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# Achieving partial nitrification by inhibiting the activity of *Nitrospira*-like bacteria under high-DO conditions in an intermittent aeration reactor

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ABSTRACT

It is generally accepted that a low dissolved oxygen (DO) concentration is more beneficial for achieving partial nitrification than high-DO. In this study, partial nitrification was not established under low-DO conditions in an intermittent aeration reactor for treating domestic wastewater. During the operational period of low-DO conditions (DO: 0.3 ± 0.14 mg/L), stable complete nitrification was observed. The abundance of *Nitrospira*-like bacteria, which were the major nitrite-oxidizing bacteria, increased from $1.03 \times 10^6$ to $2.64 \times 10^6$ cells/mL. At the end of the low-DO period, the batch tests showed that high-DO concentration (1.5, 2.0 mg/L) could inhibit nitrite oxidation, and enhance ammonia oxidation. After switching to the high-DO period (1.8 ± 0.32 mg/L), partial nitrification was gradually achieved. *Nitrospira* decreased from $2.64 \times 10^6$ to $8.85 \times 10^5$ cells/mL. It was found that suddenly switching to a high-DO condition could inhibit the activity and abundance of *Nitrospira*-like bacteria, resulting in partial nitrification.



## Introduction

Partial nitrification, which avoids the oxidation of nitrite to nitrate, is a critical step in some biological nutrient removal processes (*e.g.* SHARON-ANAMMOX, CANON, OLAND) (Van Hulle et al., 2010; Regmi et al., 2014). Compared to traditional nitrification–denitrification nitrogen removal processes, processes with partial nitrification are less expensive since they consume less oxygen and less carbon-source (Ahn, 2006). The establishment of partial nitrification is always the key to the implementation of these processes, but it is difficult to achieve. At present, several effective methods have been developed to achieve partial nitrification, which are mainly based on high temperature (Ge et al., 2015), high free ammonia concentrations (FA) (Zhang et al., 2014), high free nitrous acid (FNA) (Ge et al., 2015), aeration duration control (Ma et al., 2016), inhibitors and low dissolved oxygen (DO) concentration (Peng and Zhu, 2006).

Among these factors, low-DO concentration has been widely reported to successfully achieve partial nitrification in wastewater treatment systems (Peng and Zhu, 2006; Ma et al., 2009, 2011). It is commonly recognized that the activity of ammonium-oxidizing bacteria (AOB) is higher than that of nitrite-oxidizing bacteria (NOB) under low-DO conditions. This is because the oxygen half-saturation constant ($K_o$) of AOB is lower (Hanaki et al., 1990; Manser et al., 2005; Stenstrom, 1990), resulting in NOB being gradually selected out due to the stronger inhibition by low-DO conditions. However, Liu and Wang (2013) recently found their reactors performed complete nitrification during long-term operation

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under low-DO conditions. Similarly, Park and Nogura (2004) suggested that complete and stable nitrification could be maintained even at a low-DO level (0.12 mg/L). Moreover, Regmi et al. (2014) found that the $K_o$ of AOB (1.16 mg-$O_2$/L) was significantly higher than that of NOB (0.16 mg-$O_2$/L) in their reactor. These contradictory findings suggest that there is a high probability of not achieving partial nitrification in some low-DO reactors, which can adversely affect processes based on partial nitrification. However, there has been limited understanding of how to start-up or recover partial nitrification in a low-DO reactor performing complete nitrification.

In our study, an intriguing phenomenon was observed in an intermittent aeration nitrifying reactor treating organic-removed domestic wastewater. Complete nitrification was maintained under the low-DO concentration, but partial nitrification was started up under the high-DO concentration. In order to determine the mechanism of the start-up of partial nitrification, the nitrogen removal performance of the sequencing batch reactor (SBR) was monitored, and the activity and community of nitrifying bacteria were detected by batch experiments and biological methods.

