

## N<sub>2</sub>O EMISSION FROM A NITRITATION DOUBLE-SLUDGE SYSTEM TREATING WASTEWATER WITH A HIGH CONCENTRATION OF AMMONIUM

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### ABSTRACT

A high efficient nitrite based double-sludge system combining sequential denitrification and nitrification reactors has been developed to remove nitrogen from wastewaters containing high concentrations of ammonium. System performance was examined and also nitrous oxide (N<sub>2</sub>O) emission was measured under different aeration rates with or without the addition of nitrite nitrogen (NO<sub>2</sub>-N). The nitrification efficiency (the ratio of NO<sub>2</sub>-N to the oxidized nitrogen, NO<sub>x</sub>-N) reached to 99.8% at the ammonium loading rate of 1.47 g l<sup>-1</sup> d<sup>-1</sup>. With increasing the aeration rate, ammonium oxidizing rate increased, indicating that the activity of ammonia oxidizing bacteria was enhanced. However, N<sub>2</sub>O emission decreased with increasing the aeration rate. N<sub>2</sub>O emission was high with the addition of NO<sub>2</sub>-N. The aeration rate had a high effect on N<sub>2</sub>O emission at high initial NO<sub>2</sub>-N concentrations, with the N<sub>2</sub>O emission factor ranged from 0.59% to 1.64%. While the effect of aeration rate on N<sub>2</sub>O emission was not significantly without the addition of NO<sub>2</sub>-N, with the emission factor of only around 0.20% to 0.27%.

**Keywords:** high ammonium wastewater, nitrous oxide, nitrification, aeration rate, nitrite

### 1. Introduction

Wastewaters such as landfill leachate and anaerobically digester effluent are usually containing high concentrations of ammonium nitrogen (NH<sub>4</sub>-N) and low concentrations of biodegradable organic matters. A high efficient nitrite based double-sludge system combining sequential denitrification and nitrification reactors has been developed to remove nitrogen from such types of wastewater (Wu *et al.*, 2014). In this process, ammonia oxidizing bacteria (AOB) oxidize NH<sub>4</sub>-N to nitrite nitrogen (NO<sub>2</sub>-N) by the alternative anoxic and aerobic operating mode and activities of nitrite oxidizing bacteria (NOB) are inhibited. However, for this NO<sub>2</sub>-N based system, nitrous oxide (N<sub>2</sub>O) can be produced and emitted both in denitrification and nitrification processes during biological nitrogen removal, with the main contribution from the nitrification process. AOB have been identified as the major contributor to N<sub>2</sub>O production during nitrification (Ahn *et al.*, 2010; Law *et al.*, 2012; Gong *et al.*, 2012), with three possible pathways of nitrifier denitrification, and biological and chemical oxidation of hydroxylamine (NH<sub>2</sub>OH; Law *et al.*, 2013). NO<sub>2</sub>-N and dissolved oxygen (DO) are considered as key factors affecting N<sub>2</sub>O emission during nitrification. DO has been widely used as a control factor to achieve nitrite accumulation in the nitrification reactor. Low DO may inhibit activities of NOB and induce nitrite accumulation, because the competition ability for DO of NOB is less than AOB. In addition, under low DO conditions, NO<sub>2</sub>-N may be used as the electron acceptor for nitrifier denitrification by AOB, and this will lead to N<sub>2</sub>O emission (Zheng *et al.*, 1994; Tallec *et al.*,

2006). Aboobakar *et al.*, (2013) proposed that  $N_2O$  production had a direct correlation with DO. While Weissenbacher *et al.* (2011) suggested that DO did not affect  $N_2O$  emission obviously. By varying aeration rates in an anoxic/aerobic sequencing batch reactor (SBR), Hu *et al.* (2011) found that the aeration rate was an important factor affecting  $N_2O$  emission. A high aeration rate led to low  $N_2O$  emission (Hu *et al.*, 2010). Therefore, the influence of DO concentrations (or aeration rates) on  $N_2O$  emission during wastewater treatment is worth of further investigation.

