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# Long-term effect of dissolved oxygen on partial nitrification performance and microbial community structure

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ABSTRACT

In this study, the performance of partial nitrification via nitrite and microbial community structure were investigated and compared in two sequencing batch reactors (SBR) with different dissolved oxygen (DO) levels. Both reactors achieved stable partial nitrification with nitrite accumulation ratio of above 95% by using real-time aeration duration control. Compared with high DO (above 3 mg/l on average) SBR, simultaneous nitrification and denitrification (SND) via nitrite was carried out in low DO (0.4–0.8 mg/l) SBR. The average efficiencies of SND in high DO and low DO reactor were 7.7% and 44.9%, and the specific SND rates were 0.20 and 0.83 mg N/(mg MLSS h), respectively. Low DO did not produce sludge with poorer settling properties but attained lower turbidities of the effluent than high DO. Fluorescence in situ hybridization (FISH) analysis in both the reactors showed that ammonia-oxidizing bacteria (AOB) were the dominant nitrifying bacteria and nitrite-oxidizing bacteria (NOB) did not be recovered in spite of exposing nitrifying sludge to high DO. The morphology of the sludge from both two reactors according to scanning electron microscope indicated that small rod-shaped and spherical clusters were dominant, although filamentous bacteria and few long rod-shaped coexisted in the low DO reactor. By selecting properly DO level and adopting process control method is not only of benefit to the achievement of novel biological nitrogen removal technology, but also favorable to sludge population optimization.


## 1. Introduction

Recently, achieving higher pollutants removal efficiency with less energy consumption has become a very urgent and critical task for wastewater treatment plants (WWTPs) operation. Many biological technologies and processes have been developed for nitrogen removal from wastewater over the past few decades. Partial nitrification, as one of the cost-effective and sustainable biological nitrogen removal processes, has gained much attention currently (Yamamoto et al., 2008; Zhu et al., 2008). Compared with conventional nitrification and denitrification, shortcut nitrification and denitrification via nitrite not only reduces the aeration consumption by 25%, but also saves the carbon source dosage by 40% (Turk and Mavinic, 1987; Turk and Mavinic, 1989). Moreover, higher denitrification rate and lower wasted sludge production can be achieved by shortcut nitrification and denitrification via nitrite (Tokutomi, 2004; van Kempen et al., 2001). Similarly, simultaneous nitrification and denitrification (SND) has also attracted many research interests due to its potential to eliminate the anoxic tanks and simplify the overall process design and without the need for external carbon source dosage (Daniel et al., 2009; Keller et al., 1997; Third et al., 2003a). Particularly, SND via nitrite is a new biological nitrogen removal process with the combinational advantages of the two processes mentioned above (Chen et al., 2009; Daniel et al., 2009; Ruiz et al., 2006).

The enrichment of ammonia oxidizing bacteria (AOB) and limitation-inhibition-washout of nitrite oxidizing bacteria (NOB) is the critical point for stable maintaining of partial nitrification via nitrite (Blackburne et al., 2008a; Peng and Zhu, 2006). Several process parameters, such as dissolved oxygen (DO) concentration, temperature, sludge retention time (SRT), substrate concentration, aeration pattern, aeration duration, and inhibitors, have been found to selectively inhibit or washout NOB (Aslan et al., 2009; Peng and Zhu, 2006; Yuan et al., 2008). From the economically and practical considerations, DO concentration and aeration duration are economically feasible control parameters, since low DO concentration and appropriated aeration duration can save aeration consumption. Correspondingly, DO concentration, C/N ratio, floc size and biomass concentration are the key factors to achieve SND process. On-line monitoring DO is recommended to be an efficient means in SND process (Fuerhacker et al., 2000; Zhao et al., 1999).

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Until now, a significant amount of research has focused on the partial nitrification and SND achieved by low DO (Aslan et al., 2009; Blackburne et al., 2008b; Wang and Yang, 2004). Moreover, the short-term effect of DO on biological nitrogen removal has been discussed in many studies using batch test (Bae et al., 2001; Ciudad et al., 2005; Park and Noguera, 2004). However, limited reports are available on comparisons of partial nitrification performance under different DO for long-term operation. It is still doubtful whether high DO level would destroy the stable and high nitrite accumulation ratio (NAR) built by low DO or other operational factors. In addition, further studies are necessary to investigate the shift of microbial community structure and morphological property when the partial nitrifying sludge exposes to different DO over long-term operation. It is also not very clear whether high DO would cause the recovery of NOB after long time operation. Based on these questions above, the objective of this study is to compare the partial nitrogen removal performance and microbial community structure as well as floc morphology and floc size under different DO concentrations operation. It also aims at building the relationship between the operation conditions and sludge population structure and finding a feasible control strategy to achieve novel biotechnology and sludge population optimization.