## 1. Materials and methods

### 1.1. Experimental bioreactor set-up and operating conditions

An SBR with a working volume of 10 L was used which included two operational stages: low-DO stage (DO: 0.3 ± 0.14 mg/L) and high-DO stage (DO: 1.8 ± 0.32 mg/L). The SBR was operated at a fixed temperature of 25°C using an electric heater. The oxygen required for the nitrification process was supplied by an air pump through a flow meter. A mechanical stirrer was used to ensure a completely mixed status during the nitrification process. The pH, DO and temperature in the reactor were monitored using an on-line multi-parameter sensor (WTW 3420, Germany). The pH was not controlled in the reactor. The operational stage in each cycle included four periods, namely 10 min feeding period, variable-length intermittent aeration (10 min aeration/10 min anoxic) period, 30 min settling period, and 15 min discharging of 5 L supernatant period. The terminal point of nitrification was judged by the real-time control of DO concentration. The endpoint values were 0.7 mg/L in the low-DO stage and 3 mg/L in the high-DO stage. The SRT was maintained at 30 days by withdrawing the activated sludge regularly. The mixed liquor suspended solids (MLSS) was maintained at 1500 ± 134 mg/L, and the effluent suspended solids (SS) was 24 ± 8 mg/L.

The influent to the nitrifying SBR was domestic wastewater from which the organics had been removed in an activated sludge bioreactor by direct aeration for a short time (35 min). The characteristics of the influent were as follows: COD: 40–55 mg/L, $NH_4^+$-N: 50.2–80.4 mg/L, $NO_2^-$-N: 0.1–5.4 mg/L, $NO_3^-$-N 0.04–1.24 mg/L, and pH 7.2–7.8. Activated sludge taken from a pilot-scale SBR (volume: 7000 L) was used as the inoculum.

### 1.2. Batch experiments

Batch experiments were carried out to investigate the effect of DO fluctuation on the nitrifying performance of the activated sludge acclimated to the stable DO concentration. The experiments were conducted in a lab-scale SBR with a working volume of 2.5 L at the end of the low-DO stage and the high-DO stage, separately. The sludge was taken from the operating SBR (10 L) and washed three times with deionized water before being transferred to the 2.5 L SBR. The synthetic wastewater was prepared from a solution of $NH_4Cl$ and $NaNO_2$. The initial $NH_4^+$-N and $NO_2^-$-N concentrations were 40 mg/L and 20 mg/L, respectively. Different DO concentrations (0.3, 0.5, 1.0, 1.5 and 2.0 mg/L) were applied in the batch experiment. A variable frequency air pump was used to adjust air flow automatically according to the DO probes, in order to ensure a stable DO concentration. The initial MLSS in each 2.5 L SBR was kept at 2000 mg/L. The temperature was maintained at 24°C and the pH was kept at 7.2 by adding $NaHCO_3$ solution. The batch experiments were operated in triplicate for each DO concentration.

### 1.3. Analytical methods

$NH_4^+$-N, $NO_2^-$-N and $NO_3^-$-N concentrations were measured using a Quickchem® 8500 system (Hach Company, Lachat Instruments, USA), while the chemical oxygen demand (COD), SS and MLSS, MLVSS were analyzed using standard methods (American Public Health Association, 1998).

### 1.4. DNA extraction and quantitative PCR assay

Total DNA was extracted from 0.1 g of lyophilized sludge using a Fast DNA Spin Kit for Soil (QBIOgen Inc., Carlsbad, CA, USA), following the manufacturer's instructions. The DNA samples were stored at −20°C. The concentration of DNA was measured using a Nanodrop ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Quantitative PCR was performed with a Stratagene Mx3000P QPCR System (Agilent Technologies, USA), using the fluorescent dye SYBR-Green approach (TAKARA, Dalian, China). Three coupled primers (Table 1) were used to amplify the AOB and NOB (*Nitrobacter*, *Nitrospira*), namely amoA-1f/amoA-2f, FGPS872f/FGPS1269r and NSR1113f/NSR1264r (Park et al., 2010; Degrange and Bardin, 1995; Geets et al., 2007). Each PCR amplification mixture (20 μL) consisted of 10 μL SYBR® Premix Ex Taq™ (Takara, Dalian, China), 0.4 μL ROX Reference Dye50, 1 μL bovine serum albumin (25 mg/mL), 0.2 μL of each primer (10 μmol/L) and a 2 μL template of DNA (1–10 ng). The PCR amplification program performed denaturation for 3 min at 96°C followed by 40 cycles of