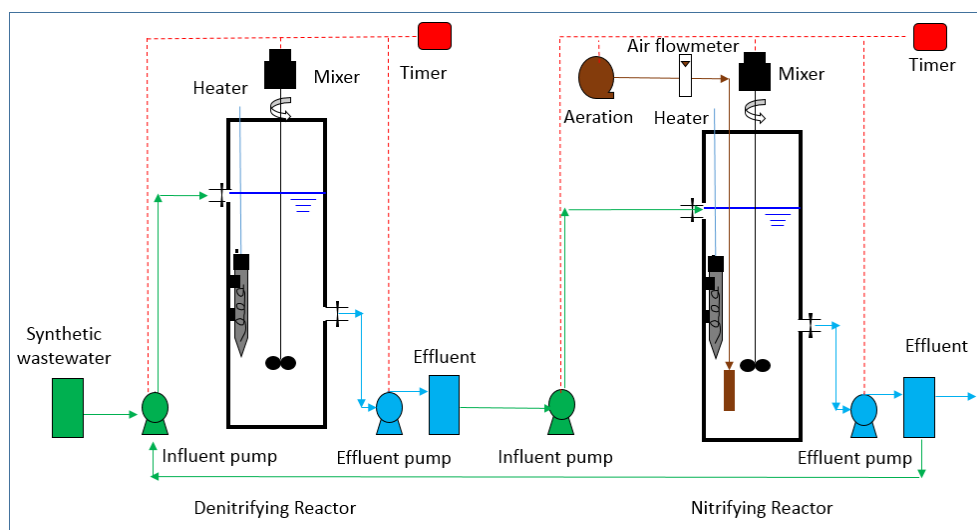
Nitrite accumulation can be achieved in the nitrification reactor, but nitrite may induce a high  $N_2O$  production. Xiao *et al.* (2014) demonstrated that  $N_2O$  emission through AOB denitrification had a positive correlation with the  $NO_2-N$  concentration, and the  $N_2O$  emission factor ranged from 0.41% to 7.25%. Law *et al.* (2013) found that  $N_2O$  production reached the highest level when the  $NO_2-N$  concentration was less than  $50 \text{ mg N l}^{-1}$ , and then decreased with the increasing  $NO_2-N$  concentrations from  $50 \text{ mg N l}^{-1}$  to  $500 \text{ mg N l}^{-1}$ . Therefore, the influences of DO concentration (or aeration rate) and  $NO_2-N$  concentrations on  $N_2O$  production in the nitrification system should be further examined during the treatment of high ammonium concentration wastewater so as to support the sustainable development of the proposed high ammonium wastewater treatment system.

In this study, nitrite accumulation was achieved in a double-sludge system, and factors affecting nitrite accumulation and  $N_2O$  emission were furthered analyzed.

## 2. Materials and methods

### 2.1. System operation

Two lab-scale SBRs (Figure 1), one for denitrification and the other for nitrification, each with a working volume of 6 l, were operated at  $27^\circ\text{C}$ .



**Figure 1.** Schematic diagram of the sequential denitrifying and nitrifying reactors

The two reactors were seeded with sludge acclimated in the laboratory, and the operation cycles were both 4 h. One cycle in the denitrifying reactor consisted of 180 min anoxic stirring, 20 min aeration, 30 min settling, and 10 min decanting and idle periods. During each cycle, 3 l of mixed wastewater (2 l synthetic wastewater and 1 l recycled nitrified wastewater from the nitrifying reactor) was fed to the reactor, and 3 l was discharged, resulting in a hydraulic retention time (HRT) of 8 h. While one cycle in the nitrifying reactor consisted of 80 min aeration-I, 40 min anoxic stirring, 80 min aeration-II, 30 min settlement, and 10 min decanting and idle periods, and the HRT was also 8 h. The effluent from the denitrifying reactor was fed to the nitrifying reactor. The solids retention time (SRT) of the denitrifying

reactor was kept at 20 days by wasting 300 ml of sludge each day, and sludge was not wasted in the nitrifying reactor under steady state. In each cycle, the net 2 l of synthetic wastewater was fed to the denitrifying and nitrifying reactors, and 2 l of treated wastewater was decanted. Therefore, the HRT of the combined system was 1 day.

