

## Characteristics of N<sub>2</sub>O production and hydroxylamine variation in short-cut nitrification SBR process

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### ABSTRACT

In order to study the characteristics of nitrous oxide (N<sub>2</sub>O) production and hydroxylamine (NH<sub>2</sub>OH) variation under oxic conditions, concentrations of NH<sub>2</sub>OH and N<sub>2</sub>O were simultaneously monitored in a short-cut nitrification sequencing batch reactor (SBR) operated with different influent ammonia concentrations. In the short-cut nitrification process, N<sub>2</sub>O production was increased with the increasing of ammonia concentration in influent. The maximum concentrations of dissolved N<sub>2</sub>O-N in the reactor were 0.11 mg/L and 0.52 mg/L when ammonia concentrations in the influent were 50 mg/L and 70 mg/L respectively. Under the low and medium ammonia load phases, the concentrations of NH<sub>2</sub>OH-N in the reactor were remained at a low level which fluctuated around 0.06 mg/L in a small range, and did not change with the variation of influent NH<sub>4</sub><sup>+</sup>-N concentration. Based on the determination results, the half-saturation of NH<sub>2</sub>OH in the biochemical conversion process of NH<sub>2</sub>OH to NO<sub>2</sub><sup>-</sup>-N was very small, and the value of 0.05 mg NH<sub>2</sub>OH-N/L proposed in the published literature was accurate. NH<sub>2</sub>OH is an important intermediate in the nitrification process, and the direct determination of NH<sub>2</sub>OH in the nitrification process was beneficial for revealing the kinetic process of NH<sub>2</sub>OH production and consumption as well as the effects of NH<sub>2</sub>OH on N<sub>2</sub>O production in the nitrification process.

**Key words** | hydroxylamine, nitrous oxide, SBR process, short-cut nitrification

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### INTRODUCTION

Nitrous oxide (N<sub>2</sub>O) is a potent greenhouse gas, and has a large negative effect on the environment (IPCC 2013). According to the report published by the United Nations Environment Programme, 0.2 Tg N<sub>2</sub>O-N were emitted from wastewater to the atmosphere every year (UNEP 2013). The biological wastewater treatment process has been identified as an anthropogenic source responsible for the increase of N<sub>2</sub>O in the atmosphere (UNEP 2013). It is generally accepted that N<sub>2</sub>O can be produced during both

aerobic nitrification and anoxic denitrification in biological nutrient removal processes (Ding *et al.* 2016). In heterotrophic denitrification, the mechanisms of N<sub>2</sub>O production and loss are quite clear and uncontested. N<sub>2</sub>O is produced in nitric oxide (NO) reduction as an intermediate, and finally is reduced to N<sub>2</sub> by heterotrophic denitrifiers. However, mechanisms of N<sub>2</sub>O production in nitrification are quite complicated. In nitrification, ammonia (NH<sub>3</sub>) is converted to nitrate (NO<sub>3</sub><sup>-</sup>) via hydroxylamine (NH<sub>2</sub>OH) and

nitrite (NO<sub>2</sub><sup>-</sup>). Two types of bacteria are responsible for this conversion process, ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). N<sub>2</sub>O production and emission can be stimulated by the increase of NO<sub>2</sub><sup>-</sup> (Wang *et al.* 2016a), and decreased NO<sub>2</sub><sup>-</sup> accumulation can reduce the N<sub>2</sub>O generation (Chen *et al.* 2014). AOB have been identified as the main contributor for N<sub>2</sub>O production in nitrification process (Chen *et al.* 2014).

N<sub>2</sub>O can be produced by chemical breakdown of the unstable nitrosyl radical (NOH) during the oxidation of NH<sub>2</sub>OH (Poughon *et al.* 2001). Meanwhile, reduction of NO produced from the oxidation of NH<sub>2</sub>OH can also produce N<sub>2</sub>O (Stein 2011). Moreover, the electrons released from the oxidation of NH<sub>2</sub>OH were used to sustain ammonium oxidation and satisfy the cell's reductant needs including reduction of CO<sub>2</sub> and generation of a proton gradient using oxygen (O<sub>2</sub>) as terminal electron acceptor (Arp & Stein 2003). Generally speaking, NH<sub>2</sub>OH is an important intermediate formed in the oxidation of ammonia/ammonium (NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>) to nitrite (NO<sub>2</sub><sup>-</sup>) by AOB (Vajrala *et al.* 2013), and it has a direct relationship with N<sub>2</sub>O production in biological wastewater treatment processes (Kim *et al.* 2010).

However, due to short-lived and extremely reactive properties of NH<sub>2</sub>OH (Liu *et al.* 2014), determination of NH<sub>2</sub>OH

in aqueous solution is not an easy task. As a result, experimental studies of effects of NH<sub>2</sub>OH on N<sub>2</sub>O production during nitrification process are scarce. Furthermore, the lack of the quantitative relation between NH<sub>2</sub>OH and N<sub>2</sub>O production caused great difficulties in understanding the mechanism of N<sub>2</sub>O production in the nitrification process.