**Table 1 – List of primers.**

| Target | Primer name | Sequence (5′-3′) | Temp. (°C) |
|---|---|---|---|
| AOB | amoA-1f/amoA-2r | ggggtttctactggtggt/cccctckgsaaagccttcttc | 53 |
| *Nitrobacter* | FGPS872f/FGPS1269r | ctaaaactcaaaggaattga/ttttttgagatttgctag | 56 |
| *Nitrospira* | NSR1113f/NSR1264r | cctgctttcagttgctaccg/gtttgcagcgctttgtaccg | 53 |
| | EUB338f/Ntspa0685M | actcctacgggaggcagc/cgggaattccgcgctc | 53 |

denaturing at 95°C for 30 sec, annealing at proper temperatures (Table 1) for 30 sec, extension at 72°C for 30 sec, and finished with a final extension at 72°C for 10 min (Park et al., 2010; Degrange and Bardin, 1995).

Standard curves were obtained using ten-fold serial dilutions of the plasmid DNAs after determining their concentrations using a Nanodrop ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The plasmid inserts were made by performing PCR, then were ligated to the pGEM-T Easy Vector System (Progmega, Madison, WI, USA) and transformed into *Escherichia coli* BMJM109 competent cells (TAKARA, Dalian, China) following the manufacture's protocol. Plasmids were extracted from *E. coli* using a Wizard Plus SV Minipreps DNA Purification System (Progmega, Madison, WI, USA). The standard curves were employed when they covered 5–6 orders of magnitude with amplification efficiency and correlation coefficients greater than 95% and 0.98, respectively (Appendix A Figs. S1–S3). Triplicate data assays were performed for the decimally diluted standard plasmids, properly diluted samples, and negative controls (Wang et al., 2015).

### 1.5. *Nitrospira 16S rRNA gene clone library analysis*

One sludge sample was taken from the reactor at the end of the low-DO period. Total DNA was extracted following the method described in Section 1.4 and used as a DNA template in the PCR reaction with primers EUB33f/Ntspa0685M (*Nitrospira*). The PCR products were ligated to the pGEM-T Easy Vector System (Progmega, Madison, WI, USA) and transformed into *Escherichia coli* BMJM109 competent cells following the manufacture's protocol. Ampicillin and x-gal were used to screen for plasmids. 100 correct-insert colonies were screened from each sample and amplified with M17f/M17r primers. Then, the positive PCR products were used for screening the genetic diversity by using RELP with Hhal (Progmega, Madison, WI, USA) restriction enzyme digestion reactions (Wang et al., 2015). Finally, one representative clone from each group was chosen for sequencing. The sequences were then compared with available database sequences by performing a BLAST search in the NCBI database. The phylogenetic trees were constructed by neighbor-joining using the MEGA 4.0 package (Wang et al., 2015; Tamura et al., 2007).

## 2. Results

### 2.1. Reactor performance

The removal efficiency of the reactor and the abundance of AOB in the reactor are shown in Fig. 1. After 4 days of adapting, the influent $NH_4^+$-N was almost completely removed during the low-DO stage (DO: 0.3 ± 0.14 mg/L), while the abundance of AOB was steady at $6.0 \times 10^6$–$8.0 \times 10^6$ cells/mL. Over the entire low-DO stage, $NO_3^-$-N was dominant in the effluent with concentration ranging from 25.34–48.6 mg/L, in comparison to $NO_2^-$-N (range: 0.64–1.02 mg/L). In a typical cycle in the low-DO stage, the influent $NH_4^+$-N was converted to $NO_3^-$-N during the intermittent aeration phase. The value of DO suddenly increased when the nitrification was accomplished (Fig. 2a). Accumulation of $NO_2^-$-N was not found in the intermittent aeration phase of the typical cycle. These results indicate that complete nitrification was achieved in the reactor under the low-DO condition.

