High-rate nitrogen removal and its behavior of granular sequence batch reactor under step-feed operational strategy

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HIGHLIGHTS

High nitrogen removal efficiency was achieved in step-feed granular SBR.
Higher nitrogen removal rate was obtained in granular SBR under step-feed mode.
Granules were well maintained under the step-feed operational strategy.

ABSTRACT

Alternating anoxic/oxic (A/O) combined with the step-feed granular sequence batch reactor (step-feed SBR) was operated in laboratory scale to investigate nitrogen removal. The results showed that when the total inorganic nitrogen (TIN) and chemical oxygen demand (COD) levels were 55 and 320 mg/L in the influent, the TIN removal efficiencies were 89.7–92.4% in the step-feed mode and 48.1–59.5% in the conventional alternating A/O single-feed mode within a 360 min cycle. The pH and dissolved oxygen (DO) were used to optimize the process of denitrification and nitrification in the step-feed mode. The optimized operational condition was achieved by shortening the cycle time to 207 min, resulting in a nitrogen removal rate of 0.27 kg N/m³d, which was much higher than those achieved using activated sludge systems. The dominant community in the aerobic granules was coccius-like bacteria, and filamentous bacteria were hardly found. Granules were well maintained throughout the 90 days of continuous step-feed operation.

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1. Introduction

Nitrogen is one of the key nutrients causing eutrophication in water, and requires to be removed before discharge. Compared with the removal of phosphorus from wastewater, nitrogen can only be effectively and economically removed by biological methods. Biological nitrogen removal (BNR) generally involves aerobic nitrification with the conversion of NH₄⁺-N to NO₂⁻-N and/or NO₃⁻-N by ammonia-oxidizing bacteria and/or nitrite-oxidizing bacteria, as well as denitrification with the conversion of NO₃⁻-N and/or NO₂⁻-N to molecular nitrogen by heterotrophic bacteria under the anoxic condition (Coelho et al., 2000). A sequence batch reactor (SBR) can carry out BNR in a single reactor based on the successive alternation of aerobic and anoxic reactions, which has attracted much interest in recent years for municipal and industrial wastewater treatment. However, during BNR, denitrification is normally the rate-limiting step because of the lack of sufficient organic carbon source to sustain a high denitrification rate, especially in treating ammonium-rich or low C/N wastewater (Chang and Hao, 1996; Chen et al., 2011; Shi et al., 2011). Consequently, a high concentration of NO₃⁻-N or NO₂⁻-N is often retained in the effluent, resulting in low nitrogen removal efficiency (Wang et al., 2009; Gao et al., 2011). To solve this problem, the step-feed SBR is proposed to improve nitrogen removal. The step-feed SBR can make good use of the influent COD as the carbon source required in the denitrification process (Wang et al., 2012), which means that the carbon source required to denitify NO₃⁻-N/NO₂⁻-N formed in each aerobic phase is provided by the subsequent anoxic period influent. A step-feed SBR is reportedly both technically and economically effective in enhancing nitrogen removal in activated sludge systems (Puig et al., 2004; Yang et al., 2007; Guo et al., 2008; Lemaire et al., 2009).