In this study, a short-cut nitrification process with different influent ammonia concentrations was operated for more than 6 months, and concentrations of NH<sub>2</sub>OH and N<sub>2</sub>O were monitored simultaneously for understanding the characteristics of N<sub>2</sub>O production and NH<sub>2</sub>OH variation. Also through the relationships between NH<sub>4</sub><sup>+</sup>, NH<sub>2</sub>OH and NO<sub>2</sub><sup>-</sup>, the half-saturation constant of NH<sub>2</sub>OH oxidation in the nitrification process is discussed and further understandings of the effects of NH<sub>2</sub>OH on N<sub>2</sub>O production can be obtained.

## MATERIAL AND METHODS

### The short-cut nitrification SBR

A laboratory-scale sequencing batch reactor (SBR) was made from transparent plexiglas with a working volume of 10 L. The schematic diagram of the reactor is shown in Figure 1.

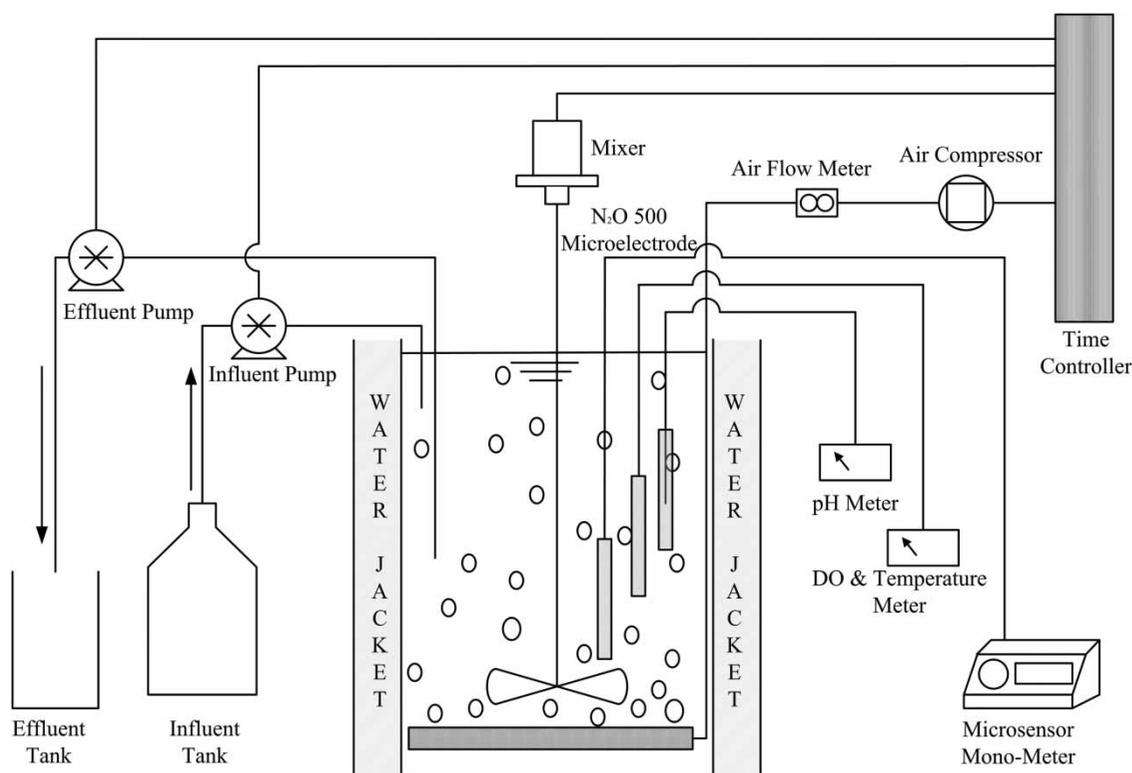


Figure 1 | The schematic diagram of the partial nitrification SBR process.

The diameter and the height were 200 mm and 400 mm respectively. The reactor was stirred with a rectangular mixing paddle. The air was supplied to the reactor by an air compressor through a diffuser placed at the bottom of the reactor, and the air flow was controlled by an air flow meter. The dissolved oxygen (DO) concentration in the reactor was maintained in the range of 0.4 mg/L–0.5 mg/L. The temperature of the SBR was controlled at 30 °C ± 2 °C by a water jacket. The drainage ratio of the reactor was 0.5.

The seeding sludge was taken from the oxic unit of the fourth wastewater treatment plant of Xi'an city, Shaanxi Province, China.