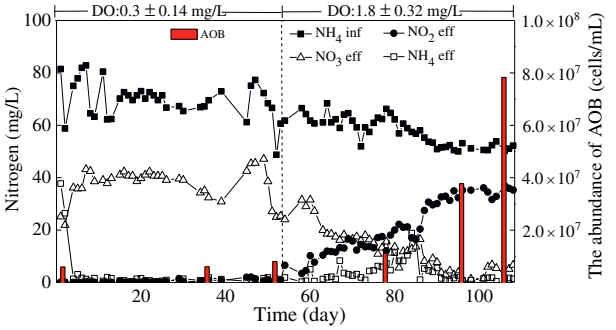


**Fig. 1 – Influent and effluent nitrogen concentration and abundance of ammonium-oxidizing bacteria.**

In the first day of the high-DO period (Day 55), the effluent $NO_2^-$-N concentration immediately increased to 6.1 mg/L. Then, the effluent $NO_2^-$-N concentration and nitrite accumulation rate (NAR) gradually increased and reached 22.22 mg/L and 60%, respectively, until Day 82 (Fig. 1). Although the effluent nitrite concentration moderately decreased due to the incomplete removal of $NH_4^+$-N, the ammonium removal efficiency rapidly reached 100% when the air flow was adjusted. Meanwhile, the accumulation of $NO_2^-$-N continued. At the end of the high-DO stage, the effluent $NO_2^-$-N and NAR were 35–36.8 mg/L and 80%–98.4%, respectively. The abundance of AOB also increased from $8.0 \times 10^6$ to $7.82 \times 10^7$ cells/mL. In a typical cycle of high-DO stage, the influent $NH_4^+$-N was converted to $NO_2^-$-N in the intermittent aeration phase, rather than $NO_3^-$-N (Fig. 2b). Partial nitrification was finally achieved under high-DO conditions in the intermittent aeration reactor.

In addition, during the low-DO stage typical cycle, the total nitrogen decreased from 49.93 to 39.20 mg/L (Fig. 2a). Similarly, during the typical cycle in the high-DO stage, the total nitrogen decreased from 44.3 to 39.01 mg/L (Fig. 2b). This suggested that the denitrification pathway was also present in the nitrifying reactor. Moreover, because the influent water was organic-removed domestic wastewater and little COD was removed during the operation (Appendix A Fig. S4), the denitrification performance of the reactor could probably be attributed to endogenous denitrification (Miao et al., 2016). However, the specific mechanism of the denitrification phenomenon of the reactor still needed further investigation.

### 2.2. Batch experiment results

The effect of DO fluctuation on the $NH_4^+$-N and $NO_2^-$-N oxidation rates was investigated using batch experiments, and the results are shown in Fig. 3. For the low-DO period, the $NH_4^+$-N and $NO_2^-$-N oxidation of the reactor increased, while the value of DO increased from 0.3 to 0.5 mg/L (Fig. 3a). Further, the $NO_2^-$-N oxidation rate was higher than the $NH_4^+$-N oxidation rate, suggesting that complete nitrification would be maintained during the low-DO period. However, the $NO_2^-$-N oxidation of the reactor decreased from 6.21 to 2.99 mg-N/g VSS/hr when the DO concentration further increased from 1.0 to 2.0 mg/L, whereas the $NH_4^+$-N oxidation continued to rise (Fig. 3a). The $NO_2^-$-N oxidation rate was markedly lower than the $NH_4^+$-N oxidation

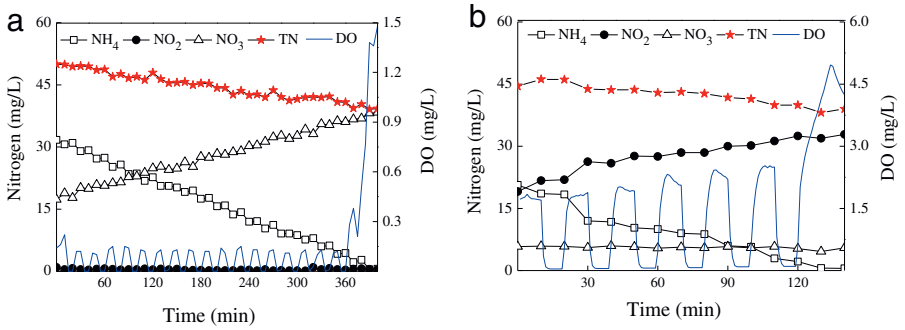


**Fig. 2 – Typical cycle: (a) low dissolved oxygen period (52 days); (b) high dissolved oxygen period (102 days).**

rate at the DO concentrations of 1.5 and 2.0 mg/L, indicating that the $NO_2^-$-N oxidation of the reactor could be inhibited by high-DO concentration and that $NO_2^-$-N would accumulate in the reactor after switching to the high-DO period.