The synthetic wastewater comprised: 3.06 g l<sup>-1</sup> of NH<sub>4</sub>Cl (0.8 g l<sup>-1</sup> NH<sub>4</sub>-N), 1.00 g l<sup>-1</sup> of sodium acetate, 7.00 g l<sup>-1</sup> of NaHCO<sub>3</sub>, 0.10 g l<sup>-1</sup> of Na<sub>2</sub>HPO<sub>4</sub>, 0.056 g l<sup>-1</sup> of CaCl<sub>2</sub>, 0.36 g l<sup>-1</sup> of MgSO<sub>4</sub>, 0.01 g l<sup>-1</sup> of yeast extract and 0.4 ml l<sup>-1</sup> of a trace element stock solution. The trace element stock solution was according to Smolders *et al.*, (1994).

## 2.2. Batch experiments

Batch experiments were carried out to examine the effect of aeration rates and initial NO<sub>2</sub>-N concentrations on N<sub>2</sub>O emission for activated sludge acclimated in the nitrifying reactor. The batch reactors were made from capped glass flasks, each with three ports on the cap, one for liquid sampling, one for gas sampling (measuring gas flow rates simultaneously), and the other for aeration.

For the effect of aeration rates on N<sub>2</sub>O emission at a high initial NO<sub>2</sub>-N concentrations, 1.2 l mixed liquor was taken from the nitrifying reactor after filling and divided into three batch reactors. The mixed liquor was then aerated at the flow rate of 0.24, 0.36 and 0.48 l min<sup>-1</sup>, respectively. Samples (both liquid and gas samples) were taken at intervals of 10 min, and simultaneously DO and pH were measured. Mixed liquor sample was also taken from the batch reactor to determine concentrations of the mixed liquor suspended solids (MLSS) and the mixed liquor volatile suspended solids (MLVSS).

The effect of aeration rates on N<sub>2</sub>O emission was also examined without a high initial NO<sub>2</sub>-N concentration. 1.2 l mixed liquor was taken from the nitrifying reactor before the end of the aeration phase. After centrifugation, supernatant was discarded and the residual sludge was re-suspended with synthetic wastewater but without the organic carbon, and the initial NH<sub>4</sub>-N concentration was controlled at around 165 mg l<sup>-1</sup>. The mixed liquor was then divided into three batch reactors and aerated at the flow rate of 0.24, 0.36 and 0.48 l min<sup>-1</sup>, respectively. Samples (both liquid and gas samples) were taken at intervals of 10 min, and simultaneously DO and pH were measured. Mixed liquor sample was also taken from the batch reactors to determine MLSS and MLVSS concentrations.

