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Cultivation of phosphorus-removal granules under low organic loading rates – a case report

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ABSTRACT

A sequencing batch reactor (SBR) seeded with flocculated sludge and fed with synthetic wastewater was used to cultivate phosphorus-removal granules. The effects of low organic loading rates (OLR) on the granulation and the phosphorus removal performance were investigated in the SBR. The experimental results indicated that the OLR required to cultivate phosphorus-accumulating microbial granules was much lower than that required for full aerobic granules. In this study, the granulation of sludge was divided into three phases based on OLRs, which each presenting a different sludge morphology. The first phase consisted mainly of activated sludge floc with a small amount of round granules (OLR = 0.5 to 1.0 kg COD/(m^3.d)). In the beginning of the second phase, the granulation accelerated, after which the granule morphology became irregular (OLR = 2.2 kg COD/(m^3.d)). In the third phase, the mature granules were cultivated successfully (OLR = 1.0 kg COD/(m^3.d)). The changes in the OLR did not affect the COD removal but did strongly affect the phosphorus removal. The phosphorus removal efficiency decreased to 73% when the OLR was increased to 2.2 kg COD/(m^3.d) and didn't recover even the OLR was decreased to 1.0 kg COD/(m^3.d) again.

Keywords: phosphorus-removal granules; organic loading rate; DO; SBR

1. Introduction

Granulation is a self-immobilization phenomenon in which fluffy biological solids assemble and agglomerate as dense and compact granular sludge under controlled loading and operating conditions. This promising technology is superior to activated sludge floc in terms of settling ability, compactness, activity and ability to withstand organic shock loading[1–6]. Aerobic granular sludge technology is not only applied to sewage treatment[7], but also applied to industrial wastewater with recalcitrant and toxic xenobiotic compounds[8–9].

Although it has been reported that the organic loading rate (OLR) has an insignificant effect on the formation of aerobic granules, it is essential to determine the granule characteristics and stability[10–11]. Tay et al. [12] reported that no granulation was observed in a sequencing batch reactor (SBR) at OLRs of 1~2 kg COD/(m^3.d). Furthermore, aerobic granular sludge was formed after 20 days under an OLR of 8 kg COD/(m^3.d); however, the granule characteristics were fairly poor, whereas mature aerobic granular sludge was successfully produced at an OLR of 4 kg COD/(m^3.d). The OLR affected the size of the granules, which increased with increasing OLR until substrate limitation occurred in the center of the granule. Large sludge granules would disintegrate, and a lower OLR would result in a smaller granules[13]. Several researchers investigated aerobic granular formation from activated sludge at high OLRs of up to 15 kg COD/(m^3.d) [3,14–16], finding that high OLRs resulted in a high biomass concentration in the reactor, which

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led to low dissolved oxygen (DO) concentrations in the aerobic feast period. Moreover, the granules disintegrated due to the severe oxygen transfer resistance in the granules, excessive filament growth or the possible presence of anaerobes in large aerobic granules[11]. Four strategies were proposed by Show et al. [4] to enhance granule stability for practical applications, applying appropriate OLR and DO, promoting slow-growing organisms, strengthening the granule core and inhibiting anaerobic growth.

To date, most aerobic granules have been successfully cultivated in pneumatically agitated reactors, in which the hydrodynamic shear force is usually represented by the superficial air velocity. In fact, the superficial air velocity affects not only the hydrodynamic shear force but also the oxygen supply. The effects of oxygen and shear force on granules were not independently assessed in most previous studies. Therefore, to satisfy the shear force requirement for the formation and structure of aerobic granular sludge, the aeration rate in high-biomass systems was greater than that in conventional biological treatment processes, causing high energy consumption and thereby limiting the application of aerobic granular sludge. The high biomass resulting from the high OLR and the high energy consumption due to the DO supply should be considered together. De Kreuk et al. [17] proposed the use of slow-growing organisms, which include phosphorus-accumulating bacteria (PAOs), to decrease oxygen demand for the stable operation of aerobic granules.