Partial nitrification was observed in the reactor at the end of the high-DO period. Therefore, the results of batch experiments suggest that the $NH_4^+$-N oxidation rate continued to increase, and was markedly higher than the $NO_2^-$-N oxidation rate at all DO concentrations. The results also indicate that AOB were dominant in the reactor, while NOB were inhibited.

### 2.3. NOB abundance and community

The changes in the NOB community and abundance are crucial for achieving partial nitrification. Therefore, the abundance of two major NOB groups (*Nitrospira* and *Nitrobacter*) was quantified using real-time PCR. During the low-DO stage, the total NOB abundance (*Nitrospira* + *Nitrobacter*) increased. The number of *Nitrospira* increased from $1.03 \times 10^6$ to $2.64 \times 10^6$ cells/mL, while *Nitrobacter* remained at $1.41 \times 10^5$–$1.96 \times 10^5$ cells/mL (Fig. 4). *Nitrospira* was the dominant group of NOB in the reactor, and could be enriched under the low-DO condition. However, once the high-DO period had been reached, the number of *Nitrospira* gradually decreased from $2.64 \times 10^6$ to $8.85 \times 10^5$ cells/mL. The decrease of *Nitrospira* abundance suggested that the activity of *Nitrospira* was continually suppressed during the high-DO period. In contrast, the number of *Nitrobacter* began to increase and finally reached $2.44 \times 10^6$ cells/mL, which indicated that *Nitrobacter* gradually overtook *Nitrospira* as the dominant group of NOB at the end of the high-DO period. Overall NOB abundance recovered to $3.32 \times 10^6$ cells/mL at the end of the high-DO period because of the increase of *Nitrobacter* abundance.

Obviously, the decrease of the low-DO dominant NOB group (*Nitrospira*) was the determining factor for achieving partial nitrification by switching from low-DO to high-DO condition. To further investigate *Nitrospira*, the sample of activated sludge that was taken on day 53 was analyzed by constructing clone libraries. As shown in Fig. 5, 100 positive *Nitrospira* clones were clustered by RELP and finally divided into 4 groups, namely LD-Group 1, LD-Group 2, LD-Group 3 and LD-Group 4. The clones from LD-Group 1, LD-Group 2 and LD-Group 3 were similar to the clone *Candidatus Nitrospira defluvii*, constituting 94% of clones. Only 6% of *Nitrospira* clones (LD-Group 4) were distributed to a branch containing uncultured bacterium clone (FJ77893), suggesting that *Candidatus Nitrospira defluvii*-like bacteria were the dominant *Nitrospira* during the low-DO period. Similar findings were also reported by the study conducted by Park and Nogura (2008), in which the majority of *Nitrospira* were *Candidatus Nitrospira defluvii*-like bacteria in the low-DO reactor.

## 3. Discussion

Partial nitrification generally starts with unbalanced overall activity of nitrifying bacteria (AOB > NOB) (Ahn, 2006; Ge et al.,

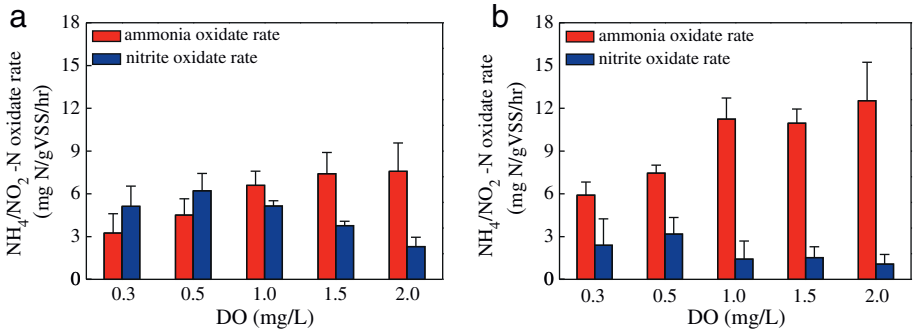


**Fig. 3 – Batch experiment on ammonia oxidation and nitrite oxidation of activated sludge with different DO concentrations: (a) tested at the end of low-DO period; (b) tested at the end of high-DO period. Error bar: standard deviation, *n* = 3.**