## 2. Methods

### 2.1. Lab-scale sequencing batch reactors (SBRs)

The experiments were performed in two identical SBRs, each with 10 l working volume. Oxygen was supplied by an air compressor through an air diffuser inside the reactor. A mechanical stirrer was used to provide liquid mixing. In addition, the pH, oxidation–reduction potential (ORP) and DO sensors were installed for monitoring pH, ORP and DO in the reactor. No pH adjustment occurred in two reactors. Each cycle consisted of 3 min feeding, aerobic reaction, 30 min settling, 30 min decanting, and idling. Aeration duration was not fixed and aeration was stopped when detecting characteristic point in pH profile. Five liters of supernatant was withdrawn from the reactor at the end of settling phase and 5 l fresh wastewater was pumped into the reactor during the filling phase. The sludge of SBR-1 was exposed under high DO (above 3 mg/l on average) and the sludge of SBR-2 was operated to low DO (0.4–0.8 mg/l). The average mixed liquor suspended solids (MLSS) concentrations in SBR-1 and SBR-2 were 2600 mg/l and 2800 mg/l during the experimental period, respectively.

### 2.2. Wastewater and sludge characteristics

The seeding sludge of SBR-1 with a mixture of heterotrophic organisms capable of oxidizing carbonaceous compounds and denitrification, autotrophic nitrifying organisms was obtained from the secondary clarifier of another pilot-scale A/O reactor under limiting DO in our lab. Partial nitrification was achieved by using aeration duration control. After operated for 92 cycles, about 2 l of the mixed liquor from SBR-1 was seeded into SBR-2. The feed to both reactors was the same and real domestic sewage from residential area near our lab and the wastewater characteristics are described as the following Table 1. After the cultivation of the activated sludge, the experiment had lasted for a year.

### 2.3. Analytical methods

The temperature and pH were detected on line using WTW level 2 pH meter (WTW Company, Germany). ORP and DO were continuously monitored by WTW, pH/oxi340i meter with ORP and DO probes (WTW Company, Germany). TN was measured by multi N/C 3000 analyzer (Analytik Jena AG). COD, $NH_4^+$–N, $NO_2^-$–N, $NO_3^-$–N, MLSS and volatile MLSS (MLVSS) were measured according to Standard Methods (APHA, 1998). Turbidity was detected by WTW Turb 555 (Germany). Microscopic examination was performed using an OLYMPUS-BX52 (Japan).

### 2.4. Fluorescence in situ hybridization (FISH)

Sample fixation and hybridization steps were carried out according to methods previously described by (Amann et al., 1990). FISH was performed with EUBmix (EUB338, EUB338-II, EUB338-III) specific for members of the domain bacteria, NSO190 specific for all ammonia-oxidizing *β-proteobacteria* except some *Nitrosomonas* strains, NIT3 specific for *Nitrobacter* and Nstpa662 specific for *Nitrospira* (Loy et al., 2003; Mobarry et al., 1996). The images of FISH samples were captured using an OLYMPUS-BX52 fluorescence microscope. FISH quantification was carried out by Image-pro plus 6.0 Software®, where the relative abundance of the interested bacteria was determined as the mean percentage of all bacteria.

### 2.5. Scanning electron microscope (SEM) observation

The morphology of the bacteria was examined with high resolution SEM (FEI QUANTA 200, FEI Company in USA). The samples were pretreated by fixing with 2.5% glutaraldehyde in a 0.1 M phosphate buffer. Subsequently, the samples were washed and dehydrated in a graded series of ethanol solution (50%, 70%, 80%, 90%, and 100%). The dewatered samples were dried by the critical point method and further sputter coated with gold for SEM observation.

### 2.6. Floc size determination

The volumetric floc size distribution was determined using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK). The Malvern Mastersizer 2000 uses light scattering and returns a volume fraction for each of the 100 size bands between 0.01 and 10,000 μm.

