved oxygen became a major limiting factor of metabolic activity of aerobic granules when bacterial-dominant granules grew to a size larger than 0.5 mm. Therefore, most of the granules in R3 would be expected to have anoxic zones, which would provide niches for denitrifying bacteria. In R3, the appropriate pH provided the environment for the growths of nitrifying and denitrifying bacteria, and the appropriate microbial structure provided the space for the livings of nitrifying and denitrifying bacteria, so the granules in R3 had the good nitrification-denitrification. The results suggested that the nitrification-denitrification of SBRs was affected by reactor pH and the microbial structure of the aerobic granules, was also affected by reactor pH. So reactor pH played an important role in the nitrification-denitrification of SBRs. Beun et al. (2001) and Adav et al. (2009) have successfully achieved aerobic granules with good nitrification-denitrification under alka- line condition. The nitrification-denitrification of R2 and R3 were quite different, suggesting that the nitrification-denitrification was not related to carbon source. Therefore, the nitrification-denitrification performance of the reactors was also closely related to reactor pH, and had nothing to do with carbon source.

In this study, it can be concluded that for glucose-fed and acetate-fed aerobic granules, the characteristics of aerobic granules, including microbial structure, MLSS, SVI and nitrification-denitrification, were affected by reactor pH, and were independent with the substrate supplied.

In the cultivations of aerobic granules with glucose and sodium acetate, the differences between the reactors were only the carbon source and the changed pH resulting from the oxidation of the carbon source. The differences between the characteristics of aerobic granules with the two carbon sources were maybe from one of them or both. The experimental results showed that the characteristics of aerobic granules were asso- ciated with reactor pH but not carbon source. So it is clear that the differences came from the changed reactor pH resulting from the degradation of the two carbon sources, which can fully support the validity of the new theory.

Conclusions

The potential explanation suggests that the cultivation of aerobic granules with glucose or sodium acetate should have more attention on reactor pH rather than carbon source itself.

The implications of this potential explanation are discussed with regards to the other common carbon sources as well as better understanding of the mechanisms of aerobic granulation.

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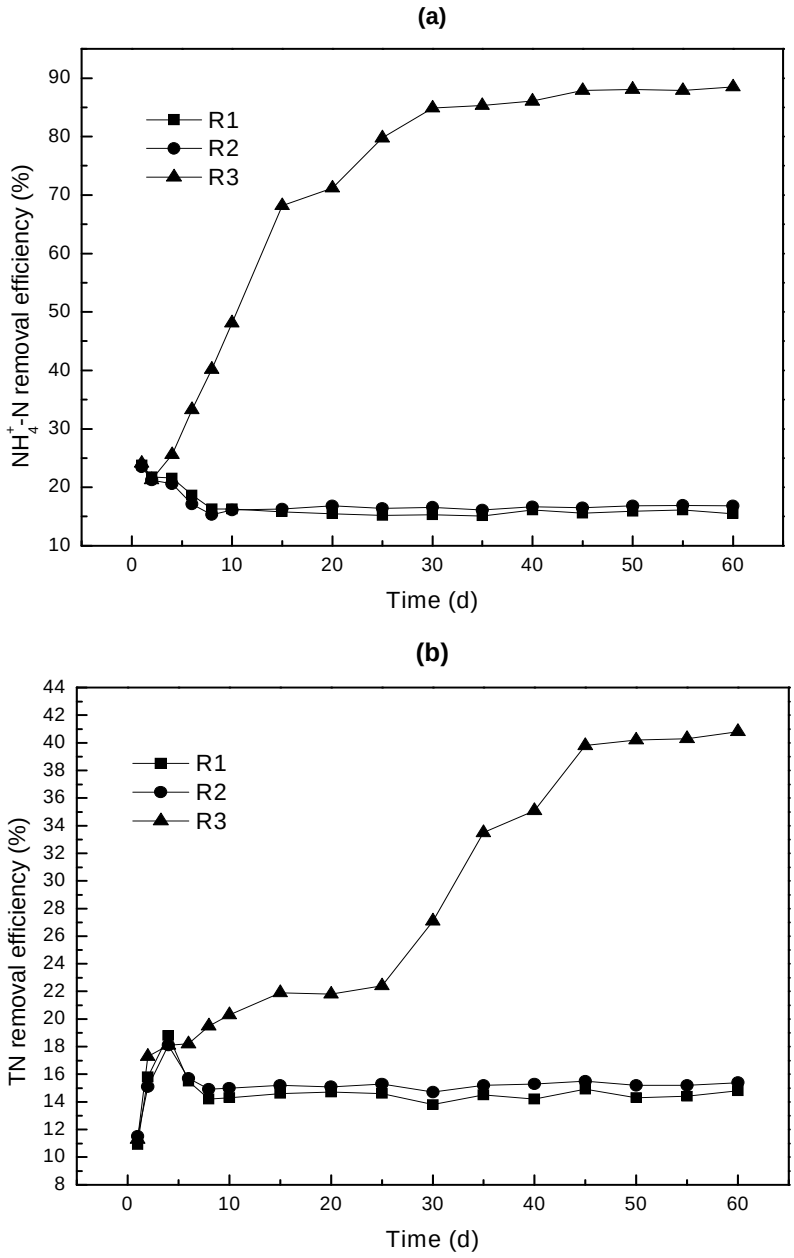

Figure 6. Nitrification-denitrification of the reactors in a 60-day operation: (a) NH_4^+-N removal efficiency; (b) TN removal efficiency; R1: acetate-fed, maintained in acid pH; R2: glucose-fed, maintained in acid pH; R3: glucose-fed, maintained in alkaline pH.

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