### Operating conditions

Three phases including cultivation phase (CP), medium ammonia load phase (MAP) and low ammonia load phase (LAP), were operated under different conditions in this study. The influent NH<sub>4</sub><sup>+</sup>-N concentrations of CP, MAP and LAP were 110, 70 and 50 mg/L respectively. The chemical oxygen demand (COD) to NH<sub>4</sub><sup>+</sup>-N (C/N) ratios of the influent in all operation phases were maintained at 2 by changing the COD concentrations in the influent of each operation phase.

In the CP, the sludge concentration of the reactor was ~3,800 mg/L, and the corresponding sludge retention time

(SRT) was 18 d. In the MAP, the sludge concentration of the reactor was ~3,300 mg/L, and the corresponding SRT was 15 d. In the LAP, the sludge concentration of the reactor was ~3,100 mg/L, and the corresponding SRT was 15 d. The sludge concentrations and SRTs of the different phases are listed in Table 1.

The influent quality and compositions of the three operating phases are shown in Table 2. In addition, NaHCO<sub>3</sub>, MgSO<sub>4</sub>·7H<sub>2</sub>O, CaCl<sub>2</sub> and trace elements were also contained in influent and their dosages were unchanged during the three phases. The dosages of NaHCO<sub>3</sub>, MgSO<sub>4</sub>·7H<sub>2</sub>O and CaCl<sub>2</sub> in the influent were 950.0, 50.0 and 20.0 mg/L respectively. The use of trace elements was the same as in Cheong & Hansen (2008).

Each phase operated with a cyclic duration of 4 hours, including a sequence of filling, aeration and mixing, settle, draw and idle. The operation procedures of the three operating phases are shown in Figure 2. In each operation phase, DO concentration was detected by real-time monitoring. Aeration duration of each operation phase was not fixed, and aeration was immediately stopped when obvious increase of DO concentration in the reactor was detected.

### Analytical methods

COD, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N, N<sub>2</sub>O-N, NH<sub>2</sub>OH-N, DO concentration, pH value and temperature during the experiments were monitored. COD, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N and NO<sub>2</sub><sup>-</sup>-N were monitored according to standard methods (APHA 1998). DO concentrations, temperatures and pH values in the reactor were measured by a DO meter (Hach) and pH meter (Rex, ShangHai).

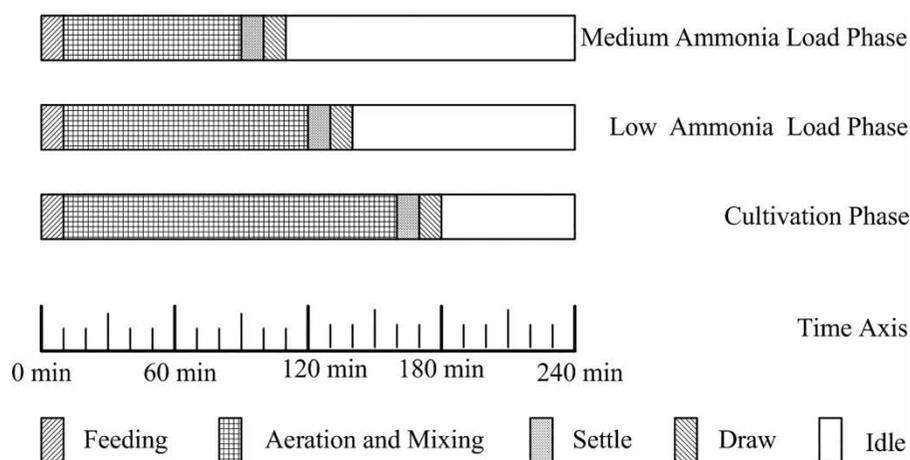
N<sub>2</sub>O-N concentration profiles in the reactor were measured using Clark-type microelectrodes continuously. The N<sub>2</sub>O 500 microelectrode and the microsensor monometer were purchased from Unisense (Aarhus, Denmark).

**Table 1** | The sludge concentrations and SRTs of different phases

	Cultivation phase (n = 8)	Medium ammonia load phase (n = 5)	Low ammonia load phase (n = 5)
Sludge concentrations (mg/L)	3,877 ± 111	3,344 ± 75	3,172 ± 72
SRT (d)	18	15	15

**Table 2** | Influent quality and main compositions of the three operating phases

Operating phase	Compounds	Concentration	COD	NH <sub>4</sub> <sup>+</sup> -N	PO <sub>4</sub> <sup>3-</sup> -P
Cultivation	CH <sub>3</sub> COON <sub>a</sub>	256 mg/L	200 mg/L	100 mg/L	4 mg/L
	NH <sub>4</sub> HCO <sub>3</sub>	564.3 mg/L			
	Na <sub>3</sub> PO <sub>4</sub>	21.2 mg/L			
Medium ammonia	CH <sub>3</sub> COON <sub>a</sub>	179.2 mg/L	140 mg/L	70 mg/L	4 mg/L
	NH <sub>4</sub> HCO <sub>3</sub>	395.0 mg/L			
	Na <sub>3</sub> PO <sub>4</sub>	21.2 mg/L			
Low ammonia	CH <sub>3</sub> COON <sub>a</sub>	128 mg/L	100 mg/L	50 mg/L	4 mg/L
	NH <sub>4</sub> HCO <sub>3</sub>	282.2 mg/L			
	Na <sub>3</sub> PO <sub>4</sub>	21.2 mg/L			