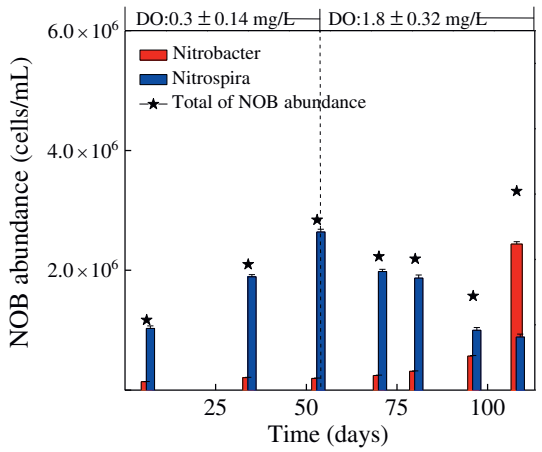


**Fig. 4 – Abundance of nitrite oxidizing bacteria (NOB) in the reactor. (Error bar: standard deviation, *n* = 3).**

2015). Maintaining the unbalanced activity results in AOB being the major group of nitrifying bacteria, with NOB being limited. Consequently, stable partial nitrification is achieved. Previous studies showed that the activity of nitrifying bacteria could be affected by high temperature, high FA/FNA, aeration duration control and low-DO concentration (Ma et al., 2016). In this study, the pH range still remained at 7.0–7.5 during the whole operation (low-DO period and high-DO period). The maximum values of FA and FNA were respectively 0.54 mg $NH_3$-N/L and 0.0038 mg $HNO_2$-N/L before partial nitrification was achieved, far below the inhibition value for NOB. The temperature of the reactor was also normal. Thus, low-DO concentration can be considered as a possible inducer for achieving partial nitrification (Peng and Zhu, 2006). In contrast, the reactor in this study still performed complete nitrification during the low-DO period, rather than partial nitrification. This phenomenon contradicted many previous research studies in which low-DO conditions successfully started up partial nitrification. However, it is similar to findings in recent research, which pointed out that complete nitrification could be maintained even under low-DO concentrations (Liu and Wang, 2013; Park and Nogura, 2008). Therefore, a low-DO concentration still had some limitations for achieving stable partial nitrification in biological nitrogen removal systems. The phenomenon might be influenced or restricted by the differences in each system, including: operation mode, concentration of substrates, or nitrifying bacteria community.

Another possible inducer of partial nitrification is intermittent aeration (Gilbert et al., 2014; Kornaros et al., 2010). Some researchers have pointed out that alternating from anoxic to aerobic conditions could inhibit NOB because of the more rapid recovery of AOB from oxygen starvation than NOB (Ge et al., 2015; Ma et al., 2016), and many wastewater treatment systems have been operated under intermittent aeration mode to implement or maintain partial nitrification (Table 2). However, partial nitrification was not observed in the intermittent aeration nitrifying reactor used in this study during the low-DO period, unlike other low-DO intermittent aeration reactors shown in Table 2 (Mota et al., 2005; Li et al., 2011, 2013). These studies used ammonium-rich wastewater such as landfill leachate or synthetic wastewater (Table 2) with a relatively higher FA concentration. This can possibly explain the conflicting observations between these research studies and this study.

Partial nitrification was reported in some intermittent aeration reactors (Regmi et al., 2014; Yang and Yang, 2011), which treated low-ammonium wastewater such as domestic wastewater or synthetic wastewater. This could be because the studies maintained a higher DO concentration in the system (Table 2), for *e.g.* Regmi et al. (2014) set a high-DO concentration (>1.5 mg/L) for their intermittently aerated