Typically, the activated sludge in the SBR is in the form of floculent. Presently, a new form known as aerobic granular sludge exists and has been extensively studied over the past ten years (Beun et al., 1999; Qin and Liu, 2006; Adav et al., 2008; Muda et al., 2010; Verawaty et al., 2012). Compared with activated sludge, aerobic
granules have a regular and compact physical structure, diverse microbial communities, good settling property, high biomass, and high resistance to organic loading shock. Thus, the aerobic granular sludge process is considered as one of the most promising processes for wastewater treatment. Generally, sufficient nitrifying biomass is difficult to obtain and maintain in a conventional activated sludge system, because nitrifying bacteria are sensitive to alterations in environmental factors and have low growth rates (Ochoa et al., 2002). However, the specific nitrification rate is higher in granular sludge than in flocculent. Aerobic granules also have a special layer of microbial structure that helps stimulate the growth of more denitrifying bacteria, eventually leading to better denitrification (Mosquera-Corral et al., 2005). Accordingly, many researchers have attempted to achieve a high nitrogen removal in the aerobic granule SBR system to treat synthetic wastewater (Kishida et al., 2006), and real municipal wastewater (Wang et al., 2009), and the synthetic removal efficiencies (50–60%) in granular SBRs to treat the real municipal wastewater (Su et al., 2011). High nitrogen removal efficiencies (>90% and 81%) have been achieved under anoxic/oxic/anoxic and A/O conditions in the granular SBRs with the influent COD/N ratios of 10 and 4–8, respectively. Unfortunately, these results are not always the real events. Indeed, some experiments have obtained low nitrogen removal efficiencies (50–60%) in granular SBRs to treat the real municipal wastewater (Wang et al., 2009), and the synthetic wastewater (Yuan and Gao, 2010; Gao et al., 2011; Zhang et al., 2011). The main reason may be the deficient carbon source for denitrification. Thus, to obtain stable and high nitrogen removal efficiency, the application of the step-feed operational strategy and its nitrogen removal characteristics in an aerobic granular SBR system must be investigated.

To date, experimental research on granular SBR systems operated under the step-feed strategy is limited. Chen et al. (2011) studied the step-feed operation strategy in two granular SBRs using different particle sizes. They found that the nitrogen removal efficiency can reach 93% for 0.7 mm-sized granules and 95.9% for 1.5 mm-sized granules under 12 h of hydraulic retention time condition. Wang et al. (2012) explored the effects of step-feed on granulation and nitrogen removal performance of partial nitrifying granules in a two-phase step-feed SBR with influent COD and NH₄–N at 800 and 300 mg/L, respectively. The system achieved a nitrite accumulation rate of 93 ± 5% and total nitrogen (TN) removal efficiency of 70%. Nevertheless, the optimization of a granular SBR system with the step-feed strategy under continuous operation is still unreported. Thus, achieving high-rate nitrogen removal in a step-feed granular SBR under continuous operation requires further investigation. Accordingly, this study aimed to achieve high-rate nitrogen removal using the step-feed strategy in a granular sludge SBR system under continuous operation condition. Denitrification and nitrification durations were optimized using DO and pH as the control parameters under a four-phase step-feed condition. The stability of aerobic granules under continuous operation was determined as well.

2. Methods

2.1. Experimental SBR and operation

Experiments were carried out in a cylindrical column SBR (16 cm in diameter and 32 cm in high) with a working volume of 4.4 L and volumetric exchange ratio (VER) of 77%. The reactor was inoculated with activated sludge from the Gaobeidian municipal wastewater treatment plant in Beijing. The seeding sludge had a sludge age of 10 days, a mixed liquor suspended solids (MLSS) concentration of 4.1 g/L, and a sludge volume index (SVI) of 93 mL/g. Good and completely mature aerobic granules formed in 75 days. After the granulation was finished, nitrogen removal experiments were then conducted. A typical experiment was divided into three different stages. In stage I (1–28 d), the conditions were as follows: alternating A/O condition in the SBR with a 360 min cycle including 8 min feed, 60 min anoxic reaction, 260 min aerobic reaction, 3 min settling, 14 min decanting, and 15 min idling. In stage II (29–89 d), the conditions were as follows: four step-feeds with 30 min anoxic phase and 50 min aerobic phase in each feed for a 360 min cycle. In stage III (90–120 days), the conditions were as follows: optimization of nitrogen removal at four step-feeds and 207 min cycle. In stages II and III, the influent was introduced four times (2 min per feed) and sequentially fed into four anoxic phases with an equal volume of 0.85 L. The operational strategy of the three stages is shown in Fig. S1. The pH and DO were used to monitor the biological reaction process in the reactor; and they were automatically recorded online using the collecting data recorder at 1 min intervals. During aerobic reaction, air was introduced into the bottom of the reactor through a fine-bubble diffuser at a flow rate of 200 L/h. A slender agitator with a crescent-shaped vane was placed in the reactor, and the stirring speed was controlled at 120 rpm to avoid the granular sludge settling in the anoxic phase.

