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The effect of dissolved oxygen concentration on long-term stability of partial nitrification process

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ABSTRACT

Dissolved oxygen (DO) concentration is regarded as one of the crucial factors to influence partial nitrification process. However, achieving and keeping stable partial nitrification under different DO concentrations were widely reported. The mechanism of DO concentration influencing partial nitrification is still unclear. Therefore, in this study two same sequencing batch reactors (SBRs) cultivated same seeding sludge were built up with real-time control strategy. Different DO concentrations were controlled in SBRs to explore the effect of DO concentration on the long-term stability of partial nitrification process at room temperature. It was discovered that ammonium oxidation rate (AOR) was inhibited when DO concentration decreased from 2.5 to 0.5 mg/L. The abundance of *Nitrospira* increased from $10^{11.5}$ to $10^{13.7}$ copies/g DNA, and its relative percentage increased from 0.056% to 3.2% during 190 operational cycles, causing partial nitrification gradually turning into complete nitrification process. However, when DO was 2.5 mg/L the abundance of *Nitrospira* was stable and AOB was always kept at $10^{10.7}$ copies/g DNA. High AOR was maintained, and stable partial nitrification process was kept. Ammonia oxidizing bacteria (AOB) activity was significantly higher than nitrite oxidizing bacteria (NOB) activity at DO of 2.5 mg/L, which was crucial to maintain excellent nitrite accumulation performance.

Introduction

Anammox technology becomes the hot topic all over the world with the advantages of high efficiency, no need of oxygen and additional carbon source and low sludge output (Chen et al., 2018; Ding et al., 2018; Kang et al., 2018). The metabolism of anammox bacteria simultaneously consumes ammonium and nitrite, causing stable nitrite supplying to be the premise of application anammox technology to wastewater treatment. Partial nitrification process has been widely investigated since decade years ago. Selective enrichment of AOB and inhibition the growth of NOB is crucial to achieve and maintain partial nitrification. Therefore various effective strategies have been conducted to achieve partial nitrification, including high temperature (Kong et al., 2013), high FA concentration (Ma et al., 2017; Qian et al., 2017), high FNA concentration (Ma et al., 2017; Miao et al., 2018), intermittent aeration (Miao et al., 2018), aerobic starving treatment (Liu et al., 2017) and low dissolved oxygen concentration (Jianlong and Ning, 2004). Among these strategies, controlling low DO concentration can further save aeration consumption. Thus, low-DO condition has been widely studied to achieve partial nitrification in wastewater treatment systems. Stable

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nitrite accumulation was kept in a continuous-flow reactor with DO of 0.4 mg/L and influent nitrogen of 1000 mg/L (Blackburne et al., 2008). When ammonium nitrogen was 200 mg/L in influent, nitrite accumulation concentration even reached to 177 mg/L with low DO concentration (Aslan et al., 2009). For above reports on achieving partial nitrification with low DO concentration, the effect of FA inhibition could not be neglected. FA inhibition is an effective strategy to control NOB activity, while it is not feasible to main-stream wastewater treatment in engineering application. Maintaining long-term and stable partial nitrification performance is also a pronounced challenge after achieving partial nitrification process, which will promote the application of anammox technology to main-stream wastewater treatment.

Some research showed that partial nitrification might not be stable at low DO condition for domestic wastewater treatment. A recent study discovered that under long-term low DO condition, NOB became a better oxygen competitor than AOB and as a result, no nitrite accumulated (Liu and Wang, 2013). In SBR partial nitrification process gradually turned into complete nitrification process at DO concentration of 0.5 mg/L (Zhou et al., 2018). Moreover, stable complete nitrification was observed during low DO period, but partial nitrification was gradually achieved when switching to the high-DO period (Bao et al., 2017). However, some study showed that stable partial nitrification process was achieved with DO concentration of 3.0~4.0 mg/L for domestic wastewater treatment, and it was kept stable at 25~15°C for more than 140 days (Guo et al., 2010). Partial nitrification process even kept stable with DO concentration of 2.0 mg/L, when temperature dropped to 11~16°C (Gu et al., 2012). Therefore, the effect of DO concentrations on the stability of partial nitrification process is also another noteworthy issue. Up to now much work has been conducted to achieve nitrite accumulation during nitrification by controlling different DO concentrations. However, these results might be incomparable, because so many different factors existed including operational condition, wastewater quality and reactor structure.

Therefore, in this study two same partial nitrification processes operated at different DO concentrations were built up to treat domestic wastewater. The objectives of this study are: (1) to reveal the effect of DO concentration on the long-term stability of partial nitrification process; (2) to analyze the mechanisms of partial nitrification process turning into complete nitrification process; (3) to explore the cause of partial nitrification keeping stable.

