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# Long-Term Effects of Trace $NO_2$ Addition on the Performance of CANON SBR

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**Abstract**: Two parallel CANON Sequencing Bach reactors were started, and 67ppm $NO_2$ was added into Sequencing Bach Reactor 2 while nothing was added to Sequencing Bach Reactor 1. The total nitrogen removal efficiency of SBR1 was 65.5±5.0% at a removal rate of 0.198±0.023 kgN/m$^3$/d. Meanwhile, the SBR2 with $NO_2$ addition showed a removal efficiency of 67.5±6.2%, with a removal rate of 0.277±0.017 kgN/m$^3$/d. The SBR2 had a higher removal efficiency and rate than the SBR1. The continuous addition of trace $NO_2$ into the CANON Sequencing Bach Reactor allows conventional aerobic ammonia oxidation with $O_2$ as the electron acceptor and ammonia oxidation of with $NO_2$ as the electron acceptor to take place simultaneously, thus improving the ammonia oxidation rate and autotrophic nitrogen removal performance.

**China Library Classification No.: X703.1 Literature Label: A Article No.:**

## 1 Introduction

The aerobic ammonia-oxidizing bacteria of Completely Autotrophic Nitrogen Removal Over Nitrite (CANON) would firstly oxidize part of $NH_4^+$ into $NO_2^-$, while the anaerobic ammonia-oxidizing bacteria then convert $NO_2^-$ and the rest $NH_4^+$ into $N_2$. Compared with traditional nitrification and denitrification, the CANON process can theoretically save 62.5% of $O_2$ required for nitrification and 100% of organic COD required for denitrification [1]. The CANON process was developed by Sliekers et al. [2] from Delft University of Dutch. Aerobic and anaerobic ammonia-oxidizing bacteria were cultured in SBR with organic matter of low concentration and limited oxygen (< 0.5% of air saturation). The removal rate of total nitrogen in the system reached 0.3kgN/m$^3$/d, with most $NH_4^+$ being converted into $N_2$ (85%) and the rest into $NO_3^-$ (15%). The autotrophic nitrogen removal involving aerobic ammonia oxidation and anaerobic ammonia oxidation is given by [2]:

$$NH_3 + 0.85O_2 \rightarrow 0.11NO_3^- + 0.44N_2 + 0.14H^+ + 1.43H_2O \quad (1)$$

In CANON SBR, aerobic ammonia-oxidizing bacteria and anaerobic ammonia-oxidizing bacteria co-exist, and trace $NO_2$ has great influence on autotrophic nitrogen removal. Schmidt et al. [3] inoculated aerobic ammonia-oxidizing bacteria and anaerobic ammonia-oxidizing bacteria, and used $NO_2$ as electron acceptor to study the effect of $NO_2$ on aerobic and anaerobic ammonia-oxidizing bacteria. In the presence of excessive $NH_4^+$ in the system, aerobic and anaerobic ammonia-oxidizing bacteria would work together, with the former using $NO_2$ as electron acceptor to oxidize part of $NH_4^+$ in the system into $NO_2^-$ for anaerobic ammonia-oxidizing bacteria, which consumes NO[4, 5] formed by the reduction of $NO_2$ and relieve the toxicity of NO to cells. In the pure culture of aerobic ammonia-oxidizing bacteria, the microbial activity was inhibited at 50ppm $NO_2$. However, the CANON system didn't show significant inhibitory effect even with a $NO_2$ concentration of up to 250 ppm. With limited $NH_4^+$, aerobic ammonia-oxidizing bacteria are in a dominant position in the competition with anaerobic ammonia-oxidizing bacteria for $NH_4^+$, most of which is oxidized to $NO_2^-$, thus inhibiting anaerobic ammonia-oxidizing bacteria. The accumulation of $NO_2^-$ leads to a decline in the total nitrogen removal rate.

Ren Hongyang [6] found that the ammonia nitrogen degradation rate of NO2 ammonia oxidation peaked at 5.36mg/(g·h) under anaerobic conditions when $NO_2$ concentration was 4.475mmol/m$^3$ (100ppm). The introduction of trace $NO_2$ can improve the activity of aerobic ammonia-oxidizing bacteria. The activity of aerobic ammonia-oxidizing bacteria peaked at 161.21mg/(g·h) with a DO concentration of 1.5-2.0mg/L and $NO_2$ concentration of 4.475mmol/m$^3$ (100ppm). The enhanced kinetic model of aerobic ammonia oxidation can be regarded as the coupling of conventional aerobic ammonia oxidation and $NO_2$ ammonia oxidation. The activity of anaerobic ammonia oxidation peaked at 4.10mg/(g·h) with the $NO_2$ concentration of 2.23 mmol/m$^3$ (50ppm). The enhancement effect of $NO_2$ on anaerobic ammonia oxidation can be described by the modified Andrews model. According to the kinetic characteristics of $NO_2$-enhanced aerobic and anaerobic ammonia oxidation, a kinetic model of $NO_2$-enhanced CANON was established, the simulation results of which showed that the total nitrogen removal rate peaked with a DO of 0.6mg/L and $NO_2$ concentration of 3.0mmol/$m^3$ (67ppm). In this paper, two parallel SBR reactors were inoculated with anaerobic ammonium oxidation sludge to enable quick start of CANON. This paper observed the long-term effects of trace $NO_2$ on reactor with or without trace $NO_2$ (67ppm), and investigated the enhancement effect of $NO_2$ on CANON SBR reactor.