**Figure 2** | The operation procedures of the three operating phases.

After polarization, the N<sub>2</sub>O microelectrode responded linearly in the range of 0.0 to 22.0 mg N<sub>2</sub>O/L. The microelectrode was calibrated with the two-point method according to the instruction provided by Unisense. By establishing the relationship between the reading voltages and the corresponding two N<sub>2</sub>O-N concentrations, a calibration curve can be acquired. The dissolved concentrations of N<sub>2</sub>O-N can be obtained by substituting reading voltages into the calibration curve.

NH<sub>2</sub>OH-N concentrations were measured by the spectrophotometric method proposed by Hu *et al.* (in press). Under acidic condition, NH<sub>2</sub>OH can be stabilized as NH<sub>3</sub>OH<sup>+</sup>. The spectrophotometric method was based on oxidation of NH<sub>2</sub>OH to N<sub>2</sub>O by Fe(III) using ferric ammonium sulfate (NH<sub>4</sub>Fe(SO<sub>4</sub>)<sub>2</sub>) as oxidation agent (Bengtsson *et al.* 2002). NH<sub>2</sub>OH was determined through formation of ferrion, a tris complex of 1,10-phenanthroline with Fe(II). Ferrion is a red-colored octahedral complex ion, and is soluble and stable in aqueous solution in the pH range of 2–9 (Adhikamsetty *et al.* 2008). By eliminating the interference of phosphate by the numerical method proposed by Hu *et al.* (in press), the spectrophotometric method can be used to determine NH<sub>2</sub>OH-N concentrations in biological wastewater treatment processes.

### Nitrite accumulation rate

Nitrite accumulation rate (NAR) was calculated by:

$$\text{NAR} = \frac{C_{\text{Nitrite}}}{C_{\text{Nitrite}} + C_{\text{Nitrate}}} \times 100\% \quad (1)$$

where  $C_{\text{Nitrite}}$  and  $C_{\text{Nitrate}}$  represent NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations in the reactor, mg/L.

## RESULTS AND DISCUSSION

### Performances of the SBR process under different operating phases

Figure 3 shows the performance of the process in the CP. In CP, parts of NH<sub>4</sub><sup>+</sup>-N in influent were converted to NO<sub>2</sub><sup>-</sup>-N at the beginning, and the NAR was lower than 40%. After 12 days, the NAR was dramatically increased and the majority of NH<sub>4</sub><sup>+</sup>-N in influent was converted to NO<sub>2</sub><sup>-</sup>-N. After 22 days, the NO<sub>2</sub><sup>-</sup>-N concentration in the effluent of the reactor was higher than 33.0 mg/L and the NO<sub>3</sub><sup>-</sup>-N concentration in the effluent of the reactor was lower than 2.0 mg/L. Consequently, the NAR was higher than 95%.

When the short-cut nitrification process was successively achieved in the SBR, the operation of the reactor was changed to MAP and then LAP. The operation periods of both MAP and LAP lasted for 1 to 2 months for achieving the steady performance. After the reactor achieved steady state in the LAP and the MAP, measuring campaigns were carried out in each operation phase. The performances of the short-cut nitrification SBR process during continuous 9 days of the steady states under the LAP and the MAP are shown in Figure 4.

In the MAP and the LAP, all NO<sub>3</sub><sup>-</sup>-N concentrations in effluent were lower than 2.0 mg/L, and NARs were always higher than 90%. Under the three operation phases, the SBR achieved stable short-cut nitrification and high NARs.