1. Materials and methods

1.1. *Reactors setup and operation*

In this study, two same lab-scale SBRs with a working volume of 22 L were operated from 18.1 to 28.8°C. By adjusting the rotor flow meter DO concentrations of SBR1 and SBR2 were controlled at 0.5 and 2.5 mg/L, respectively. On-line DO and pH probes (3420, WTW, Germany) were used to control constant DO concentration and monitor the variations of pH value. For SBR1 and SBR2, each cycle was consisted of feeding, aeration, anoxic phase (adding methanol in the first min of anoxic period), decanting and idling. According to the pH curve, aerobic and anoxic period were terminated when the "ammonia valley" point appeared and the value of pH began to decrease, respectively (Yang et al., 2007). In each cycle 14 L clarified supernatant was decanted after settling. Over the entire study, the MLSS was kept at 3000–3500 mg/L in both two SBRs with SRT of 40-45 days.

1.2. *Characteristics of domestic wastewater and seeding sludge*

The domestic wastewater was taken from a septic tank. The concentrations of COD, NH_4^+-N, NO_2^--N and NO_3^--N in the domestic wastewater were 128.7 ± 36.8 mg/L, 55.59 ± 11.1 mg/L, 0.42 ± 0.33 mg/L, 0.35 ± 0.58 mg/L, respectively. The seeding sludge of two SBRs was taken from a pilot-scale SBR with a working volume of 7 m^3, which was fed with the same domestic wastewater and operated at DO > 2.5 mg/L by programmable logic controller controlling. Partial nitrification performance has been stabilizing for more than two years in the pilot-scale SBR with the same operation sequence to the lab-scale SBR.

1.3. *Analytical methods*

Wastewater samples were collected during each operation cycle of two SBRs. The concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were measured with a Lachat Quik Chem 8500 Flow Injection Analyzer (QC8500, Lachat Instruments, USA). COD was analyzed using a COD quick-analysis apparatus (5B-1, Lian-hua Tech. Cooperation, China). MLSS was measured according to the Standard Methods (APHA, 2017). DO, pH and temperature were measured by DO and pH probes (3420, WTW, Germany).

1.4. *Half-saturation constants for substrate*

Half-saturation constant testing was conducted in batch-test reactor. Before batch test, 500 mL activated sludge collected from SBR was washed by distilled water three times to eliminate the influence of remaining substrate from SBR. Reaction mediums containing 25 mg/L NH_4^+-N or NO_2^--N with major element and tract element were prepared. Constant DO at the concentrations of 0.2, 0.4, 0.8, 1.2, 2.0 mg/L was controlled by adjusting the flow of nitrogen gas and air. DO concentrations were monitored on-line by DO probe (3420, WTW, Germany). Samples were collected at intervals to measure the reaction rates of AOB and NOB. After each test MLSS was measured. Triplicate batch tests were carried out at each DO concentration for NH_4^+-N and NO_2^--N. The recorded results were analyzed and fitted to a modeled Monod curve to calculate oxygen half-saturation constants.

1.5. *DNA extraction, PCR, and high-throughput sequencing*

Five sludge samples were collected from SBR1 and SBR2 at different periods, and then were freeze-dried in Labconco 183 Freezone (Freeze one, Labconco, USA). Genomic DNA was extracted from about 0.2 g of freeze-dried sludge using the Fast DNA SPIN Kit for Soil (QBIOgene, Carlsbad CA, USA). The concentrations of extracted DNA were measured with Nanodrop ultraviolet-visible spectrophotometry (NANODROP ONE, Thermo, USA). Bacterial 16S rRNA genes were PCR-amplified with primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′) for the V3~V4 region. The genes sequencing were carried out on Illumina Miseq PE300 platform. Low quality sequences were removed, and high quality sequences were processed to generate OTUs at 97% sequence similarity threshold by the Mothur software program. Taxonomy was assigned via the RDP classifier with the Silva databases.

1.6. Real-time Quantitative PCR

Real-time Quantitative PCR was performed in a Stratagene Mx3005P thermocycler (Mx3005P Agilent Technologies, USA). Six coupled primers were used to quantify the copy numbers of ComAOB, AOA, AOB, NOB-*Nitrobacteria*, NOS-*Nitrospira*, and AnAOB. The reactions were carried out in a volume of 20 μL mixture that were consisted of 10 μL SYBR Premix Ex TaqTM II (RR820A Takara, China), 0.8 uL of each primers (10 mmol/L), 2 μL DNA templates and 6.4 μL sterile water. The annealing temperatures were also listed in Appendix A Table S1. The plasmids that contained target genes were taken in 10-fold serial dilution to conduct standard curves. All the standard DNA and samples were run in triplicate. The amplification curves with correlation coefficients above 0.95 and amplification efficiencies in range of 90%–110% were employed.