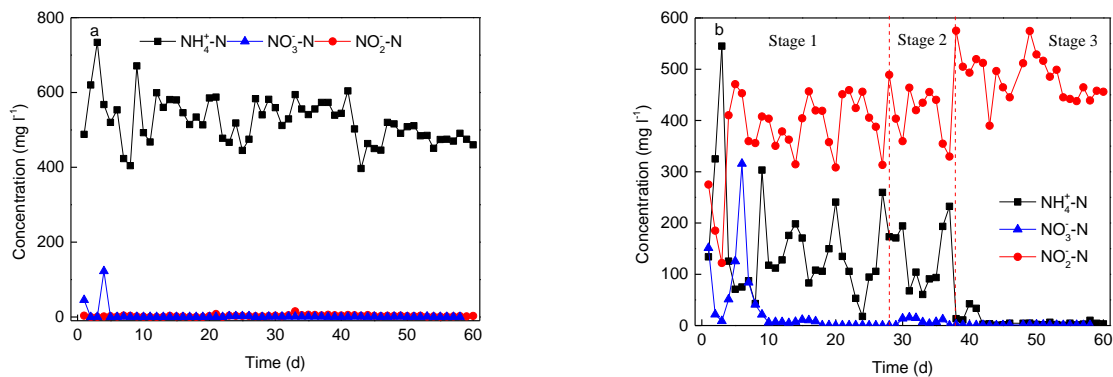
## 2.3. Analytical methods

NH<sub>4</sub>-N, NO<sub>2</sub>-N, nitrate nitrogen (NO<sub>3</sub>-N), MLSS and MLVSS were analyzed according to standard methods (APHA, 1999). DO and pH were determined by a DO meter (oxi 315i, WTW, Germany) and a pH meter (pH3110, WTW, Germany), respectively. Free ammonia (FA) and free nitrous acid (FNA) were calculated according to Anthonisen *et al.* (1976). N<sub>2</sub>O concentration in gas phase was measured with a gas chromatograph (Agilent 6820, Agilent Technologies, USA). To compare conveniently, the produced N<sub>2</sub>O in gas phase was represented as mg l<sup>-1</sup>, which was mg N<sub>2</sub>O (gas) produced from the unit volume (l) of mixed activated sludge liquor.

## 3. Results and discussion

Dynamics of effluent nitrogen parameters during the long-term operation of both denitrifying and nitrifying reactors are shown in Figure 2. The denitrifying reactor reached to steady state after operation for 46 days. The average concentrations of NH<sub>4</sub>-N, NO<sub>2</sub>-N and NO<sub>3</sub>-N in effluent were 489.8 mg l<sup>-1</sup>, 2.2 mg l<sup>-1</sup> and 0.4 mg l<sup>-1</sup>, respectively (Figure 2a). During the initial 28 days, the nitrifying reactor was operated under an aeration rate of 0.9 l min<sup>-1</sup>, and one cycle consisted 50 min aeration-I, 30 min anoxic stirring-I, 50 min aeration-II, 30 min anoxic stirring-II, 40 aeration-III, 30 min settling and 10 min discharging periods. As shown in Figure 2b, NH<sub>4</sub>-N existed in effluent during this stage, and it indicated that complete nitritation was not achieved. Therefore, the aeration rate was increased to 1.8 l min<sup>-1</sup> to enhance the ability of nitritation in Stage 2. However, NH<sub>4</sub>-N was still not converted completely. In Stage 3, the operation mode was changed to consist 80 min aeration-I, 40 min anoxic stirring-I, 80 min aeration-II, and 30 min settling and 10 min discharging periods on Day 38. Nitrite accumulation was achieved steadily, and

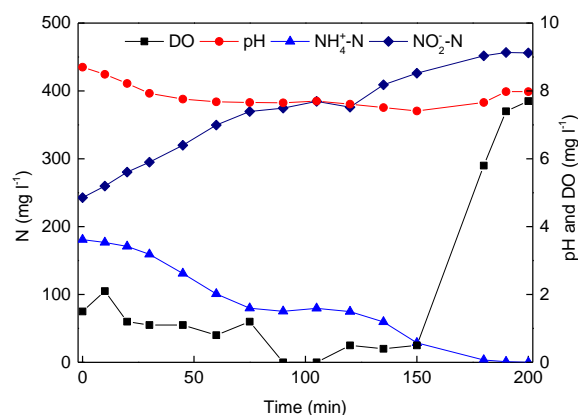
the average concentrations of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  in the effluent were  $3.9 \text{ mg l}^{-1}$ ,  $445.7 \text{ mg l}^{-1}$  and  $0.9 \text{ mg l}^{-1}$ , respectively.



**Figure 2.** Long-term dynamics of the effluent nitrogen concentrations in the denitrifying reactor (a) and the nitrifying reactor (b)

The ammonium loading rate was  $0.8 \text{ g l}^{-1} \text{ d}^{-1}$  of the whole system, and it reached to  $1.47 \text{ g l}^{-1} \text{ d}^{-1}$  with a nitrification efficiency of 99.8% in the nitrifying reactor. The loading rate was three times higher than that of Wu *et al.* (2014), and a high nitrification efficiency was still maintained. The ammonium loading rate and the nitrification efficiency were also both higher than those of Scaglione *et al.* (2013), who obtained an ammonium loading rate of  $0.5 \text{ g l}^{-1} \text{ d}^{-1}$  and a nitrification efficiency of 80% in a pilot SBR treating high ammonium wastewater. In the present double-sludge system, ammonium loading rate could be further increased on the basis of high nitrification efficiency by modifying the operating conditions.