### 2.7. Calculation of NAR and SND efficiency

The nitrite accumulation ratio (NAR) was calculated as follows:

$$\mathrm{NAR} = \frac{NO_2^- - N}{NO_2^- - N + NO_3^- - N} \times 100\% \tag{1}$$

The efficiency of SND and the specific rate of SND were calculated according to the following equations (Third et al., 2003a), respectively:

$$\text{SND Efficiency} = \frac{NH_4^+ - N_{(\text{oxidized})} - NO_x^- - N_{(\text{produced})}}{NH_4^+ - N_{(\text{oxidized})}} \times 100\% \tag{2}$$

**Table 1**
Wastewater characteristic.

| | pH | COD (mg/l) | $NH_4^+$–N (mg/l) | $NO_2^-$–N (mg/l) | $NO_3^-$–N (mg/l) | TN (mg/l) | Alkalinity ($CaCO_3$ mg/l) |
|---|---|---|---|---|---|---|---|
| Range | 7.0–7.8 | 160–320 | 46.8–75.6 | 0.04–0.26 | 0.12–1.08 | 50.3–89.7 | 280–400 |
| Average value | | 215 | 59.7 | 0.08 | 0.46 | 67.9 | 320 |

$$\text{Specific SND rate (mgN/mgMLSS/h)} = \frac{NH_4^+ - N(\text{oxidized}) - NO_x^- - N(\text{produced})}{\text{MLSS} \times t} \quad (3)$$

where $NH_4^+$–$N_{(oxidized)}$ represents the total amount of $NH_4^+$–N oxidized after nitrification reaction, $NO_x^-$–$N_{(produced)}$ is the concentration of $NO_x^-$–N (including $NO_2^-$–N and $NO_3^-$–N), $t$ is the aerobic time.

## 3. Results and discussion

### 3.1. Typical nitrogen conversion via nitrite at high DO

According to nitrification reactions, there is no hydrogen ion produced in the course of converting nitrite to nitrate, which is contrary to the process of ammonia conversion to nitrite. After achieving ammonia oxidation, pH will start to ascend and then a valley point will appear in pH profile. Accordingly, it can be available to indicate nitritation process completion by identifying "ammonia valley" (Alghusain and Hao, 1995; Guo et al., 2007) and hence avoid excess aeration and the further transformation from nitrite to nitrate. After a period operation with real-time aeration duration control, the nitrite accumulation could be obtained. In this study, partial nitrification was achieved by the real-time aeration duration control built before (Yang et al., 2007). During the start-up and stable operation period, the average DO was always kept above 3.0 mg/l.

Typical variations of nitrogen species and control parameters including DO, pH in SBR-1 reactor are shown in Fig. 1. From the nutrient profile, it can be seen that $NH_4^+$–N was completely oxidized within 240 min aerobic phase, where there was a corresponding increase of the nitrite but nitrate concentration was almost kept constant (always lower than 2 mg/l). The initial rise in the pH curve was caused by carbon dioxide stripping from the system and the rapid consumption of COD (Ra et al., 1998). The decrease of pH was caused by the reduction of alkalinity and the acid production during the first step of nitrification. After completion of ammonia oxidation, the pH started to increase. The point marked with a circle in pH profile represented the end of ammonia oxidation process and it was known as the "ammonia valley". Constant aeration was applied during the aerobic period. DO increased slowly and then held at constant value during nitrification. DO concentration ascended sharply after the completion of ammonia oxidation. A break point named as "DO break point" showed in DO profile (Pavšelj et al., 2001). The average DO during a cycle was more than 3 mg/l. By detecting these break points (generally "ammonia valley" in pH profile), the duration of aerobic could be controlled stably, which had the advantages of achieving high ammonia removal efficiency and avoiding the transformation from nitrite to nitrate.

### 3.2. Typical nitrogen conversion via nitrite at low DO

The seed sludge to SBR-2 was directly from SBR-1 when it achieved stable partial nitrification via nitrite after 92 cycles' operation. Because most NOBs were eliminated from SBR-1 after a period of real-time aeration duration control, partial nitrification via nitrite in SBR-2 could be directly started up and maintained under low DO. Typical variations of nitrogen species and control parameters including DO, pH in SBR-2 reactor are shown in Fig. 2.

The variations of nitrogen species were similar with those results attained at high DO. Nitrite concentration increased with time as ammonia was converted through nitrification, but nitrate concentration was nearly negligible. $NH_4^+$–N was completely oxidized to $NO_2^-$–N during longer aeration time than that under high DO. Although low DO was adopted, those particular points including "ammonia valley" and "DO break point" were also easily detected. DO increased slowly during nitrification and the average DO during a cycle was less than 0.5 mg/l. At the end of ammonia oxidation process, DO increased sharply, then aeration was stopped in order to prevent from excess aeration.