Unlike purely aerobic or anaerobic granular systems, biological phosphorus removal (BPR) granular systems require alternation between anaerobic and aerobic conditions. Lin[18] reported the successful development of BPR granules in an SBR and investigated the effects of the substrate P/COD ratio on performance, granule size, density and aerobic activity. Although the performance of phosphorus-accumulating microbial granules has been investigated extensively, the effect of low OLR on the formation and characteristics of aerobic granules with slow-growing organisms, such as PAOs has received little attention.

In this paper, the feasibility of the formation of P-accumulating granules under low OLR, and the characteristics of the P-accumulating granules were investigated. The shear force in the reactor was provided by aeration and mechanical mixing, which differentiated the supply of shear force from the DO supply. A DO concentration of approximately 1 to 2 mg/L was maintained during the aerobic feast period by reducing the aeration rate, while sufficient shear force was supplied by increasing the mixing intensity.

2.Materials and methods

2.1. Experimental setup and seeding

A completed stirred laboratory-scale sequencing batch reactor (SBR) was set up and operated at 25ºC. The working volume of the SBR was 10 L, and air compressors were used for aeration. The DO concentration was maintained at 1.0~2.0 mg/L by adjusting the airflow rate and the pH was adjusted to 7.5±0.1 by adding NaOH and HCl. During the operation at low aeration levels or if the compressors were not working, a mechanical stirrer was used to ensure sufficient shear force to cultivate the aerobic granular sludge. Peristaltic pumps were employed for feeding and withdrawal. The SBR was operated with a cycle of 6 h during the operation. The cycle consisted of 5min feeding, 120min anaerobic period, 205min aeration, 30min settling, 15min drainage and 5 min idle. The solids retention time of biological phosphorus removal period was 10 or 15d controlled by biomass wasting. 6 L or 7 L synthetic wastewater was pumped into the reactor during each feeding. The seeding sludge was taken from a local municipal wastewater treatment plant in xi'an, China. The seeding concentration of the activated sludge was about 3000 mg/L.

2.2. Medium

In this study, sodium acetate was used as the sole carbon source for the synthetic wastewater. NH_4Cl and KH_2PO_4 were used to provide the N and P source. The COD, NH_4^+–N and PO_4^{3-}–P concentrations were 200~800 mg/L, 10 mg/L, 10~25 mg/L during phosphorus removal period, respectively. Other compositions of the synthetic wastewater were 0.03 mg/L$CaCl_2$, 0.02 mg/LMg $Cl_2.6H_2O$ and 1ml trace-element solution. The trace-element solution contained $FeCl_3.6H_2O$ (700 mg/L), $MnCl_2.4H_2O$ (190 mg/L), $ZnCl_2.2H_2O$ (25 mg/L), $CuCl_2.4H_2O$ (23 mg/L) and $NaMoO_4.2H_2O$ (0.43 mg/L).

2.3. Chemical analyses

Effluent samples were withdrawn periodically and immediately filtered through a 0.45-μm syringe filter. COD, NH_4^+–N, NO_2^-–N, NO_3^-–N and PO_4^{3-}–P were determined according to the standard methods issued by the Environmental Protection Agency (EPA) of China (1989).

2.4. Microscopy and image analysis

Images of sludge were taken with a digital camera and the diameter was measured with a laser particle analyzer. Scanning electron microscopic images of the granular sludge harvested from the SBR.

2.5. Operation procedure

The SBR operating procedures were given in Table 1. The OLRs were controlled by the COD concentration in the influent and the HRT of the reactor. The SBR was operated for 90 days, which were divided into phase I, phase II and phase III according to the OLRs.

3. Results

3.1. Development of granulation at low OLR

During the reactor initialization period, granules began to appear on the 5th day and then proceeded to slowly grow. After 55 days of operation, mature granules were formed and

Table 1
SBR operation phases

Phase	HRT (h)	OLR (kg COD/(m^3·d))	SRT (d)	Operating duration (d)
I	10	0.5~1.0	20	40 (day 1 to day 40)
II	8.6	2.2	10	30 (day 41 to day 70)
III	10	1.0	20	20 (day 71 to day 90)

stabilized in the reactor, and the MLSS increased to 7 g/L. Granulation was observed in the SBR in three different morphology: a mixture of granules and floc sludge in the initial phase, larger granules that ultimately disintegrated in the second phase and recombining granules in the final phase. The variation of granule morphology corresponded well with the SBR operating phases.