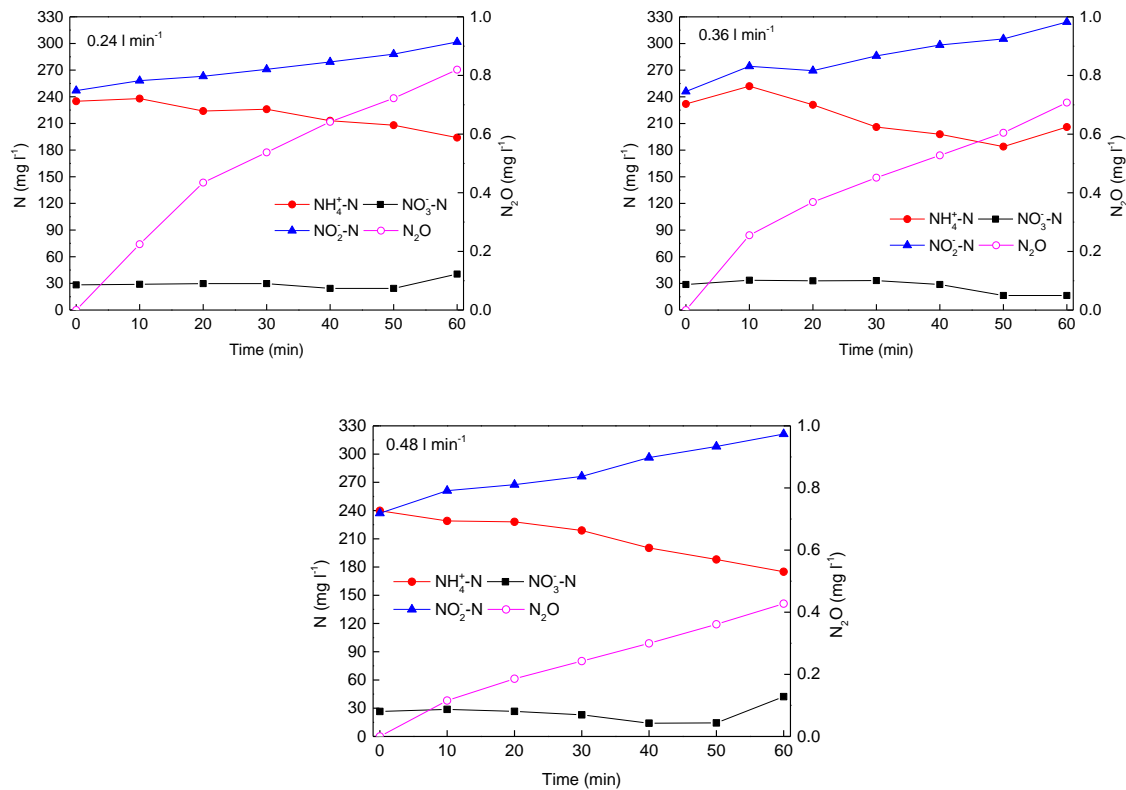
Figure 3 shows dynamics of pH, DO,  $\text{NH}_4\text{-N}$  and  $\text{NO}_2\text{-N}$  concentrations during a typical SBR cycle under steady state in the nitrifying reactor. During the first aeration period, DO concentration was around  $1.0 \text{ mg l}^{-1}$ , and the nitrification efficiency kept at steady state, indicating that nitrification could be achieved stably under a low DO condition. This was also confirmed from some previous studies (Ruiz *et al.*, 2003; Garrido *et al.*, 1997; Hu *et al.*, 2013).