Furthermore, according to Figs. 1 and 2, the real-time monitoring of pH and DO had significant effects on stable partial nitrification and energy saving. Because of the tandem of ammonia and nitrite oxidation, an initial nitrite accumulation could be accomplished at the start-up period of a reactor. However, the initial nitrite accumulation was often transitory and instable due to the rapid conversion of nitrite to nitrate by NOB, which resulted in the rarely successful application of partial nitrification. Because the real-time aeration duration control based on monitoring pH and DO could detect the completion of ammonium oxidation, the nitrite accumulation could be maintained after the long time acclimation. On the other hand, aeration consumption would be saved by preventing the transformation of nitrite to nitrate by real-time monitoring pH and DO.

### 3.3. Effect of DO on partial nitrification performance

Although the seed sludge of SBR-2 was from SBR-1, the system performance showed some differences between the two reactors after a period of operation under different DO concentrations. Figs. 3 and 4 show the nitrogen removal performance in SBR-1 and SBR-2, respectively. In order to compare distinctly, NAR, the SND

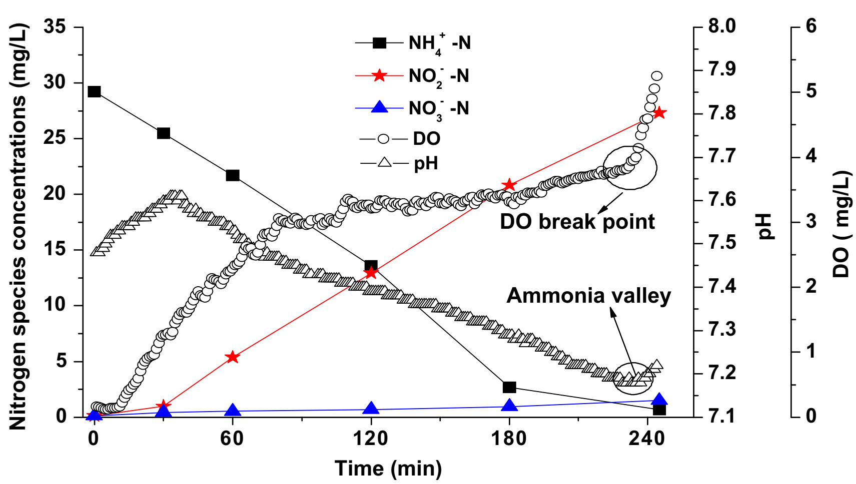


**Fig. 1.** Typical profiles of nitrogen species and control parameters in a cycle under high DO.

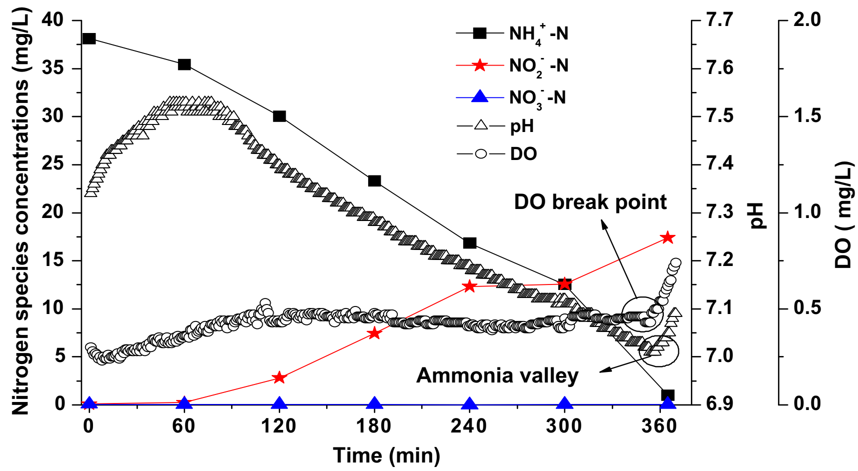


**Fig. 2.** Typical profiles of nitrogen species and control parameters in a cycle under low DO.

efficiency and nitrogen removal efficiency in both SBRs are given in Table 2.