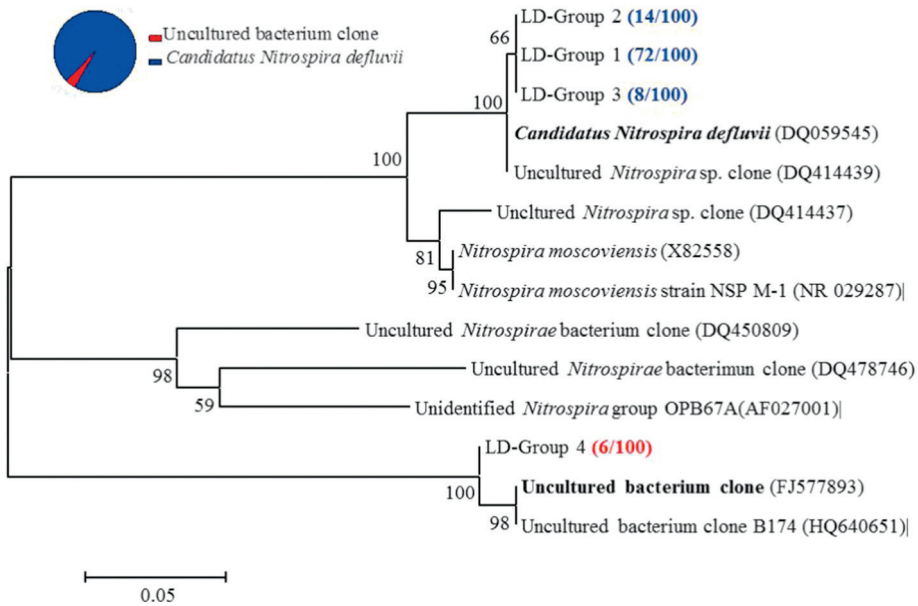


**Fig. 5 – Community of *Nitrospira* at the end of low-DO period.**

pilot-scale process ($V = 0.34\ m^3$) to maintain a sustained nitrite accumulation. Similarly, partial nitrification was gradually achieved in this study during the high-DO period. Therefore, intermittent aeration under the high-DO condition could be considered to be a factor causing the partial nitrification in the reactor.

However, the main factor for achieving partial nitrification was attributed to the sudden switching to high-DO conditions from a low-DO level. The variation of DO conditions could decrease the activity of NOB and increase the activity of AOB (Fig. 3a), and long-term operation under high-DO conditions could increase the abundance of AOB (Fig. 1). Some research found that reactors performed partial nitrification when AOB were the dominant nitrifying bacteria, even though NOB were not completely selected out (Li et al., 2011; Yang and Yang, 2011). In this study, the ratio of AOB abundance to NOB abundance increased from 3:1 to 23:1 during the high-DO period. Compared to the low-DO period, AOB were more competitive for dissolved oxygen during the high-DO period, which caused the dissolved oxygen to be preferentially used in ammonium oxidation, and the activity of NOB was inhibited. The inhibition of NOB activity due to DO competition gradually increased with increasing AOB abundance, and reached the maximum at the end of the high-DO period. The result was that overall NOB activity declined at the end of the high-DO period even though overall NOB abundance slightly increased (Fig. 4).

Moreover, a positive relationship between the variation in the dominant NOB groups (*Nitrospira* and *Nitrobacter*) and partial nitrification has also been observed. *Nitrospira* and *Nitrobacter* are two dominant types of NOB in biological nutrient removal reactors (Grady and Lim, 1980; Spieck et al., 2006; Siripong and Rittmann, 2007). The K/r hypothesis suggests that *Nitrospira* is a K-strategist that prefers low nitrite concentrations, while *Nitrobacter* is an r-strategist mostly adapted to abundant substrate (Kim and Kim, 2006; Nogueira et al., 2002). *Nitrospira* also has a competitive advantage over *Nitrobacter* for the available oxygen in oxygen-limited environments (Huang et al., 2010). Consistent with previous research, during the low-DO period, the major NOB type present in the reactor for treating domestic wastewater was *Nitrospira* (Fig. 4). The long-term low-DO operation enriched the population of *Nitrospira*, and focused mostly on *Candidatus Nitrospira defluvii*-like bacteria (Fig. 5).

At the end of the low-DO period, the dominant NOB group of the reactor was *Candidatus Nitrospira defluvii*-like bacteria. This group of *Nitrospira* differs dramatically from other known nitrite oxidizers in the key enzyme nitrite oxidoreductase (NXR) and the composition of the respiratory chain (Lucker et al., 2010). The special biological characteristics of *Candidatus Nitrospira defluvii*-like bacteria provided high activity and the ability to grow in microaerobic conditions (Lucker et al., 2010; Park and Nogura, 2008). However, it was unclear whether it could adapt to a sudden transformation to oxygen-rich conditions. In this study, after switching to high-DO operation, the number of *Nitrospira* gradually decreased from $2.64 \times 10^6$ to $8.85 \times 10^5$ cells/mL (Fig. 4), while the activity of NOB was inhibited. Based on the results of batch tests and the reactor performance, it was hypothesized that *Candidatus Nitrospira defluvii-like* bacteria preferring a low-DO environment could not adapt to the sudden change to high-DO concentration. High-DO affects its special biological nitrifying system to inhibit its activity and growth. This would lead to partial nitrification when the *Candidatus Nitrospira defluvii*-rich reactor switched to the high-DO environment, as in the case of this study. However, further investigation is necessary to verify this hypothesis, especially on the identification of key enzymes that are influenced by the high-DO condition.