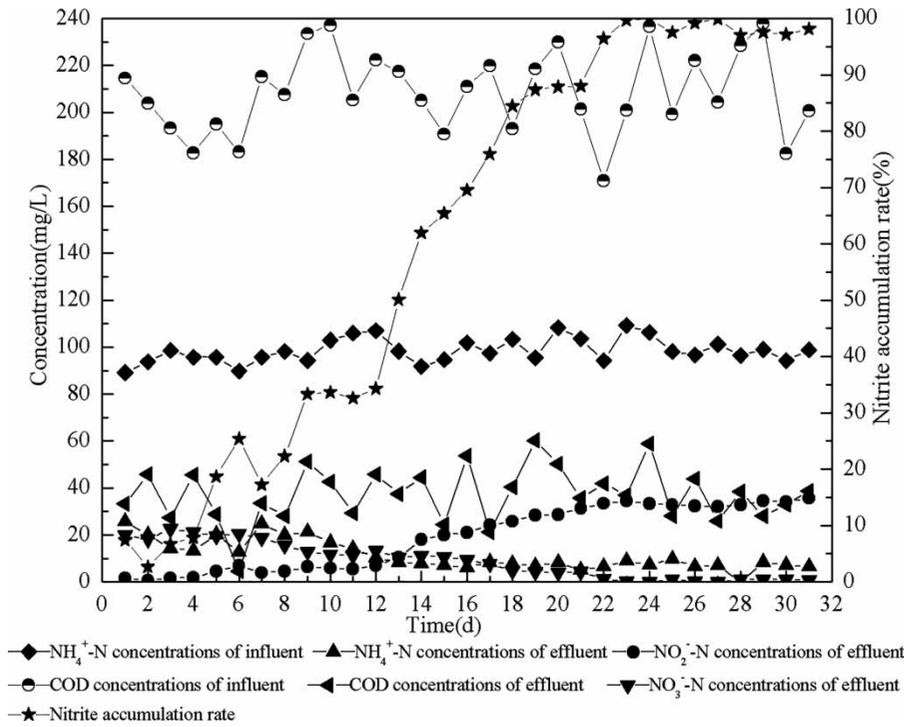


Figure 3 | The performances of the short-cut nitrification SBR process in the CP.

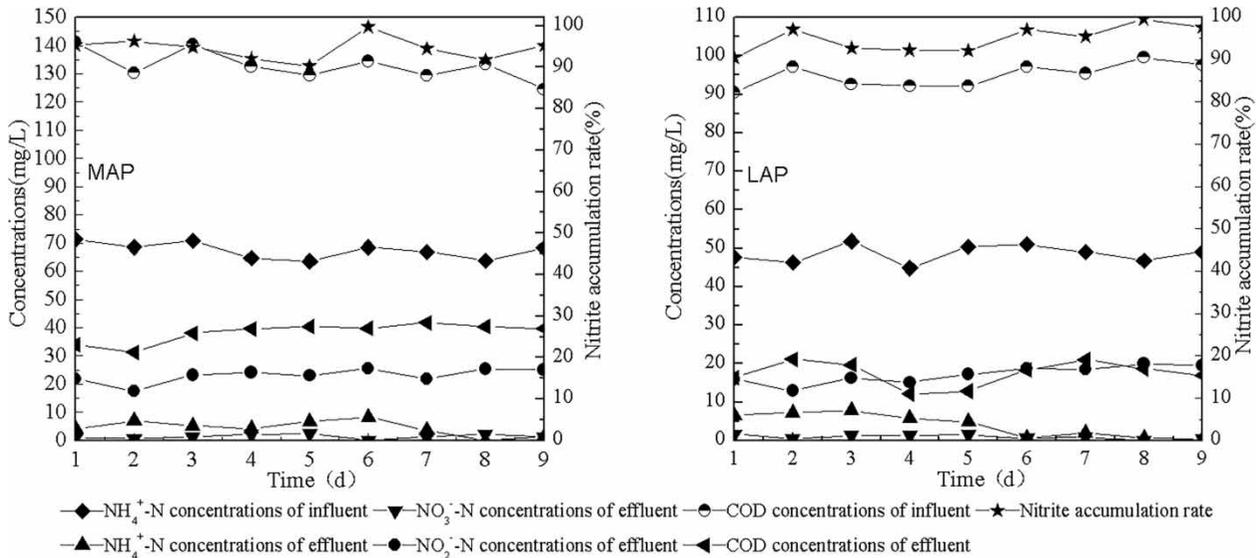


Figure 4 | The steady performances of the short-cut nitrification SBR process in the MAP and LAP.

In order to achieve the short-cut nitrification quickly, high influent  $\text{NH}_4^+\text{-N}$  concentration (110 mg/L), high temperature (30 °C), high pH value (~8.0) and low DO concentration (0.4–0.5 mg/L) were adopted in the CP in this study. Also, real-time monitoring for DO concentration in the reactor was carried out, which eliminated excessive

air supply. A broad range of operating parameters and factors are essential for achieving short-cut nitrification, such as pH, DO, temperature and free ammonia (FA) (Sinha & Annachhatre 2007). It is generally accepted that FA inhibits the activity of nitrite oxidoreductase (NOR) (Anthonisen et al. 1976; Yang & Alleman 1992) or NOB (Ahn et al.