The average ammonia removal efficiencies in both SBRs were more than 90%, while TN removal efficiencies were 51.6% and 68.6%, respectively. The average efficiencies of SND in both the reactors was 7.7% and 44.9%, and the specific SND rates were 0.20 and 0.83 mg N/(mg MLSS h), respectively. The differences of TN and SND efficiency were mainly attributed to different DO concentrations. It was widely acknowledged that low DO had positive effects on SND and TN removal, since the presence of anoxic zone was very possible in reactor or inside of the sludge floc under low DO condition (Zhu et al., 2008). Some studies suggested that SND was driven by stored poly-β-hydroxybutyrate (PHB) and the percentage of nitrogen removed via SND increased at lower DO concentrations (Third et al., 2003b). Higher PHB production coefficient could be obtained under DO limitation, as opposed to lower value under DO excess conditions, where a higher fraction of substrate was utilized for biomass growth. Up to 78% SND was achieved under DO of 0.5 mg/l using acetic as carbon source (Third et al., 2003a). A pertinent increase of wastewater propionic/acetic acid ratio was also found to be favorable for SND at low DO (Li et al., 2008). Compared with results from the literature (Li et al., 2008; Third et al., 2003a), the SND efficiency and specific SND rate were lower in our results, which might be attributed to lower PHB production from the real domestic wastewater. Oppositely, the influents in these studies mentioned were artificial wastewaters with more degradable substrates such as acetic or/and propionic, more synthesized PHB could be available as the electron donor for effective SND. Besides, it should be noted that the increased percentage of SND at a low DO concentration was compromised by a 1.7-times slower specific ammonia oxidation rate. The average specific ammonia rates were 0.078 and 0.046 g $NH_4^+$-N/(g MLSS d) in high DO and in low DO reactor, respectively.

It was interesting that DO concentration had no distinct influences on NAR, which were 96.3% and 96.6% in SBR-1 and SBR-2, respectively. Many studies achieved partial nitrification via nitrite by using low DO to enrich AOB or washout NOB due to their different oxygen affinity constants (Blackburne et al., 2008b; Wang and Yang, 2004). Additionally, some studies reported that high DO would destroy or decrease stable nitrite accumulation ratio after