2.2. Synthetic wastewater

NH₄Cl and glucose were used as the nitrogen and carbon sources in the synthetic wastewater, respectively. The synthetic wastewater contained COD of approximately 320 mg/L, with the following typical composition: NH₄–N, 40 mg/L; PO₄³⁻–P, 5 mg/L; NO₃–N, 15 mg/L (the NO₃–N in synthetic wastewater was mainly from groundwater); NaCl, 10 mg/L; and TIN, 55 mg/L. NaHCO₃ was used as buffering agent and potential inorganic carbon source for the nitrification process.

2.3. Analytical methods

COD, NH₄–N, NO₃–N, NO₂–N, MLSS, and SVI were tested according to the standard methods (APHA-AWWA-WEF, 2005). DO and pH were continuously monitored using Hach DO and pH meters. The particle size distribution was determined by a laser particle size analysis system (Malvern MasterSizer series 2000, Malvern instruments Ltd., Malvern, UK) within 0.02–2000 μm. The extracellular polymeric substances (EPS) content was qualified following the method described by Li and Yang (2007). The spatial structure of aerobic granules was observed by a scanning electron microscopy (SEM) system (FEIQUANTA 200).

3. Results

3.1. Overall performance of granular SBR

After complete granulation, the granular SBR system was operated for 120 days and the overall performance of the reactor operation is shown in Fig. 1. Stable and high COD and NH₄–N removal efficiencies were achieved at 92.4–95.2% and almost 100% in all three stages, respectively, regardless of changes in the operational strategy. This result demonstrated that the granular SBR technology can satisfy the strict effluent quality requirement for efficient COD and NH₄–N removal. However, high concentration of NO₃–N remained in the effluent, which resulted in low nitrogen removal of 48.1–59.5% in stage I. Subsequently, the TIN removal efficiency improved to 89.7–92.4% and 88.7–92.6% in stages II and III, respectively, after applying the step-feed operation mode. A quick and high nitrogen removal was observed on day 29, and TIN decreased to 5.8 mg/L in the effluent. No significant difference was observed between the nitrogen removal in stages II and III, although the
cycle time decreased from 360 min to 207 min in the experiment. The distinct improvement in nitrogen removal in stages II and III indicated that the step-feed operation effectively promoted nitrogen removal for wastewater treatment.

3.2. Nitrogen removal under A/O mode

To investigate the detailed process of nitrogen removal in the granular SBR system, the typical property of one cycle test was examined in the experiment. Fig. 2 shows the behavior of COD, NH₄⁻N, NO₂⁻N, NO₃⁻N, TIN, DO and pH under alternating A/O operational mode on day 28. The DO and pH values decreased during the feeding process, mainly because of the degradation of glucose and fermentative intermediate production. In the anoxic phase, the DO concentration was maintained at almost zero, and the pH continuously increased because denitrification generated alkalinity. Then, the pH started to decrease after reaction completion. During this period, the NO₃⁻N was completely denitrified in 16 min. The nitrate apex (point A) on the pH profile indicated the completion of denitrification. After COD depletion, NH₄⁻N concentration rapidly declined mainly because of nitrification, and was identified by the break-point in the pH curve (point B) in the aerobic phase. During the nitrification process, the pH slightly decreased and DO was stabilized at 4.3 mg/L. Meanwhile, NH₄⁻N continuously decreased from 25.1 mg/L to zero within 60 min and the NO₃⁻N concentration reached the peak. After COD and NH₄⁻N removal, the pH and DO simultaneously increased and the DO remained constant at 7.4 mg/L until the end of the aerobic reaction. The end of nitrification was denoted by the distinct increase in pH and DO (point C). Most NH₄⁻N was converted to NO₃⁻N but TIN removal was only 53.8%. The DO and pH curves showed that COD and NH₄⁻N depletion was completed in about 120 min under the aeration condition. This result suggested that the aerobic granules had a very high degradation activity for COD and NH₄⁻N removal. However, nitrogen removal was unsatisfactory under this operational strategy in the granular SBR.