In phase I, from day 1 to 40, a low OLR of 0.5~1.0 kg COD/(m^3·d) was used in the SBR. The sludge morphology of different times in the SBR in phase I was shown in Fig. 1. Several granules were observed after 5 days, and the percentage of biomass granules in the SBR and the granule size increased from day 5 to day 35.And then the granulating process slowed down from day 35 to day 40. The sludge in the SBR of this phase was a mixture of mainly activated sludge floc with a small quantity of round granules. The average granule size was only 0.3 mm, and the percentage of biomass granules was approximately 60%. The granulation of this phase showed that the microorganisms tended to self-immobilize under certain conditions, such as high shear force and feast-famine in the SBR cycle. However, the granulation was not completed, and the granules in the SBR were very small due to the low biomass and the low OLR.

The sludge morphology of various times in the SBR in phase II was shown in Fig. 2. The activated sludge floc rapidly turned into granular sludge from day 48 to day 55, while the shape of the granules became irregular after day 63. The higher OLR was achieved by increasing the COD concentration in the influent and increasing the exchange ratio from 60% to 70%. Biomass built up quickly due to the sufficient availability of carbon sources and was able to withstand wash-out from the SBR. Therefore, the granule content and size initially increased under an OLR of 2.2 kg COD/(m^3·d), but the granule disintegrated, and granule wash-out occurred on day 63. This phenomenon most likely occurred because the organism in the granule core could not obtain enough oxygen due to the depletion of DO by outer bacteria.

In phase III, the COD concentration in the influent was lowered, and the exchange ratio was returned to 60%. The OLR in this phase was set as 1.0 kg COD/(m^3·d). Fig. 3 shows the sludge morphology in the SBR with time in phase III. The percentage of biomass granules and the size of the granules increased under the OLR of 1.0 kg COD/(m^3·d), and mature granules existed for over a month. In this study, it was suggested that the OLR in our experiment suitable for stable phosphorus-accumulating granules was 1.0 kg COD/(m^3·d).

3.2. Performance and characteristics of aerobic granules

The seeding sludge was taken from a local WWTP using the oxidation ditch process. During the reactor initialization period, the removal efficiency of COD and PO_4^{3-}–P increased slowly. The evolution of the organic removal (COD) in the SBR was shown in Fig. 4, and the evolution of phosphorus removal (PO_4^{3-}–P) in the SBR was shown in Fig. 5. The removal efficiency of COD and PO_4^{3-}–P both increased during phase I, reflecting the growth of heterotrophic organisms, especially PAOs. However, the removal efficiency of COD and