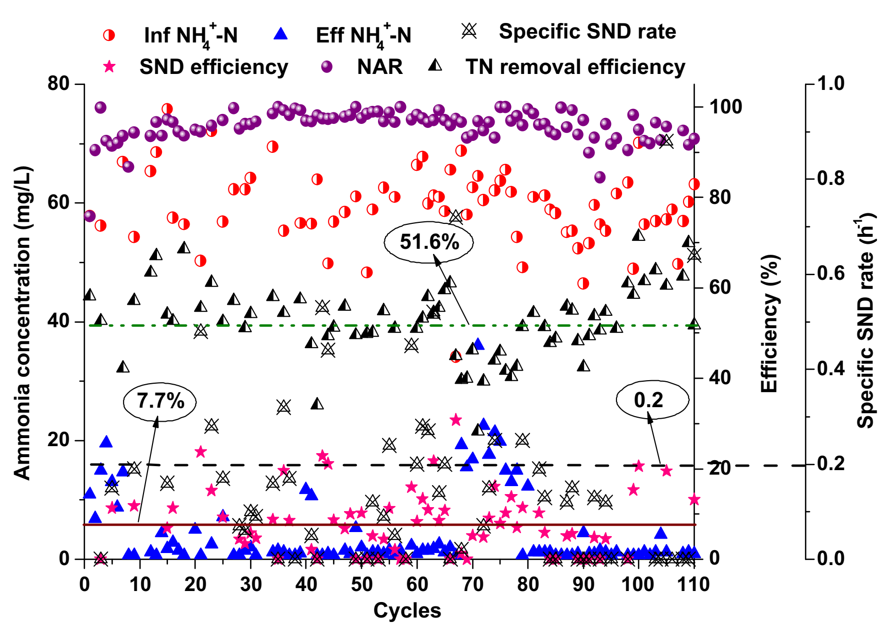


**Fig. 3.** Nitrogen removal performance at high DO in SBR-1 (cycle 1 in this figure was actual cycle 93 for SBR-1).

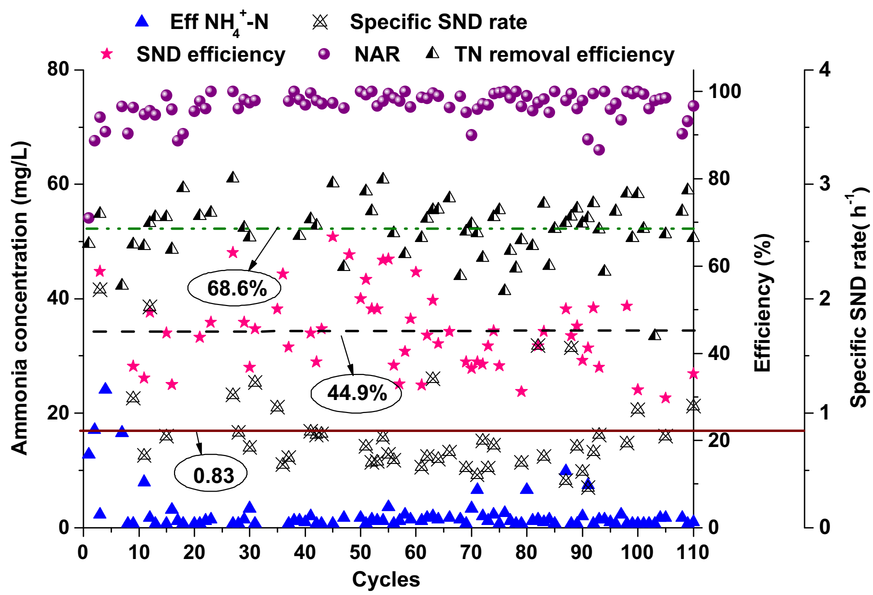


**Fig. 4.** Nitrogen removal performance at low DO in SBR-2.

**Table 2**
Comparison of SND, nitrogen removal in two SBRs.

| Item | SBR-1 | | | SBR-2 | | |
|---|---|---|---|---|---|---|
| | Min. | Max. | Aver. | Min. | Max. | Aver. |
| $NH_4^+$-N removal efficiency (%) | 44.3 | 99.0 | 92.3 | 75.3 | 99.1 | 96.4 |
| TN removal efficiency (%) | 28.3 | 71.4 | 51.6 | 44.0 | 80.1 | 68.6 |
| SND efficiency (%) | 0 | 30.8 | 7.7 | 29.7 | 66.7 | 44.9 |
| Specific SND rate (mgN/mgMLSS/h) | 0 | 1.0 | 0.20 | 0.35 | 2.08 | 0.83 |
| NAR (%) | 84.5 | 100 | 96.3 | 71.0 | 100 | 96.6 |
| $\gamma_N$ (g $NH_4^+$–N/gMLSS/d) | 0.064 | 0.091 | 0.078 | 0.023 | 0.067 | 0.046 |

long-term operation (Ciudad et al., 2005; Garrido et al., 1997). However, the results from this study did not support this point, which was mainly attributed to limited (even negligible) number of NOB in the reactor. More explanations in detailed can be found later.

### 3.4. Effect of DO on floc size and sludge settling property

In accordance with physical mechanism (Baumann et al., 1996; Hibiya et al., 2003), SND occurred as the consequence of DO gradients within activated sludge flocs, particularly with large floc size. The large floc diameter was likely to promote the SND due to diffusion limitation of oxygen in the floc. To investigate the effect of DO on floc size, the activated sludge floc sizes were measured. The average floc size under lower DO in SBR-2 was 175.25 μm, as opposed to 198.91 μm under high DO in SBR-1 (Fig. 5). The average floc size in both reactors was significantly bigger than the mean floc size reported by other authors (Pochana and Keller, 1999). Large floc diameter in both reactors should be attributed to the influent characteristic. Compared with the synthetic wastewater, the real municipal sewage had more complex in constitute and content, particularly with some visible particles, which could promote to form large sludge flocs. It was found that no clear correlation between DO concentration and average floc diameter and there was only a trend towards larger flocs at higher DO concentrations (Wilén and Balmér, 1999), which was consistent with the results in this study. Moreover, the distribution of flocs also well met to log-normal distribution functions. It should be noted that the number of smaller floc in the supernatant effluent from SBR-1 was lower than SBR-2. The volume percent of floc with the diameter lower than 50 μm in SBR-1 and SBR-2 was 10.3% and 6.39%, respectively.