Moreover, the simultaneous nitrification and denitrification (SND) efficiency was low during the aeration phase. Upon deduction of the nitrogen assimilation of NH₄⁻N during the COD consumption period, almost no TIN was removed by denitrification. SND did not occur in the subsequent aeration duration because of the lack of organic carbon. This result confirmed that the nitrogen removal process was mainly limited by the denitrification process in the alternating A/O granular SBR. To improve nitrogen removal, denitrification must be enhanced by adjusting the operational strategy.

3.3. Nitrogen removal profile under the step-feed mode

To enhance the nitrogen removal of the reactor system, operational strategy was changed to the step-feed mode on day 29. Fig. 3a shows the profile of NH₄⁻N, NO₂⁻N, NO₃⁻N, TIN, DO, and pH in the step-feed granular SBR under the cycle time of 360 min on day 80.

In the anoxic phases, the DO concentration decreased to near zero in 2 min, which indicated that the granules had a high oxygen consumption rate. Complete denitrification of NO₃⁻N from the previous aerobic stage and influent was achieved in 30 min, using the influent organic substrates as a carbon source. In this experiment, 17.2, 29.9, 33.5, and 34.1 mg NO₃⁻N were denitrified in four anoxic phases in one cycle, respectively. This finding indicated that the anoxic condition with sufficient carbon source by step feeding influent wastewater dramatically improved denitrification. Similarly, points A2, B2, C2, and D2 represented the completion of denitrification in the four anoxic phases, as shown in Fig. 3a. In the four aerobic phases, the DO concentration was maintained at 4–5 mg/L, and NH₄⁻N was completely oxidized without NO₂⁻N accumulation. Correspondingly, 16.3, 22.6, 23.2, and 23.3 mg NO₃⁻N were generated. Similar to the stage I results, the end of the nitrification was distinctly observed in the DO and pH curves (point A1, B1, C1, and D1). By detecting the break-points, the duration of nitrification and denitrification can be controlled. Apparently, both nitrification and denitrification proceeded well under the respective aerobic and anoxic conditions, thereby enhancing the nitrogen removal efficiency. A considerable amount of time remained from the 360 min cycle time after nitrification and denitrification were completed for each aerobic and anoxic phase.
To achieve high-rate nitrogen removal in the step-feed granular SBR, the durations of the anoxic and aerobic phases were reduced according to the break-points in stage II in the subsequent experiment. Fig. 3b shows the profiles of NH₄-N, NO₂-N, NO₃-N, DO and pH in the step-feed granular SBR on day 120 after optimization. Compared with stage II, the cycle time was reduced from 360 min to 207 min. Interestingly, the nitrogen and COD removal efficiencies did not significantly change. TIN was reduced to a final level of 6.4 mg/L, which was a little higher than that in stage II, and NH₄-N was completely removed. The NO₂-N concentration was a little higher at the end of each aeration phase compared with stage II. The durations of the four aerobic phases were set to 28, 21, 18, and 16 min, with nitrification rates of 0.735, 0.610, 0.624, and 0.549 kg NH₄-N/m³d, respectively. In each anoxic phase with substrate feeding, denitrification benefited from the sufficient COD supply by the step feeding. The result showed that 17.6, 27.8, 28.8, and 27.7 mg NOₓ-N were denitrified within 20, 22, 25, and 27 min in the four anoxic phases. Correspondingly, specific denitrification rates were 0.684, 0.674, 0.467, and 0.336 kg NOₓ-N/m³d, respectively. Based on the high specific nitrification and denitrification rates of granules in each aerobic and anoxic phase, a high nitrogen removal rate of 0.27 kg N/m³d was achieved. This high nitrogen removal rate enabled a much shorter time to complete the nitrogen removal in the step-feed granular SBR system, revealing the advantages of step-feed granular SBR in the process of BNR.