However, the sudden increase of *Nitrobacter* that occurred at the end of the high-DO period should also not be ignored. Because *Nitrobacter* is an r-strategist mostly adapted to abundant substrate (Kim and Kim, 2006; Nogueira et al., 2002), the high-DO concentration and the high level of nitrite accumulation at the end phase of the high-DO period were the main reasons for the increase in *Nitrobacter*. Although the

**Table 2 – Features of intermittent aeration reactors.**

| Reactor | Wastewater | Influent $NH_4^+$-N (mg/L) | Intermittent mode (aeration: non-aeration) | DO level | Nitrification | Reference |
|---|---|---|---|---|---|---|
| IA-reactor | Digested swine wastewater | 197 ± 111 | 0.5–1 hr: 1–4 hr | 1.4–5.0 mg/L | Partial nitrification (the 4 hr non-aeration reactor) Complete nitrification (the other reactors) | Cesar Mota et. al |
| SBR | Synthetic wastewater | 300 | 30 min: 10 min for maintain | 4,5 mg/L for start-up 0.2 mg/L for maintain | Partial nitrification | Jianping Li et. al |
| SBR | Landfill leachate | 688–1748 | 1.5 min: 0.7–4 min | 0.5–5.0 mg/L | Partial nitrification | Huosheng Li et. al |
| MBMBR | Synthetic wastewater | 42.2–57.5 | 2 min: 2 min/4 min | SADm*: 0.75 $m^3/m^2/hr$ | Partial nitrification | Shuai Yang et. al |
| AVN CSTR | Domestic wastewater | 29.7 ± 3.9 | 4 min: 8 min or 8 min: 4 min | >1.5 mg/L | Partial nitrification | Regmi et al |
| SBR | Domestic wastewater | 50.2–80.4 | 10 min: 10 min | Period I: 0.3 ± 0.14 mg/L Period II: 1.82 ± 0.32 mg/L | Period I: complete nitrification Period I: partial nitrification | This study |

SADm*: the specific aeration demand per membrane area; SBR: sequencing batch reactor; MBMBR: moving bed membrane bioreactor; AVN CSTR: AOB *versus* NOB continuously stirred tank reactor.

reactor still performed partial nitrification because the activity of *Nitrobacter* was limited by its relatively low abundance and the inhibition due to DO competition from AOB, the continued growth of *Nitrobacter* would probably result in a recovery of NOB activity, and then destroy the partial nitrification. This is a new problem that should be further considered: how to maintain the stable partial nitrification in this reactor after a successful start-up? Therefore, two methods will be tested in our further investigation: 1. Adjust the SRT of the reactor to select out the NOB group (specifically *Nitrobacter*); 2. Add highly active anammox bacteria to compete with *Nitrobacter* for $NO_2^-$-N, which can inhibit the activity of NOB as well as remove the nitrogen effectively.

## 4. Conclusions

The conclusions of this study were: (1) complete nitrification could be achieved during the low-DO period (DO: 0.3 ± 0.14 mg/L) in the intermittent aeration nitrifying reactor for treating organic-removed domestic wastewater. (2) Partial nitrification was successfully achieved in the reactor with high-DO (1.8 ± 0.32 mg/L) intermittent aeration. Inhibition of the nitrite oxidation due to the sudden transformation from low-DO to high-DO in this reactor was the major inducer of this phenomenon. (3) Real-time PCR and Gene library analysis showed that the majority of NOB were *Nitrospira*, most of which belong to *Candidatus Nitrospira defluvii*-like bacteria. This special NOB community might be the determining factor in achieving partial nitrification by switching from low-DO to high-DO conditions.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2016.09.004.

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