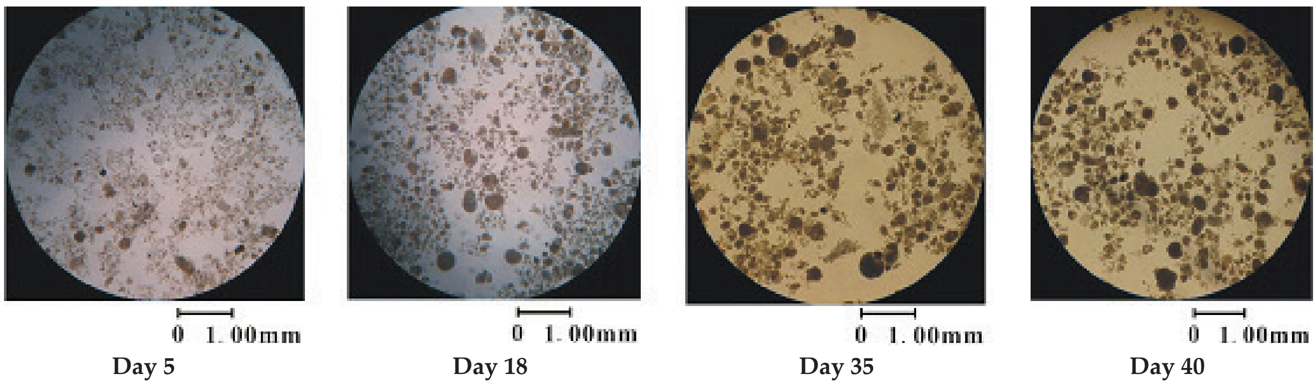

Fig. 1. Sludge morphology in the SBR with operation time in phase I.

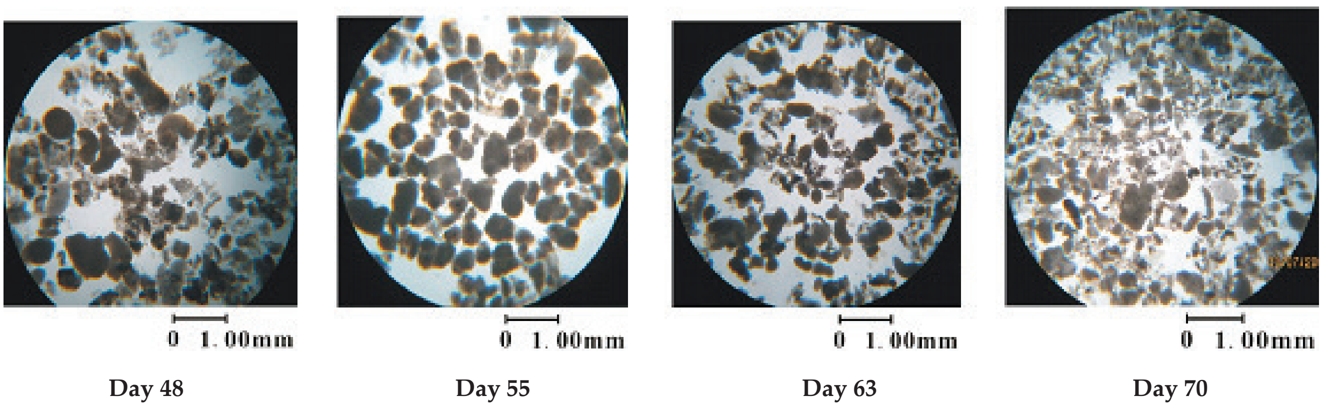

Fig. 2. Sludge morphology in the SBR with operation time in phase II.

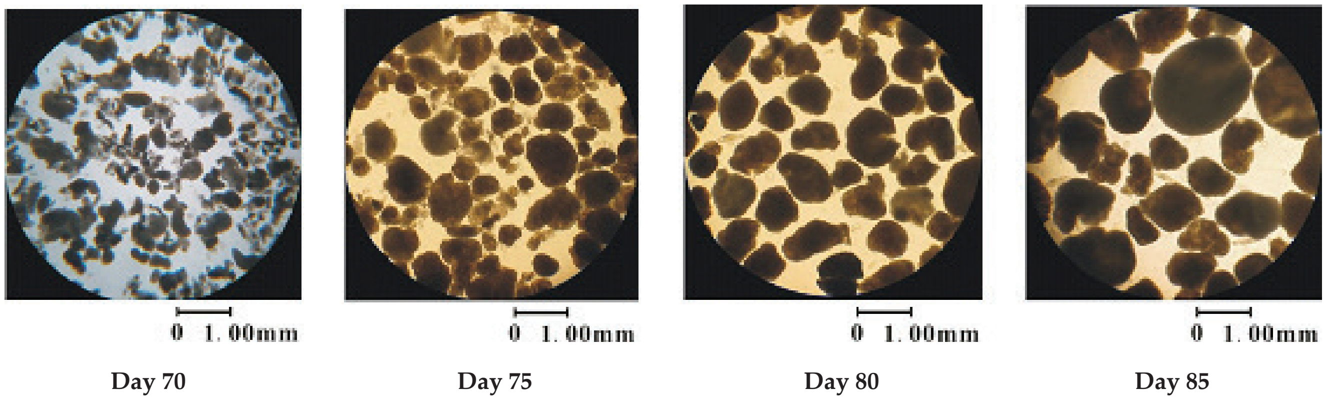

Fig. 3. Sludge morphology in the SBR with operation time in phase III.