2011). Also, most of the published literature related to nitrite accumulation using pH as a decisive factor stated that pH in the range of 7.5–8.5 was most suited to inhibit the nitrite oxidizers (Sinha & Annachhatre 2007). FA and pH had a great effect on nitrite accumulation. According to the study of Anthonisen et al. (1976), the ratio of NH<sub>4</sub><sup>+</sup>-N to NH<sub>3</sub>-N would decrease with the increasing of pH value; consequently, the activity of NOR would become more inhibited as the content of FA would increase when pH was increased. Temperature also was an important parameter for nitrite accumulation. Although, short-cut nitrification or partial nitrification can be fulfilled at low temperature (Gu et al. 2012), the short-cut nitrification process can be more easily achieved at high temperature (30–35 °C) or mild temperature (20–30 °C) (Gu et al. 2012). Furthermore, according to the study carried out by Guo et al. (2009), a stable short-cut nitrification process was achieved for a long time in the SBRs operated with high DO (above 3.0 mg/L on average) and low DO (0.4–0.8 mg/L) concentrations, and DO was not the crucial factor for achievement of short-cut nitrification. However, AOB seemed to be more robust towards low DO than were NOB (Sinha & Annachhatre 2007) and nitrite oxidation was strongly inhibited by low DO (<0.5 mg/L) (Hanaki et al. 1990). Thus, low DO concentration was beneficial for quick achievement of short-cut nitrification.

From the ecological concept, *r*-strategist microorganisms can grow quickly on easily available substrate (Andrews & Harris 1986). During CP, the residual NH<sub>4</sub><sup>+</sup>-N concentrations were relatively high at the end of the cycle, which was beneficial for the enrichment of an *r*-strategist

AOB population (Terada et al. 2013). Furthermore, *Nitrospira* sp. and *Nitrobacter* sp. are widely regarded as the two major types of NOB present in biological wastewater treatment plants (Wang et al. 2016b). Oxygen affinity of *Nitrospira* sp. belonging to *r*-strategist was low; however, that of *Nitrobacter* sp. belonging to *K*-strategist was high (Regmi et al. 2014). High DO level could provide competitive advantage for AOB over *Nitrospira* sp., and DO limitation can suppress the growth of *Nitrobacter* sp. (Wang et al. 2016b). Low DO levels were beneficial for washing out of *Nitrobacter* sp. (Wang et al. 2016b). In this study, low DO concentrations were maintained in the reactor, which made AOB dominant in the reactor instead of NOB. Consequently, high NARs were obtained.

According to the operation results, the operation strategy for achieving the short-cut nitrification in this study was effective. The short-cut nitrification was completely achieved on day 22 after the start-up of the SBR. Also, as NOB were washed out from the system in the CP, the short-cut nitrification process did not deteriorate when the operation phase was shifted to the LAP and the MAP from the CP.

### N<sub>2</sub>O production and NH<sub>2</sub>OH variation in typical cycles of LAP and MAP

Figure 5 shows the variation profiles of different contaminations in the reactor during three typical cycles of the LAP and the MAP.

Variation trends of COD, NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N, PO<sub>4</sub><sup>3-</sup>-P, NH<sub>2</sub>OH-N, dissolved N<sub>2</sub>O-N and NAR in the

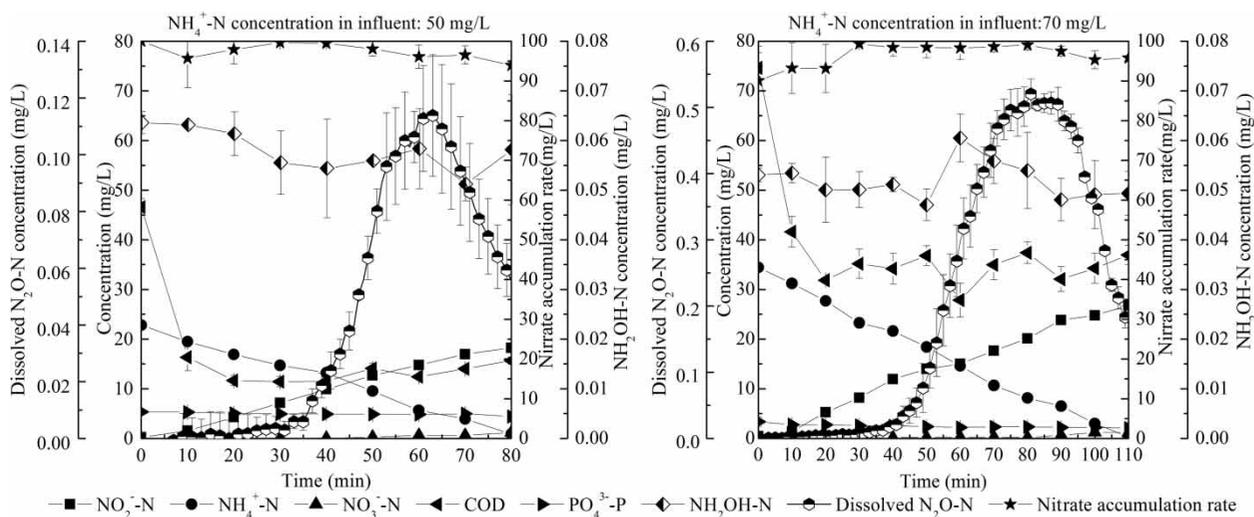


Figure 5 | Concentration variations of different contaminants in typical cycles of the short-cut nitrification SBR process under the MAP and LAP.