1.7. Calculations

NAR and TINRE were calculated using Eqs. (1) and (2):

$$NAR = NO_2^- - N_{eff} / (NO_3^- - N_{eff} + NO_2^- - N_{eff}) \times 100\% \quad (1)$$

$$TINRE = (NH_4^+ - N_{inf} + NO_3^- - N_{inf} + NO_2^- - N_{inf} - NH_4^+ - N_{eff} - NO_3^- - N_{eff} - NO_2^- - N_{eff}) / (NH_4^+ - N_{inf} + NO_3^- - N_{inf} + NO_2^- - N_{inf}) \times 100\% \quad (2)$$

The $NH_4^+-N_{-inf}$, $NO_3^- - N_{inf}$, $NO_2^- - N_{inf}$ is the influent NH_4^+-N, $NO_3^- - N$, $NO_2^- - N$ concentrations (mg/L) of SBR; $NH_4^+-N_{-eff}$, $NO_3^- - N_{eff}$, $NO_2^- - N_{eff}$ is the effluent NH_4^+-N, $NO_3^- - N$, $NO_2^- - N$ concentrations (mg/L) of SBR.

2. Results

2.1. Performance of partial nitrification process with DO of 0.5 mg/L

Fig. 1 presented the variations of NH_4^+-N, $NO_2^- - N$, $NO_3^- - N$, NAR, TINRE and Temperature of SBR1. Nitrite accumulation performances varied during the 190 operation cycles with DO of 0.5 mg/L. According to the variations of NAR, 190 cycles could be divided into four phases. In the first 30 cycles (phase 1: stable partial nitrification) the NAR was higher than 90%, and no $NO_3^- - N$ was detected in effluent. Aeration duration was extended with the increasing NH_4^+-N concentration in influent. From the 31st to 70th cycle (phase 2: decreasing partial nitrification), the NAR gradually decreased from 92% to 80%. Correspondingly, 1.0-3.2 mg/L $NO_3^- - N$ was detected, and 0.7-8.5 mg/L NH_4^+-N was remained in effluent with aeration time of 188~287 min. From the 71st to 130th cycle (phase 3: destructive partial nitrification), the NAR sharply declined from 80% to 5%. Accumulated $NO_2^- - N$ concentration was only 0.7~5.4 mg/L, and $NO_3^- - N$ concentration increased to 21.2 mg/L on the 130th cycle. Stable partial nitrification was completely destroyed in this phase. After 130 cycles (phase 4: complete nitrification) the NAR was lower than 10% in SBR1, indicating the conversion of partial nitrification into complete nitrification at DO of 0.5 mg/L. However, the ammonium oxidation

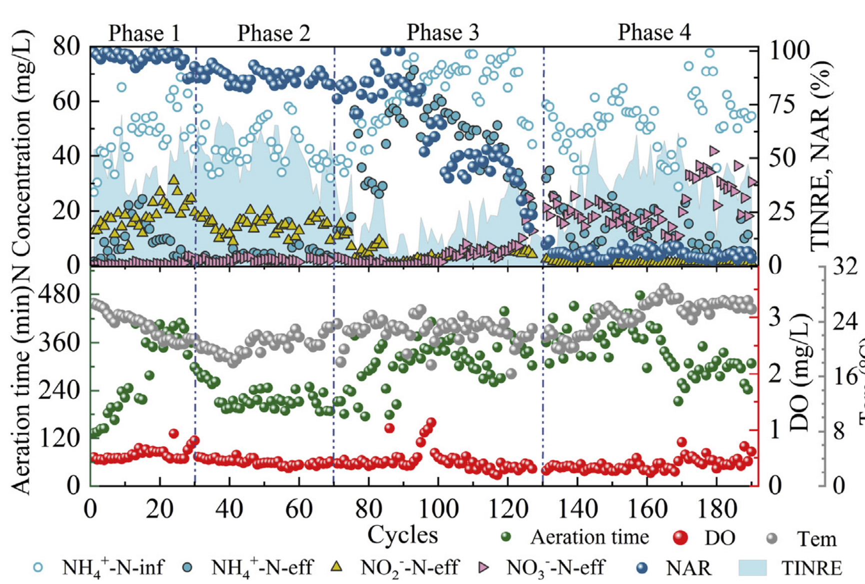

Fig. 1 – Variations of NH_4^+-N, $NO_2^- - N$, $NO_3^- - N$, NAR, TINRE and Temperature of SBR1 for long time.

ability slowly revived, which led to shortening aeration duration after 170 cycles. Apart from changing nitrite accumulation performance, nitrogen removal effect also varied in the 190 cycles. In phase 3 TINRE was only 15%, while it reached higher than 40% in other phases.