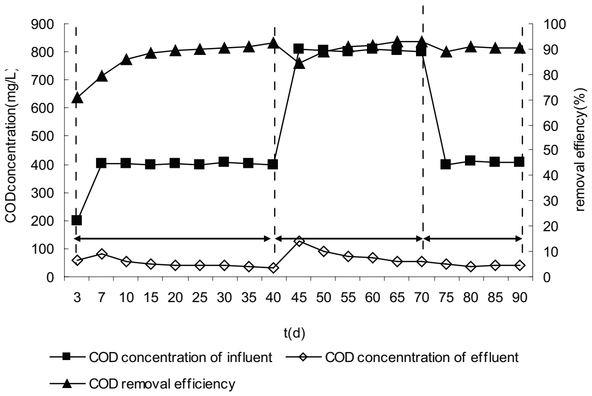

Fig. 4. Evolution of the removal efficiency of COD in the SBR.

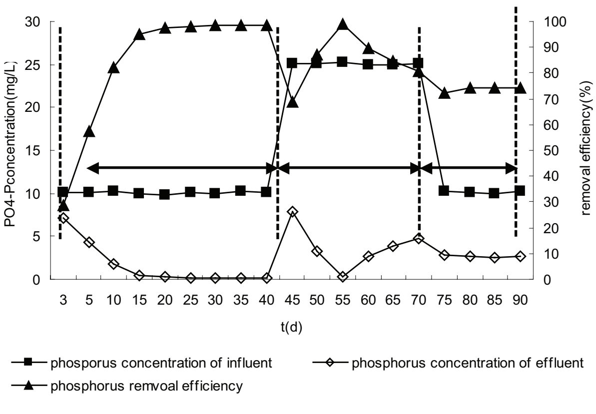

Fig. 5. Evolution of the removal efficiency of PO_4^{3-}-P in the SBR.

phosphorus decreased in the early part of phase II relative to phase I. After that the efficiency increased gradually again due to the growth of bacteria, which was attributed to the need of bacteria to adapt to the different OLR. On day 55, the removal efficiency of phosphorus decreased, reaching 73% by the end.

3.3. Granule physical characteristics

The variation of MLSS in the SBR is shown in Fig. 6. The initial MLSS in the SBR was 3300 mg/L. Firstly, the fluffy suspended solids (SS) were washed out, leaving only the

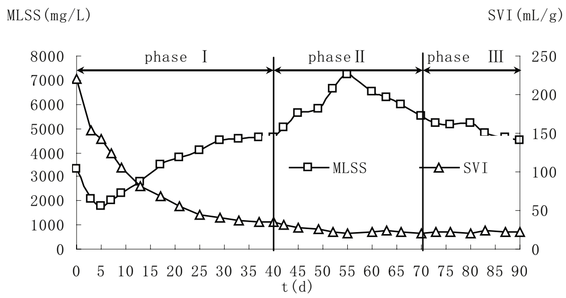

Fig. 6. Evolution of MLSS and SVI in the SBR.

heavy biomass fraction with high settling velocities in the reactor. During the first 5 days, the MLSS decreased to 2100 mg/L. Next, the granules started to accumulate in the reactors, and the MLSS increased to 7200 mg/L in the middle of phase II. Finally, the MLSS in the SBR began to decrease due to the wash-out of disintegrated fragments, and by the end of phase III, the MLSS was only 4500 mg/L.

The changes in the SVI were also shown in figure 6. The initial seed sludge for the SBR operation had an SVI of 225 mL/g. The SVI then decreased dramatically to 50 mL/g over the next 25 days, ultimately stabilizing at 20~30 mL/g. These values were much lower than the values of 80~100 mL/g reported by Peng [19], whereas the 30-min settling time was in line with this study. A shorter settling time was reported in other studies.