LAP and the MAP were almost same, except that the maximum dissolved N<sub>2</sub>O-N concentration in MAP was almost five times higher than that in LAP. During the typical cycles, COD concentrations in the reactor under two phases decreased to the lowest content within 20 min with the effects of dilution and oxidation. Also, NH<sub>4</sub><sup>+</sup>-N concentrations were decreased linearly, NO<sub>2</sub><sup>-</sup>-N concentrations were increased linearly, and NO<sub>3</sub><sup>-</sup>-N concentrations in cycle durations were lower than 2.0 mg/L. Correspondingly, NARs in whole cycle durations were higher than 90%, which meant the short-cut nitrification process was dominant in the SBR. Specifically, the NH<sub>2</sub>OH-N concentrations fluctuated around 0.06 mg/L in a small range during all monitoring operation cycles under the LAP and the MAP, and dissolved N<sub>2</sub>O-N concentrations were increased in more than half of the cycle durations, and decreased in the latter part of the cycle durations.

In all operation phases, C/N ratio of the influent was maintained at 2 which did not inhibit the nitrification process (Campos et al. 1999), and consequently a stable short-cut nitrification process was successively achieved. During the short-cut nitrification process, N<sub>2</sub>O production was suppressed to some extent during the first 30 min of operation cycles in LAP and MAP owing to the storage of COD to PHB which used for the reduction of NO<sub>2</sub><sup>-</sup>-N (Zhao et al. 2016) and NO production (Pocquet et al. 2016). At the latter part of the monitoring cycle durations, the

concentrations of N<sub>2</sub>O-N were decreased rapidly as the NH<sub>4</sub><sup>+</sup>-N in influent was closed to depletion.

In order to distinguish the differences in nitrification reaction rates before and after the maximum dissolved N<sub>2</sub>O-N concentration in the reactor, the concentrations of NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup>-N were divided into two groups respectively in each operation phase. The variations of nitrification reaction rates are shown in Figure 6.

In Figure 6, NH<sub>4</sub><sup>+</sup>-N (I) and NH<sub>4</sub><sup>+</sup>-N (D) represent the concentrations of NH<sub>4</sub><sup>+</sup>-N corresponding to the increase and the decrease periods of N<sub>2</sub>O-N concentration in the reactor respectively. NO<sub>2</sub><sup>-</sup>-N (I) and NO<sub>2<sup>-</sup>-N (D) represent the concentrations of NO<sub>2</sub><sup>-</sup>-N corresponding to the increase and the decrease periods of N<sub>2</sub>O-N concentration in the reactor respectively. Before the peak concentrations of dissolved N<sub>2</sub>O-N in operation cycles under two phases, the oxidation rates of NH<sub>4</sub><sup>+</sup>-N and the production rates of NO<sub>2</sub><sup>-</sup>-N were higher than those after the peak concentrations of dissolved N<sub>2</sub>O-N, which can be explained by the effects of low NH<sub>4</sub><sup>+</sup>-N concentration on the nitrification reaction rate.</sub>

These phenomena indicated that N<sub>2</sub>O production was decreased in the latter period of the monitoring cycles. During nitrification, two pathways were considered to be the major processes responsible for N<sub>2</sub>O emissions, namely AOB denitrification process, also called nitrifier denitrification, and incomplete hydroxylamine oxidation