Full-scale performances of typical cycles at different phases were shown in Fig. 2. The rates of NH_4^+–N oxidation, NO_2^-–N generation and NO_3^-–N generation were linearly fitted after COD being degraded. The fit equations were also shown in Fig. 2, and the slopes represented the rates. On the 3rd cycle, nitrogen removal concentration reached to 18.0 mg/L with aeration time of 135 min. In the first 30 min biodegradable COD was quickly depleted, and 12.8 mg/L nitrogen was removed. According to the slope of linear fitting, AOR was obviously higher than NiGR from the 30th min to 135th min and no nitrate was detected. After 30 min 5.2 mg/L nitrogen was removed without COD consumption. On the 45th cycle, AOR decreased to 0.119 mg N/(L·min), and aeration time being extended to 212 min. NO_3^--N concentration reached to 3.2 mg/L at aeration end-point. On the 102nd cycle, ammonium oxidation activity was seriously inhibited, which declined to 0.0431 mg N/(L·min), causing only 16.5 mg/L ammonium being utilized during 360 min. It was clear that linear growth tendency of NO_3^--N concentration was presented at this phase. On the 145th cycle, almost no nitrite accumulation was detected in effluent. In contrast, AOR and NaGR increased to 0.105 and 0.059 mg N/(L·min), respectively. At this operation cycle, 37.2 mg/L nitrogen was consumed in 360 min. Furthermore, nitrogen removal concentration increased to 8.91 mg/L after COD being depleted.

When DO concentration was 0.5 mg/L, AOB activity was first suppressed, and improving NOB activity caused effluent NO_3^-–N increasing. Then AOB rate gradually revived, leading to decreasing effluent NH_4^+–N concentration. This was similar to previous study that the ammonium oxidation rate greatly decreased, once DO concentration declined from a high level (4-6 mg/L) to a low level (0.2-0.3 mg/L). However, NOB community was less sensitive to the low DO concentrations (Liu and Wang, 2013). When AOB gradually adapted to the low DO concentration, high AOR and NaGR were both achieved that led to the complete nitrification process.

2.2. *Performance of partial nitrification process with DO of 2.5 mg/L*

SBR2 was operated at DO concentration of 2.5 mg/L. Activated sludge was regularly discharged during the 150 cycles. Stable partial nitrification performance was maintained in SBR2 with temperature of 18.3~27.8°C (Fig. 3). NAR was always kept higher than 90%, and almost no NO_3^-–N was detected in the end-point of aeration process. During the 150 cycles less than 5 mg/L NH_4^+–N was remained in effluent with aeration duration of 150–270 min. In addition, averaged TINRE reached to 53%, which was obviously higher than that of low DO condition.

The full-scale performances of typical operation cycles in SBR2 were showed in Fig. 4. On the 3rd cycle effluent NO_2^-–N reached to 24.1 mg/L and NO_3^-–N was undetectable with temperature of 27°C. In the first 30 min biodegradable COD was depleted, and 10.2 mg/L nitrogen was removed via denitrification pathway. Hereafter, AOR reached to 0.196 mg N/(L·min), which was a bit higher than NiGR. On the 147th cycle due to the decline of temperature, AOR and NiGR decreased from 0.196 to 0.128 mg N/(L·min), and 0.158 to 0.106 mg N/(L·min), respectively. Aeration duration was 260 min with influent NH_4^+–N of 45.1 mg/L. 15.2 mg/L nitrogen was removed in aeration process.

2.3. *Microbial community structure and abundance*

Microbial community structures under different DO concentrations were also measured. Sludge samples were collected