Fig. 7 shows the diameter distribution profiles of mature granules. The diameter distribution of the granules was fit well by a normal distribution, which is in agreement with previous studies [20–22].

3.4. Granule microbial characteristics

Two scanning electron microscopy (SEM) images of the mature granules were shown in Fig. 8: (a) the outside of a granule and (b) a slice of (a) showing the inside of the granule. As illustrated in figure 8, the granules had spherical or elliptical shapes and were yellow in color. Some protozoa, such as *Epistylis*, were attached to the surface of the granules. Many bacilli and cocci were located outside and inside of the granules, and no filamentous bacteria were observed, although filamentous bacteria were almost invariably in full

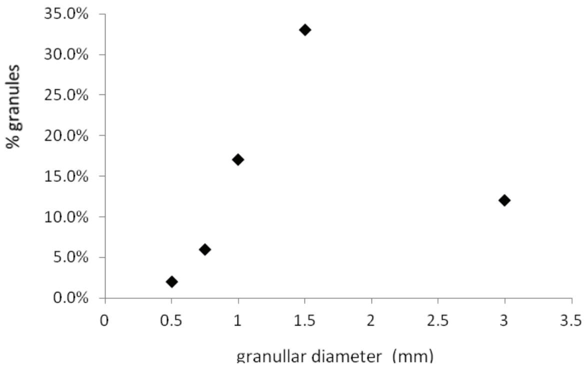

Fig. 7. Granule diameter distribution in the SBR.

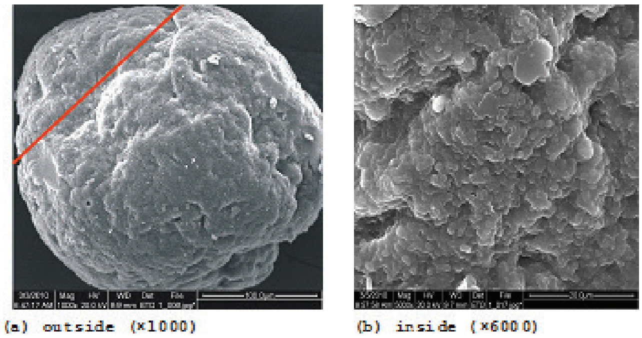

Fig. 8. Scanning electron microscopy (SEM) images of mature granules.

aerobic granules[1]. The granules demonstrated high microbial activities. The SOUR was as high as 45.32 mg/(g·h), and the ratio of MLVSS to MLSS was 0.91. Thus, in terms of biological activity, the granules were far superior to activated sludge.

4. Discussion

Granule formation using full aerobic operation was usually reported with a high OLR, ranging from 1 kg COD/(m^3·d) to 15 kg COD/(m^3·d) [12,14, 22], because a sufficient supply of substrates was required for rapid biomass growth. In addition, granulation may not be achieved under a relatively low organic loading of below 1 kg COD/(m^3·d), and a low influent concentration often results in a more filamentous growth, leading to granules deterioration and breakage. Introducing an anaerobic period in the cycle of the aerobic granular sludge reactor was advantageous for granule stability. Thus, biological phosphorus removal, unlike full aerobic operation, produced stable granules at a low loading rate. Wu et al. [23] started an SBR with a cycle of 4min filling and 120min anaerobic mixing followed by an aerobic period, resulting in the maximum simultaneous COD (98.5%) and phosphorus removal (94%) . The biomass concentration that could be maintained in this type of SBR was up to 7 g/L, and the SVI was low as 21 mL/g. This finding was similar to the results of this study, in which stable phosphorus removal granules were successfully developed at an OLR of 1.0 kg COD/(m^3·d) after accelerating OLR of 2.0 kg COD/(m^3·d) for 30 days, and the DO in the aerobic feasting period of SBR was controlled at 1~2 mg/L. Two relationships were found to be important for aerobic granules: the balance between the bacteria growth and OLR and the balance between the DO concentration and OLR. In this study, because of the slow growth rate of PAOs, granules were successfully cultivated at low OLR and low DO.