**Figure 3.** Dynamics of parameters during a typical SBR cycle.

Figure 4 shows  $\text{N}_2\text{O}$  emission with a high initial  $\text{NO}_2\text{-N}$  concentrations under the aeration rate of  $0.24 \text{ l min}^{-1}$ ,  $0.36 \text{ l min}^{-1}$  and  $0.48 \text{ l min}^{-1}$ , respectively. The effect of aeration rates on biokinetics of nitrifiers are given in Table 1.  $\text{NH}_4\text{-N}$  were mostly oxidized to  $\text{NO}_2\text{-N}$ , and the  $\text{NH}_4\text{-N}$  oxidizing rate increased from  $10.57 \text{ mg g}^{-1} \text{ h}^{-1}$  to  $18.42 \text{ mg g}^{-1} \text{ h}^{-1}$  with increasing aeration rates. It indicated that the activity of AOB could be enhanced by increasing aeration rates. The  $\text{N}_2\text{O}$  production rate and the  $\text{N}_2\text{O}$  emission factor were both decreased with increasing aeration rates, which was likely due to that AOB denitrification pathway was

inhibited with increasing aeration rates, as also confirmed by some other previous studies (Aboobakar *et al.*, 2013).



**Figure 4.** Dynamics of NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N concentrations and N<sub>2</sub>O emission under different aeration rates with a high initial NO<sub>2</sub>-N concentration during batch nitrification

**Table 1.** Effect of aeration rates on biokinetics of nitrifiers under high initial NO<sub>2</sub>-N concentrations

Aeration rate (l min <sup>-1</sup> )	r <sub>NH4-N</sub> (mg g <sup>-1</sup> h <sup>-1</sup> )	r <sub>N2O-N</sub> (mg g <sup>-1</sup> h <sup>-1</sup> )	r <sub>N2O-N</sub> /r <sub>NH4-N</sub> (%)
0.24	10.57±3.36	0.17±0.03	1.64
0.36	12.82±3.72	0.15±0.01	1.14
0.48	18.42±3.04	0.11±0.02	0.59

Note: r<sub>NH4-N</sub> indicates the NH<sub>4</sub>-N oxidizing rate, r<sub>N2O-N</sub> indicates the N<sub>2</sub>O-N emission rate, and r<sub>N2O-N</sub>/r<sub>NH4-N</sub> indicates the N<sub>2</sub>O-N emission factor.

Figure 5 shows N<sub>2</sub>O emission at a low initial NO<sub>2</sub>-N concentration under the aeration rate of 0.24 l min<sup>-1</sup>, 0.36 l min<sup>-1</sup> and 0.48 l min<sup>-1</sup>, respectively.

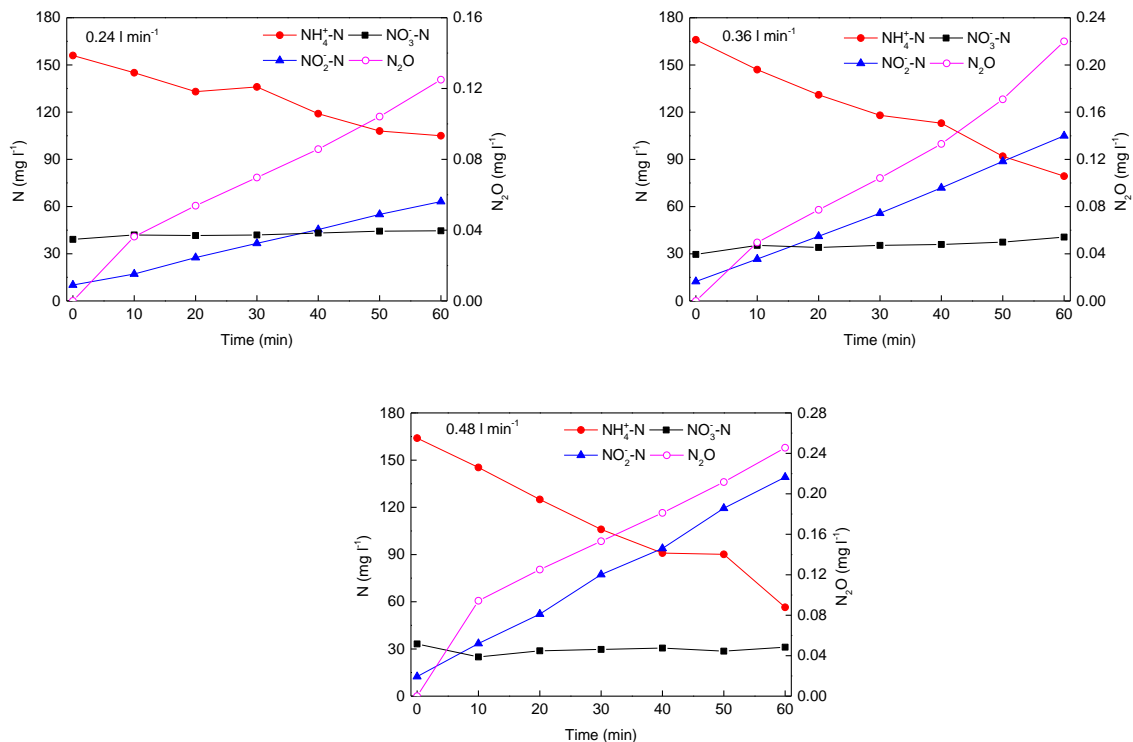
**Table 2.** Effect of aeration rates on biokinetics of nitrifiers under a low initial NO<sub>2</sub>-N concentration