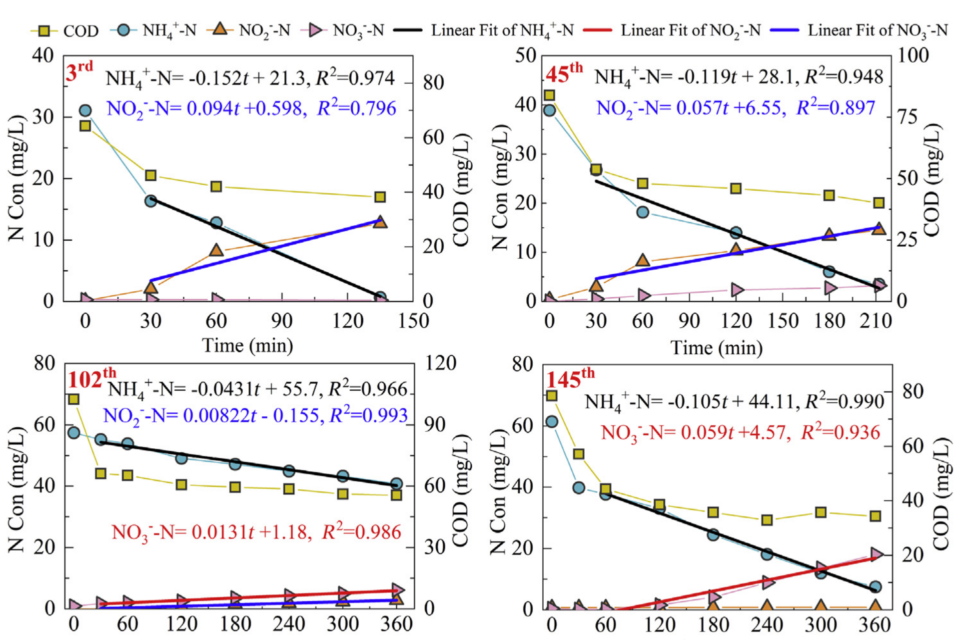

Fig. 2 – Variations of COD, NH_4^+–N, NO_2^-–N, NO_3^-–N concentration and the linear fit of NH_4^+–N, NO_2^-–N and NO_3^-–N in typical cycles for SBR1.

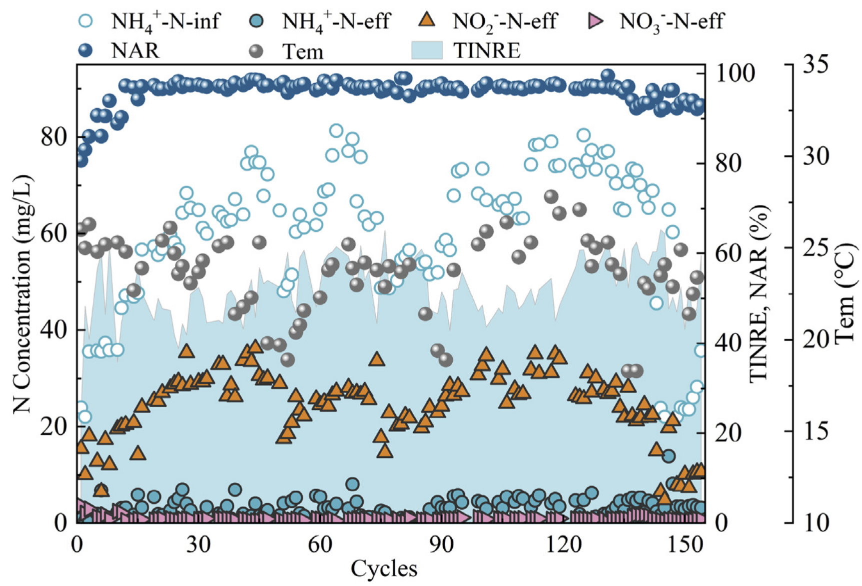

Fig. 3 – Variations of NH_4^+–N, NO_2^-–N, NO_3^-–N, NAR, TINRE and Temperature of SBR2 for long time.

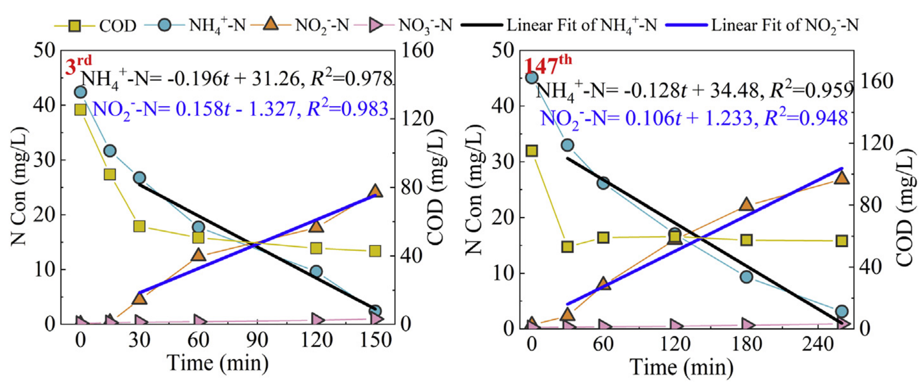

Fig. 4 – Variations of COD, NH_4^+–N, NO_2^-–N, NO_3^-–N concentration and the linear fit of NH_4^+-N and NO_2^--N in typical cycles for SBR2.