The granule diameters in this study were concentrated in the range of 1 mm to 2 mm. These values were smaller than the diameters of aerobic granules under high COD loading [14]. In general, it is recognized that high COD loading results in larger granules than low COD loading. Thus, smaller granules should be formed at lower COD loading, as demonstrated by our experiment. The phosphorus granules had two of same characteristics as the full aerobic granules: a lower COD loading and a smaller diameter. In fact, the results of this study illustrated the balance between the growth rate and the COD loading rate. As the growth rate of PAOs was lower than that of heterotrophic bacteria, a lower COD loading rate was required in anaerobic/aerobic granule SBR than in the full aerobic granule reactor. Therefore, the phosphorus-accumulating granules had smaller diameters than the other heterotrophic granules.

It was reported[24] that two groups of bacteria exist in the EBPR processes, as differentiated by the use of internally stored energy sources for anaerobic substrate uptake: PAOs (polyphosphate-accumulating organisms) and GAOs (glycogen-accumulating organisms). GAOs show similar traits to PAOs, except that they produce a different PHA and lack the EBPR ability or polyP accumulation of PAOs [25]. From a phosphorus removal perspective, GAOs are perceived as undesirable microorganisms, as they do not contribute to the biological P-removal process but compete with PAO for volatile fatty acids (VFA). Many factors affect the competition between PAOs and GAOs, such as the type of VFA present in the influent, pH, temperature and the phosphorus (P) to VFA ratio [26]. Liu et al. [27] found that the ratio of P/C (wt/wt) was a key factor influencing the "internal-energy-based" competition between PAOs and GAOs. When excessive P was provided (P/C=20/100), the PAOs could accumulate a high polyphosphate content and, with a higher and faster acetate uptake ability, successfully out-competed GAOs. In contrast, a reduction of the ratio to 2/100 caused the depletion of the polyphosphate content in PAOs, eventually leading to GAOs becoming the majority.

In our study, the COD removal efficiency was always greater than 90% after the bacteria adapted to the new OLR. However, the OLR strongly influenced the efficiency of phosphorus removal. When the OLR was elevated to 2.2 kgCOD/(m^3·d), the efficiency of phosphorus removal declined, and the removal efficiency was unable to recover, even if the OLR was decreased again. Throughout the experiment, the OLR was elevated from 0.5 kg COD/(m^3·d) to 2.2 kg COD/(m^3·d) and then lowered to 1.0 kg COD/(m^3·d) by increasing the sodium acetate concentration in the influent and altering exchange ratio of SBR. The phosphorus in the influent was increased from 10 mg/L to 25 mg/L, and then decreased to 10 mg/L. Thus, we can calculate the P/C (wt/wt) at different LORs. The P/Cs were 5.0/100, 2.5/100 and 3.1/100 at OLRs of 0.5 kg COD/(m^3·d), 1.0 kg COD/(m^3·d) and 2.2 kg COD/(m^3·d), respectively. The P/Cs were much lower than 20/100 reported by Liu et al. [27], the decrease of the phosphorus removal efficiency maybe was related to the low ratio of P/C, while the phosphorus removal efficiency decreased accompanied with the disintegration of granules, so the declination of phosphorus removal efficiency maybe also had something to do with morphology of granules.

5. Conclusions

In this study, dense phosphorus-accumulating granules were successfully developed at low OLR and low DO owing to the low growth rate of PAOs. Comparing these produced by the alternating anaerobic/aerobic technique with full aerobic granules, certain characteristics, such as morphology, diameter and microbiology, were distinct. The diameters of granules in our study were concentrated in the range of 1 mm to 2 mm, which were much smaller than the diameters of full aerobic granules. The SEM images of mature granules showed that the granule was mainly constituted of bacilli and cocci, no filamentous bacteria, which were different from full aerobic granules. The decrease in phosphorus removal efficiency was maybe related to competition between GAOs and PAOs or caused by the disintegration of granules.

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