Aeration rate (l min <sup>-1</sup> )	r <sub>NH4-N</sub> (mg g <sup>-1</sup> h <sup>-1</sup> )	r <sub>N2O-N</sub> (mg g <sup>-1</sup> h <sup>-1</sup> )	r <sub>N2O-N</sub> /r <sub>NH4-N</sub> (%)
0.24	13.26±1.88	0.036±0.045	0.27
0.36	20.32±2.92	0.046±0.037	0.23
0.48	26.03±1.87	0.051±0.043	0.20

Note: r<sub>NH4-N</sub> indicates the NH<sub>4</sub>-N oxidizing rate, r<sub>N2O-N</sub> indicates the N<sub>2</sub>O-N emission rate, and r<sub>N2O-N</sub>/r<sub>NH4-N</sub> indicates the N<sub>2</sub>O-N emission factor.

NH<sub>4</sub>-N was also mainly oxidized to NO<sub>2</sub>-N. The N<sub>2</sub>O emission was 0.12 mg l<sup>-1</sup> at the aeration rate of 0.24 l min<sup>-1</sup>. With increasing aeration rates, oxidization of NH<sub>4</sub>-N increased and the N<sub>2</sub>O emission was at a range from 0.12 to 0.25 mg l<sup>-1</sup>. As shown in Table 2, the N<sub>2</sub>O emission rate increased a little while the emission

factor decreased slightly from 0.27% to 0.20% with increasing aeration rates, indicating that the effect of aeration rate on N<sub>2</sub>O emission was insignificant at a low initial NO<sub>2</sub>-N concentration.



**Figure 5.** Dynamics of NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N concentrations and N<sub>2</sub>O emission under different aeration rates with a low initial NO<sub>2</sub>-N concentration

As shown in Figures 4 and 5, both the N<sub>2</sub>O emission rate and the N<sub>2</sub>O emission factor at the high initial NO<sub>2</sub>-N concentration were higher than those at a low initial NO<sub>2</sub>-N concentration at the same aeration rate, indicating that high initial NO<sub>2</sub>-N concentrations might lead to a high N<sub>2</sub>O emission. The reason could be due to that high NO<sub>2</sub>-N concentrations might stimulate AOB denitrification by promoting the expression of the nirK gene.

#### 4. Conclusions

In the double-sludge system, a high ammonium loading rate and nitrification was achieved by optimizing the operation mode and the reaction cycle. The nitrification efficiency reached to 99.8% at the ammonium loading rate of 1.47 g l<sup>-1</sup> d<sup>-1</sup>. The aeration rate had a high effect on N<sub>2</sub>O emission under high initial NO<sub>2</sub>-N concentrations, and the N<sub>2</sub>O emission factor decreased with increasing aeration rates, ranging from 0.59% to 1.64%. While the effect of aeration rates on N<sub>2</sub>O emission was not obvious without the addition of NO<sub>2</sub>-N, with the emission factor of around 0.20% to 0.27%.

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