from SBR1 on the 45th cycle, 102nd cycle and 145th cycle, and SBR2 on the 120th cycle. Furthermore, seeding sludge was also analyzed. As shown in Fig. 5, *Thauera* was always the dominant genus at different phases of two systems. For the compositions of AOB and NOB, the preponderant genus was *Nitrosomonas* and *Nitrospira*, respectively. In SBR1 *Nitrosomonas* percentage declined from 0.82% to 0.23% after 145 cycles. For the genus *Nitrospira*, its proportion kept increasing. From the first cycle to the 102nd cycle effluent NO_3^-–N concentration increased from 0.2 to 7.2 mg/L. Correspondingly, *Nitrospira* proportion increased from 0.056% to 0.30%. Hereafter, *Nitrospira* ratio quickly increased, which was significantly higher than *Nitrosomonas* ratio on the 145th cycle. However, there was no obvious variation of microbial community structure being detected in SBR2 after 120 cycles. The relative percentage of *Nitrospira* was distinctly lower than that of *Nitrosomonas* at different periods, which maintained the stable partial nitrification behavior in SBR2. Pearson correlation analysis for two SBRs showed the significant correlations between NAR and *Nitrosomonas* percentage ($P < 0.05$), between effluent NO_3^--N concentration and *Nitrospira* percentage ($P < 0.01$) (Appendix A Table S2). *Nitrosomonas* decreasing led to the low NH_4^+–N oxidation activity, and *Nitrospira* increasing could consume more NO_2^-–N, which caused the higher effluent NO_3^-–N concentration.

The presence of some functional bacteria with low percentage might not be resolved from 16s rRNA gene amplification sequencing alone (Roots et al., 2019). Thus, QPCR was used to quantify AOA (targeting *amoA*), AOB (*amoA*), *Nitrospira* (16S rRNA), ComAOB (*amoA*), and AnAOB (*hzsB*). As shown in Fig. 6, the abundances of AOB and *Nitrospira* in SBR2 kept stable during the 150 operation cycles, and the abundance of AnAOB was always higher than 10^9 copies/g DNA with sludge discharging.

Obviously, the abundance of *Nitrospira* increased from $10^{11.5}$ to $10^{13.7}$ copies/g DNA during 145 cycles of SBR1, which was coincided with the growth tendency resulting from 16S rRNA sequencing. For AOB, due to the suppression of low DO condition, the abundance of AOB first declined from $10^{10.7}$ copies/g DNA to $10^{10.4}$ copies/g DNA in early phase and then gradually revived to $10^{10.7}$ copies/g DNA. In addition, AOA also survived, while its abundance was significantly lower than

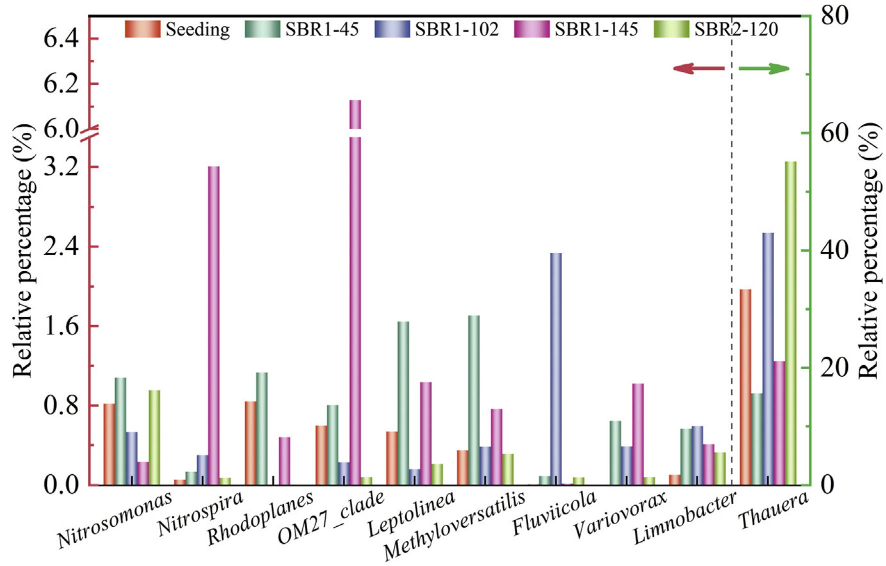

Fig. 5 – Compositions of dominant bacteria in SBR1 and SBR2 at different phases.