3.4. Aerobic granular sludge properties throughout the entire operation

The profiles of MLSS, SVI and average granule size throughout the entire operation are summarized in Fig. 4. On day 1, the average particle size was 658 μm, MLSS in the reactor was about 7.1 g/L, and SVI was 19.8 mL/g. MLSS decreased to 5.5 g/L after 7 days of operation, and further decreased to 4.3 g/L on day 28. At the end of stage I, the average particle size of the granules decreased to 428 μm and the percentage of particle size larger than 400 μm decreased to 53.2% because of the use of a mechanical stirrer. The decrease in particle size led to a low settling velocity. Consequently, small size particles (the size between 0.1 and 0.2 mm) and flocculating sludge were washed out from the reactor under the given settling time. However, the decrease in MLSS had less effect on the nitrogen and COD removal in stage I. This result demonstrated the high bioactivity of granules in SBR. On the other hand, the EPS content increased from 14.75 mg/g MLSS at the end of stage I to 33.98 mg/g MLSS at the end of stage III. The increase in EPS may have resulted from the decrease in starvation time under the step-feed mode. EPS can mediate the cohesion of cells and play a crucial role in maintaining the structural integrity of granules. Therefore, EPS increase can simulate the growth of sludge particle size. More importantly, the anoxic growth of heterotrophic bacteria in the inner layers of granules can promote aggregate densification in the anoxic phase of stage II and III. Both these situations were likely to enhance the strengthening of the granule structure and improve granular stability. Consequently, from day 49, MLSS exhibited a slight recovery and was maintained at around 6.4 g/L at the end of stage III. Meanwhile, the average particle size gradually increased from 428 μm on day 28 to 693 μm on day 120.

Fig. 4 shows the photographic and SEM images of the aerobic granules at the end of the operation period. The aerobic granules were highly compact and mainly consisted of coccus-like bacteria (the filamentous bacteria were absent). This finding may be due to the remarkably higher DO than the 2 mg/L in the aerobic phase, which prevented the growth of filamentous bacteria. These results illustrated that the granules can be well maintained under the step-feed operational strategy throughout the 90 days of continuous operation.
4. Discussion

4.1. Nitrogen removal characteristic and comparison with other systems

BNR involves two consecutive steps, i.e., nitrification and denitrification. Fig. 2 shows that complete denitrification can be achieved in the anoxic phase within 16 min. Most NH$_4^+$-N was oxidized to NO$_3^-$-N in the aerobic phase, resulting in a high concentration of NO$_3^-$-N in the effluent. This phenomenon has often been observed in previous studies (Yang et al., 2003; Qin and Liu, 2006). Meanwhile, many studies have shown that SND occurs in a granular SBR because of the layer structure of granules (Cassidy and Belia, 2005; Coma et al., 2012). However, in the present study, a low SND efficiency caused by the deficient of carbon source in the aerobic phase was observed. The accumulation of poly-beta-hydroxybutyrate during the short COD degradation period in the aerobic phase may be insufficient for the subsequent denitrification (Kishida et al., 2006). Consequently, low nitrogen removal efficiencies of 48.1–59.5% were obtained in stage I. In contrast, denitrification was remarkably enhanced under the step-feed mode. The COD/NO$_3^-$-N ratio decreased from 15.7 in the first anoxic phase to 9.3 in the last anoxic phase, as shown in Fig. 3. With glucose as the carbon substrate, the theoretical value obtained from the stoichiometric denitrification equation was 4.9 mg COD/mg NO$_3^-$-N (Matějů et al., 1992). On one hand, the high COD/NO$_3^-$-N ratio guaranteed high denitrification rates in the four anoxic stages. The COD removal efficiency was similar to that found by Lemaire et al. (2009) and higher than those found by Guo et al. (2007) and Yang et al. (2007), because the latter added the ethanol as an external carbon to enhance denitrification before discharge. In addition, Chen et al. (2011) achieved slightly higher nitrogen removal without adding an external carbon resource because of the higher COD/N ratio in the feed and the low aeration rate in the last aerobic phase. On the other hand, the low aeration rate resulted in the low nitrogen removal rate in the step-feed granular SBR. Table 1 showed that the nitrogen removal capability of this experiment exhibited the maximum ability in all systems, which was mainly attributed to the maintenance of granular sludge and operation conditions in the reactor. Also, there were some other variations of parameters including wastewater and VER. Generally, synthetic wastewater was considered to easily reach a higher nitrogen removal than practical wastewater just due to the simple composition and degradation ability. The VER might also play an impact on the nitrogen removal behavior but to less extent. The exact effects of other operational parameters on nitrogen removal need more detailed investigation in the future. The above discussions demonstrated that retrofitting the existing activated sludge SBR