Low DO was a critical factor to achieve SND and favorable to higher oxygen transfer rate, so it is recommended to be employed in WWTPs. However, DO deficiency was believed to be one of the most frequent causes responsible for most filamentous bacteria proliferation in activated sludge processes. Therefore, the effect of DO on sludge settleability was investigated. Diversely, low DO did not deteriorate settling property although some few filamentous

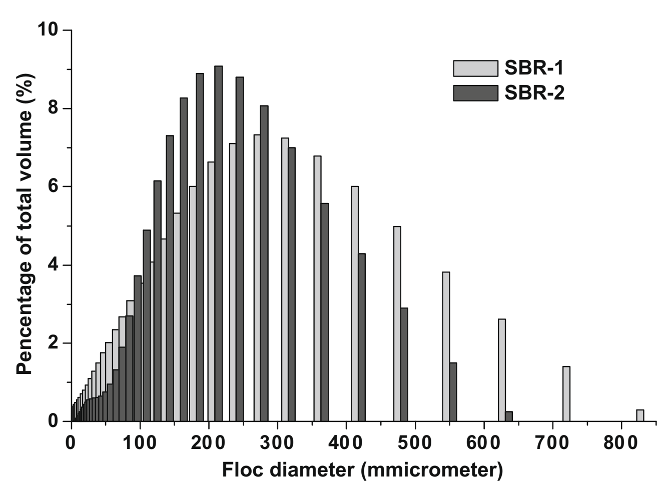


**Fig. 5.** Floc size distributions in two reactors.

bacteria occurred in SBR-2. SVI was always lower than 100 ml/g in both reactors (Fig. 6). Such good settling property might be associated with the pulse feeding strategy. According to the literature (Martins et al., 2003), the fill time ratio and the corresponding feast period had a strong effect on the sludge settleability. Good sludge settleability could be attained by promoting a strong substrate gradient in the SBR with the lower fill time ratio. No clear relationship between DO concentration and SVI could be found in this work, there was only a trend towards lower SVI value at higher DO concentrations. Moreover, the real domestic wastewater with much suspended solids was another reason for good sludge settleability. Additionally, it was interesting that the turbidity of supernatant in SBR-2 was lower than that in SBR-1. It presents that lower DO produced clearer effluent but did not lead to poorer settling properties. The effluent with lower turbidity was also related to the fact that the number of smaller floc in the supernatant effluent from SBR-2 was lower than SBR-1. On the other hand, clearer effluent during filamentous bulking was often mentioned and also used as predict phenomena of sludge bulking, which is associated with filaments that have higher A/V ratio and capture tiny particles or free flocs in the effluent. This result was not consistent with the report that the turbidity of supernatant in reactors with low DO was higher than that with high DO (Wilén and Balmér, 1999).

### 3.5. Effect of DO on microbial community structure

Microbial population shift in two reactors during operation period was investigated and compared using SEM and FISH. Sludge samples were taken from both reactors for SEM examination to observe the morphology of sludge. Sludge images from SBR-1 showed that thick clusters of small rod-shaped cells were the dominant population structure. Although the seed sludge of SBR-2 was inoculated from SBR-1, clusters of filamentous form and few long rod-shaped were observed except small rod-shaped and spherical cells after low DO operation. SEM pictures indicated the microbial morphology of sludge in both reactors showed some changes at different DO levels after long-term operation. The morphology of dominant bacteria was mainly short rod-shaped in two partial nitrification reactors. The similar phenomenon was found in a partial nitrification reactor treating high ammonia concentration wastewater (Sinha and Annachhatre, 2007). SEM observations in their study indicated that the inner structure of the granular sludge showed a shift towards spherical and small rod-shaped clusters. Nevertheless, it was difficult to identify these clusters of bacteria by SEM viewing alone. In order to investigate whether DO would influence on bacteria community structure, the composition of the microbial population was characterized using FISH method.