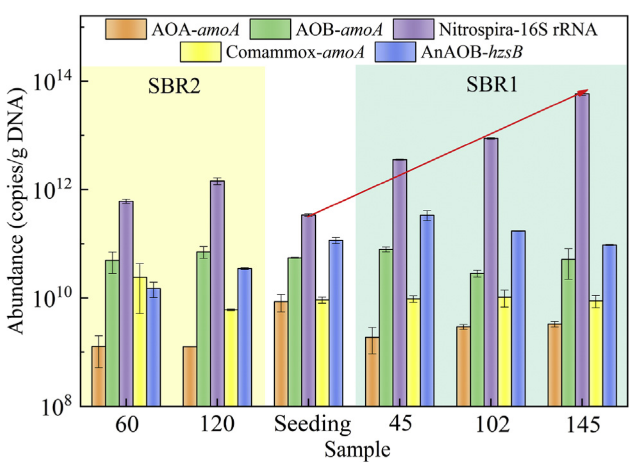

Fig. 6 – Variations of microbial abundance in SBR1 and SBR2 at different phases.

other bacteria. ComAOB was also detected, which can directly nitrify ammonium into nitrate (Daims et al., 2015). The abundance of ComAOB was constant during long-term operation regardless DO concentration, which suggested the growth of ComAOB in SBRs, but it was not the key bacteria to destroy partial nitrification process. Moreover, the abundance of AnAOB reached to 10^{11} copies/g DNA in SBR1, which was obviously higher than that of SBR2.

3. Discussion

3.1. *Mechanisms of keeping stable partial nitrification*

When DO concentration was controlled at 2.5 mg/L, nitrite accumulation was stable for long time in SBR2. The relative percentage of *Nitrosomonas* was distinctly higher than that of *Nitrospira* in the 150 cycles. For the results of QPCR, although the abundance of *Nitrospira* was higher than that of AOB, which was inconsistent to the relative abundance of *Nitrospira* and *Nitrosomonas* resulting from 16S rRNA sequencing, stable nitrite accumulation performance was maintained. It was likely because that the targeting gene segment of *Nitrospira* was 16S rRNA gene, while the functional gene *amoA* of AOB was quantified. Previous study also presented the higher abundance of *Nitrospira* than AOB via QPCR in partial nitrification system (Miao et al., 2017; Yang et al., 2018). Therefore, keeping stable microbial community structure was crucial to remain steady partial nitrification performance.

AOB:

$$NH_4^+ + 1.382O_2 + 0.09HCO_3^- \rightarrow 0.982NO_2^- + 1.036H_2O + 0.018C_5H_7O_2N + 1.892H^+$$

NOB:

$$NO_2^- + 0.003NH_4^+ + 0.485O_2 + 0.015HCO_3^- + 0.012H^+ \rightarrow NO_3^- + 0.009H_2O + 0.003C_5H_7O_2N$$

According to the stoichiometric reaction equations, the metabolism of AOB requires more oxygen than NOB. Oxygen half-saturation constant (K_{O2}) was often used by many researchers to evaluate the requirements of oxygen for AOB and NOB metabolism (Blackburne et al., 2008; Kaelin et al., 2009; Regmi et al., 2014; Wett et al., 2013). Oxygen half-saturation constant for AOB ($K_{O2,AOB}$) and NOB ($K_{O2,NOB}$) were evaluated by batch tests for two SBRs on the 120th cycle. The Monod curves were given in Fig. 7. AOB rate was always higher than NOB rate at any DO concentration in SBR2. The activity discrepancy between AOB and NOB was much larger at high DO concentration, indicating the excellent nitrite accumulation performance with higher DO. For the cause of long-term stability of partial nitrification effect,

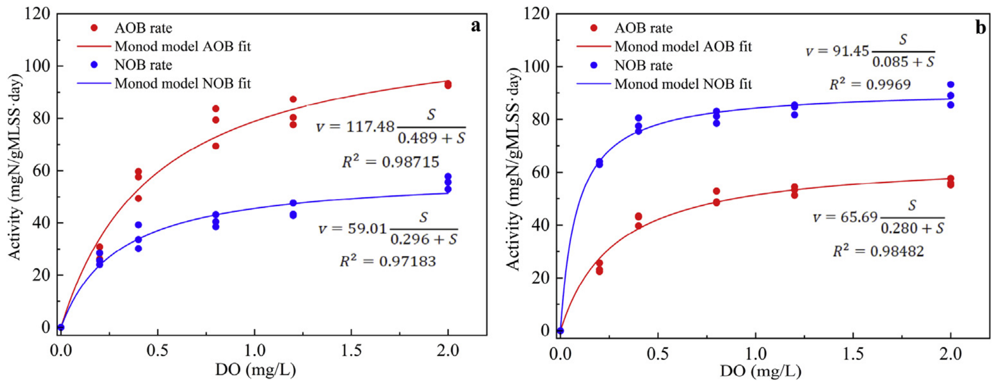

Fig. 7 – Dissolved oxygen Monod curves for AOB and NOB in high DO condition and in low DO condition (a: SBR2, b: SBR1).