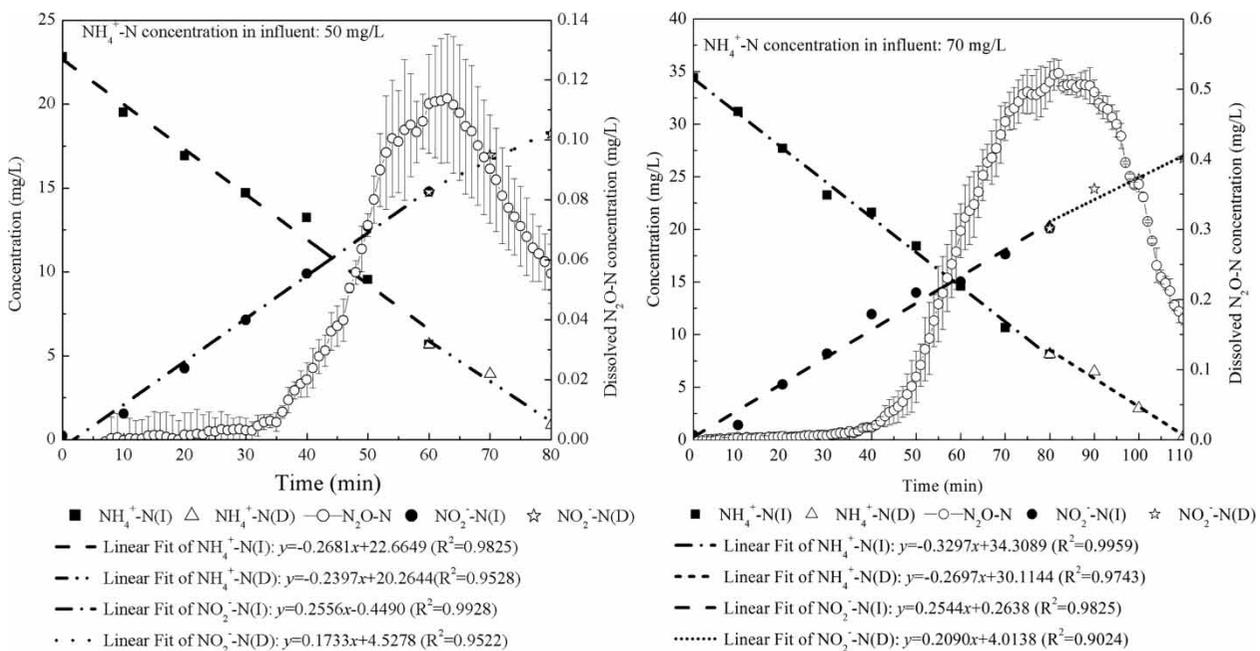


Figure 6 | Nitrification rate variations before and after the maximum concentration of dissolved N<sub>2</sub>O-N.

by the hydroxylamine oxidoreductase (Pocquet *et al.* 2016). AOB denitrification was favored by limited oxygen conditions (Tallec *et al.* 2006) such as the DO level in this study. In the AOB denitrification process, NH<sub>4</sub><sup>+</sup>-N can be converted to NO and NO<sub>2</sub><sup>-</sup>-N via NH<sub>2</sub>OH (Ni *et al.* 2011; Pocquet *et al.* 2016), and NH<sub>2</sub>OH can enhance the N<sub>2</sub>O emission (Kim *et al.* 2010). Based on the determination, concentrations of NH<sub>2</sub>OH-N in all monitoring cycles were around 0.06 mg/L, and NH<sub>2</sub>OH did not accumulate under two NH<sub>4</sub><sup>+</sup>-N concentrations (50 mg/L and 70 mg/L). As the presence of high concentration of NH<sub>2</sub>OH are toxic to AOB (Vajrala *et al.* 2013), it was speculated that maybe there was a detoxification mechanism in bacteria for avoiding accumulation of NH<sub>2</sub>OH. Consequently, NH<sub>2</sub>OH in the nitrification process cannot accumulate to a high level.

From the perspective of biochemical reaction dynamics, NH<sub>2</sub>OH might be the limitation substrate for the nitrification reaction because of its low concentration. However, according to Figure 6, although production rates of NO<sub>2</sub><sup>-</sup>-N were smaller than consumption rates of NH<sub>4</sub><sup>+</sup>-N in monitoring cycles under the LAP and the MAP, the differences between them were not distinct, which meant the low NH<sub>2</sub>OH-N concentration in the two operation phases did not obviously result in the reduction of the production rate of NO<sub>2</sub><sup>-</sup>-N. According to the determination results of NH<sub>2</sub>OH-N and the toxicity of high NH<sub>2</sub>OH concentration in nitrification, it was reasonable that the half-saturation of NH<sub>2</sub>OH ( $K_{\text{NH}_2\text{OH}}$ ) in the biochemical conversion process of NH<sub>2</sub>OH to NO<sub>2</sub><sup>-</sup> was very small. The value of  $K_{\text{NH}_2\text{OH}}$  estimated by Law *et al.* (2012) (0.05 mg NH<sub>2</sub>OH-N/L) was more accurate than the estimated values used by Ni *et al.* (2011) (2.4 mg NH<sub>2</sub>OH-N/L) and Pocquet *et al.* (2016) (0.9 mg NH<sub>2</sub>OH-N/L).

## CONCLUSIONS

In a short-cut nitrification SBR, the characteristics of N<sub>2</sub>O production and NH<sub>2</sub>OH variation were studied in this study.

Combined with real-time monitoring of DO, the short-cut nitrification process can be quickly achieved with the operation parameters of high influent NH<sub>4</sub><sup>+</sup>-N concentration, high temperature, high pH value and low DO concentration. The amount of N<sub>2</sub>O production was increased with the increasing of the influent NH<sub>4</sub><sup>+</sup>-N concentration. Furthermore, according to the determination results of NH<sub>2</sub>OH-N concentrations, the half-saturation constant of NH<sub>2</sub>OH in the biochemical conversion process of NH<sub>2</sub>OH

to NO<sub>2</sub><sup>-</sup>-N was very small, and the value of 0.05 mg NH<sub>2</sub>OH-N/L of  $K_{\text{NH}_2\text{OH}}$  in the published literature was accurate.

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