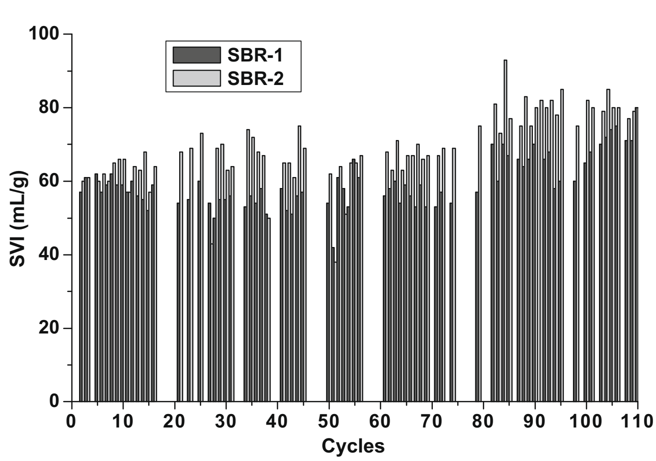


**Fig. 6.** Sludge settleability under low and high DO.

Before the start-up of SBR-2, SBR-1 had achieved stable partial nitrification via nitrite by using the real-time aeration duration control. AOB had become the dominant nitrifying bacteria, while NOB were gradually washed out from SBR-1 reactor. Correspondingly, the AOB and NOB population sizes were about 6–7% and 1–2%, respectively. Over long-term operation under different DO levels, FISH measurements showed that AOB population proliferated a little in both reactors and the phenomenon was more distinct in SBR-1 with high DO. At Cycle 108, about 9–12% of AOB in SBR-1 and 6–8% of AOB could be seen in SBR-2 by FISH analysis. Higher nitrification efficiency in SBR-1 was not only caused by high DO, but also might be due to more AOB percent. Correspondingly, NOB population decreased along operation period under low DO. After operation of 100 cycles, the NOB population in SBR-2 was seldom to be detected. Moreover, high DO did not promote the growth or recovery of NOB and lower than 0.5% of NOB population could be seen in SBR-1.

According to FISH results, it can be speculated that the competition mechanism between microbial species was different in both the reactors. In order to carry out stable partial nitrification via nitrite, it is the critical point to achieve the enrichment of AOB and the elimination of NOB. In SBR-1, the bacteria were exposed to higher DO, the dominant competition relationship occurred between AOB and NOB. Generally, high DO might destroy high nitrite accumulation ratio and it is difficult to get the AOB accumulation under high DO. But aeration was turned off at the point when ammonia oxidation proceeded completely. Therefore, the further transformation from nitrite to nitrate could be avoided effectively by the real-time aeration duration control. SBR-1 achieved and maintained more than 95% nitrite accumulation ratio for long term even under high DO condition.

Differently, the main competition relationship existed between AOB and filamentous bacteria at limited DO condition. Compared with NOB, AOB with higher oxygen affinity would outcompete NOB under low DO, thereby resulting in partial nitrification. In SBR-2, NOB would be inhibited under low DO except that they had no enough time to further oxidize nitrite. Consequently, it was easier to attain the AOB accumulation and the NOB washout under low DO concentrations. On the other hand, it was very critical for SBR-2 to achieve an optimized microbial community between floc-former bacteria and filamentous bacteria under low DO. In the famine phase of SBR-2, the condition of lower DO and lower substrate would make filamentous bacteria get the competition dominance. However, filamentous bulking did not occur in SBR-2 although some limited amount of filamentous bacteria could be found by microscopic examination, which was attributed to controlling DO concentration and fill mode. In SBR-2, domestic wastewater was pumped into the reactors in a short filling period (3 min) under non-aeration condition, which had a negative effect on the growth of filaments. After the substrate was exhausted, filaments should theoretically outcompete floc-formers due to double limiting factors. But AOB had more energetic growth ability by utilizing ammonia compared with filaments using very low concentration biodegradable COD under low DO in SBR-2.

Sludge population optimization, as an emerging concept and a new dimension to the control of biological wastewater treatment systems, firstly proposed by (Yuan and Blackall, 2002). Recently, many studies have shown that control system and process design have significant effects on microbial community (Yuan et al., 2008). Some control systems have been designed and exploited to achieve an optimized microbial community. Based on the comparisons of nitrogen removal performance, SEM and FISH analysis in two reactors, the combination of real-time aeration duration control with low DO was not only favorable for novel biological nitrogen removal technology such as partial nitrification, but also benefit for the achievement of sludge population optimization.

Furthermore, it should make very high emphasis on sludge population optimization between floc-formers and filaments or AOB and NOB through real-time process control and operation mode. The balance between interested bacteria would be the critical point for novel processes in order to attain good effluent quality and good settling property.

## 4. Conclusions

Nitrogen removal performance, sludge settling properties, floc size and microbial community structure were investigated and compared in two SBR reactors with different DO levels over long-term operation. DO limitation was of benefit to SND via nitrite and total nitrogen removal. Moreover, low DO did not produce sludge with poorer settling property but attained lower turbidities of the effluent than higher DO concentration. FISH analysis in both the reactors showed that AOB became the dominant nitrifying bacteria compared to NOB by through the control of aeration duration. High DO did not destroy partial nitrification and did not lead to the recovery of NOB. Energy saving by low DO would be technically feasible if sludge settleability did not become too weak to affect separation of sludge and effluent. In addition, optimizing the microbial community structure, such as the case between AOB and NOB, filaments and floc-formers should be a further research aim for the design and operation of a WWTP.

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