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<td><strong>Comparison of performances of step-feed granular SBR with step-feed activated sludge SBR.</strong></td>
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<td>Denitrification rates (kg NO$_3^-$-N/m$^3$d)$^a$</td>
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<td>Nitrogen removal rate (kg N/m$^3$d)</td>
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AS, activated sludge; GS, granular sludge.

$^a$ The nitrification and denitrification rates were calculated by the performance in each aerobic and anoxic phase in one cycle, respectively.
to granular SBR operated under the step-feed mode combined with pH and DO parameters to control the duration improved nitrogen removal efficiency. The treatment capacity was also dramatically enhanced at the same working volume.

4.2. Stability of the granules under step-feed operational strategy

The stability of physical properties is important for continuous operation of a granular sludge SBR. A mechanical stirrer is often used in a granular SBR to achieve well-mixing situation under anoxic conditions (Cassidy and Belia, 2005; Yuan and Gao, 2010; Mosquera-Corral et al., 2011; Zhang et al., 2011). In the present experiment, the decrease in granule size mainly resulted from the increased hydrodynamic force around the impeller. The large granules lacked nutrients and had to utilize granular constituents for survival, which weakened the overall dense structure of the granules (Wang et al., 2007). Consequently, the larger-sized granules were easier to break into flocculating sludge than the smaller ones under high hydraulic shear force. However, on one hand, NO₃⁻–N was totally denitrified in the four anoxic phases in stages II and III. The nitrate respiration of heterotrophic bacteria allows them to grow deep inside the granules without oxygen under the step-feed mode (Wan et al., 2009). On the other hand, the degradation of organic carbon by denitrifying bacteria in the four anoxic stages caused the microorganisms to be starved in the four aerobic stages, resulting in the improved microbial aggregation (Su et al., 2012). As aforementioned, the increase in EPS content simulated the growth of sludge particle size. Based on these factors, the granular size gradually increased and reached the initial level on day 80. In stage III, the average COD volumetric loading rate was much higher than those in stages I and II because of the decrease in cycle time (0.99 kg COD/m³d in stages I and II, and 1.72 kg COD/m³d in stage III). Generally, a high COD volumetric loading rate is favorable for granule stability, increase in sludge concentration, and sludge particle size (Wang et al., 2009). At the same time, increased MLSS and particle size were observed in stage III. Therefore, the granules were well maintained and the granule size increased to 693 μm at the end of operation. The stability of the granules guaranteed a high nitrogen removal rate under the step-feed operational strategy.

5. Conclusions

The TN removal efficiencies were 48.1–59.5% and 88.7–92.6% in single-feed and step-feed granular SBRs, respectively, for treating synthetic wastewater under a 360 min cycle. DO and pH were reliable parameters for identifying the endpoints of nitrification and denitrification in the step-feed granular SBR. The TN removal efficiency and nitrogen removal rate were 89.7–92.4% and 0.27 kg N/m³d, respectively, after optimization in the four-phase step-feed granular SBR under a 207 min cycle. Granules can be well maintained under the step-feed strategy throughout the 90 days of continuous operation period.

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