it was mainly attributed to the higher AOB rate and the lower abundance of *Nitrospira*. The microbial growth curve includes adjustment period, logarithmic growth period, stationary period and decay period. The growth of AOB might retain in logarithmic growth period or stationary period, while *Nitrospira* might retain in the adjustment period or the initial logarithmic growth period in each operation cycle. Slow growth of *Nitrospira* was kept to resist the discharging of activated sludge, maintaining a constant abundance in SBR2. On the other hand, aeration duration was accurately controlled via on-line pH curve, and the higher AOB rate led to the shorter aeration duration, which was beneficial to inhibit *Nitrospira* enriching. Therefore, long-term and stable partial nitrification process was maintained with DO of 2.5 mg/L.

3.2. *Mechanisms of partial nitrification destruction*

After seeding stable partial nitrification sludge, satisfactory nitrite accumulation behavior was kept in SBR1 during the first 70 cycles. It was mainly due to the delay of microbial response to the changing growth condition, and low abundance of *Nitrospira* in seeding sludge. Because the more oxygen was required by AOB than NOB, after 70 cycles, AOB activity and abundance both declined, causing aeration time to be prolonged. Longer aeration duration was favorable to the growth of NOB. Because *Nitrospira* sp. are K-strategists with higher oxygen affinity than *Nitrobacter* sp. (Wang et al., 2016), and genus *Nitrospira* tended to enrich in the condition of low nitrite concentration (Dong-Jin Kim, 2006), the abundance of *Nitrospira* kept increasing. Moreover, previous studies also found that the enrichment of *Nitrospira* was fast under low DO case (Liu and Wang, 2013; Park and Noguera, 2008). Consequently, NiGR gradually declined and NaGR began to increase. On the 120th cycle, AOB and NOB rate were displayed in Fig. 7b. Obviously, NOB rate was always higher than AOB rate at any DO concentration. Partial nitrification process might not revive anymore by adjusting DO concentration in SBR1. Therefore, the enrichment of *Nitrospira* and the declining of AOB rate at low DO condition led to the complete nitrification process. Keeping the largest discrepancy between AOB rate and NOB rate was crucial to achieve nitrite accumulation.

3.3. *The pathways of nitrogen removal under different DO conditions*

Obvious nitrogen removal phenomena were found at both DO conditions. *Thauera* was prevalent in two SBRs, which was the ubiquitous heterotrophic denitrifier in wastewater treatment plant (Lu et al., 2014; Trine Rolighed Thomsen, 2007), indicating that heterotrophic denitrification was the main nitrogen removal pathway. Nevertheless, nitrogen loss also occurred after biodegradable COD being depleted. Recently, it is discovered that AnAOB distribute widely in ecosystem. Partial nitrification process is more beneficial for AnAOB growing. AnAOB was detected in both SBRs, and it displayed a first increasing and then decreasing tendency in SBR1. Therefore, anammox should also contribute to nitrogen removal. In the early stage of SBR1, AOB kept a high activity and NOB maintained a low abundance, and AnAOB was mainly out-competed by AOB. Then nitrogen removal concentration improved to 9.0 mg/L via anammox with AOR decreasing. When it came to complete nitrification phase, AnAOB competed with AOB for NH_4^+–N, and simultaneously competed with NOB for NO_2^-–N (Laureni et al., 2016). The improving of NOB reaction activity not only reduced NO_2^-–N accumulation concentration, but also reduced nitrogen removal ability in SBR1. In SBR2, the competition between AnAOB and AOB was dominant. However, due to the inhibition of high DO, the copy number of AnAOB was obviously higher in SBR1 than SBR2, causing that more nitrogen was removed via anammox pathway in SBR1. Thus, it is potential to improve nitrogen removal via anammox pathway in complete nitrification reactor by promoting the competition among various microorganisms.

4. Conclusions

The effect of DO concentration on the stability of partial nitrification process was studied in two same SBRs at room temperature. (1) AOB rate declined and *Nitrospira* abundance increased at low DO condition, resulting in partial nitrification converting into complete nitrification process. (2) High AOB rate and stable microbial community structure led to the activity discrepancy between AOB and NOB at high DO condition, keeping stable partial nitrification process. (3) Anammox and heterotrophic denitrification pathways contributed to nitrogen removal in both two SBRs.

Conflict of interest

There is no known competing financial intrests or personal relationships.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2019.12.012.

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