## 2 Experiment

### 2.1 Test equipment, sewage and sludge

The SBR is made of cylindrical plexiglass, with an inner diameter of 11cm, height of 50cm and effective volume of 2.5L. There is a constant temperature water bath layer outside the reactor set at 31±1°C. The exterior is wrapped with black shading material for inhibition of light-induced anaerobic ammonium oxidation bacteria. The temperature of the reactor is controlled at 31±1°C by constant temperature water bath layer. The pH value is adjusted by adding $NaHCO_3$ into the inlet water, and is left alone during operation. The addition of $NO_2$ into the reactor was enabled by a set of gas flow control device, which introduces a proper amount of air and $NO_2$ with a concentration of 5000ppm (the balanced gas consists of $N_2$ and $CO_2$ with a concentration of 300ppm), which obtained $NO_2$ with a concentration of 67ppm in the SBR. See Figure 1 for the diagram of enhanced SBR with trace $NO_2$. The SBR reactor without trace $NO_2$ showed the same performance as previously reported [7].

The inflow of SBR was synthetic wastewater consisting of $(NH_4)_2SO_4$ as the substrate and $NaHCO_3$ as inorganic carbon source with a concentration of 1.5mg /L. Based on existing literatures, the trace elements include two types (trace element liquid I and II) [8, 9]. 1.25mL of trace element liquid I and II were added to 1L of synthetic wastewater.

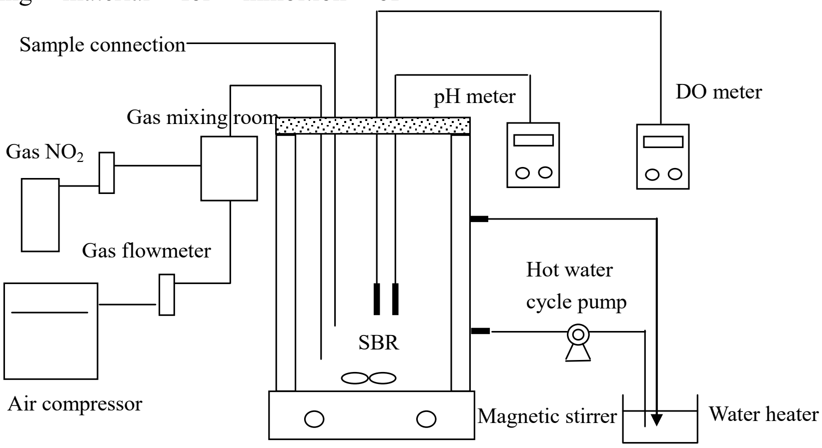



**Fig.1** Schematic diagram of the experimental set-up

### 2.2. Experiment Plan

The anaerobic ammonia oxidation sludge was inoculated and two parallel CANON SBRs (SBR1 and SBR2) were started synchronously. The operation cycle of SBR lasts for 240min (4h), including 12min of water inflow (a volume of about 1L), 138min of oxygen-limited aeration, 66min of anaerobic digestion, 12min of sedimentation, and 12min of discharge. In the first step, the ammonia nitrogen and aeration in the influent gradually increased, with the aeration rate rising from 100mL/min on the 16th day to 150mL/min (on the 46th day), which remained constant till the end. On the 16th day of operation, a small amount of $NO_2$ was introduced into SBR2, while nothing was done to SBR1. Meanwhile, the other operating conditions were kept the same. Then, the paper studied the long-term effect of $NO_2$ on CANON SBR.

### 2.3. Method for analysis

The pH value was determined by online pH electrode (pH2100e, METTLER TOLEDO). The concentration of $NH_4^+$-N was determined by Nessler's reagent spectrophotometry [10], while that of the $NO_2^-$-N was tested by N-(1-naphthyl)-ethylenediamine dihydrochloride spectrophotometric method [10]. Phenol disulfonic acid was used to measure the concentration of $NO_3^-$-N [10], and gravimetric analysis was adopted to determine the MLSS and MLVSS of sludge [10].

## 3 Result and Discussion

### 3.1. Long-term effect of trace $NO_2$ on the operation of reactors

See Figure 2 for the start-up of CANON SBR with or without trace $NO_2$. The operation of the reactor (SBR1) without adding trace $NO_2$ are shown in Figure 2a and 2b. The operation of the gas reactor (SBR2) with 67ppm $NO_2$ added after 16 days of operation is shown in Figure 2c and 2d. The $NO_2$-enhanced SBR2 had a removal efficiency of total nitrogen of 67.5±6.2% and a removal rate of total nitrogen of 0.277±0.017kgN/$m^3$/d (The average values from 32d to 64d are shown in Figure 2d). Meanwhile, SBR1 without $NO_2$ had a removal efficiency of total nitrogen of 65.5±5.0% and a removal rate of total nitrogen of 0.198±0.023kgN/m$^3$/d (The average values from 39d to 52d are shown in Figure 2b), which were lower than the former. According to Figure 2a and 2b, when the aeration rate increased to 150mL/min in the late operation (54d-64d) of SBR1 without $NO_2$, the concentration of effluent nitrate nitrogen rocketed along with a sharp fall in the total nitrogen removal rate and total nitrogen removal efficiency. However, the $NO_2$-enhanced reactor didn't have the problem in the same period (54d-64d).

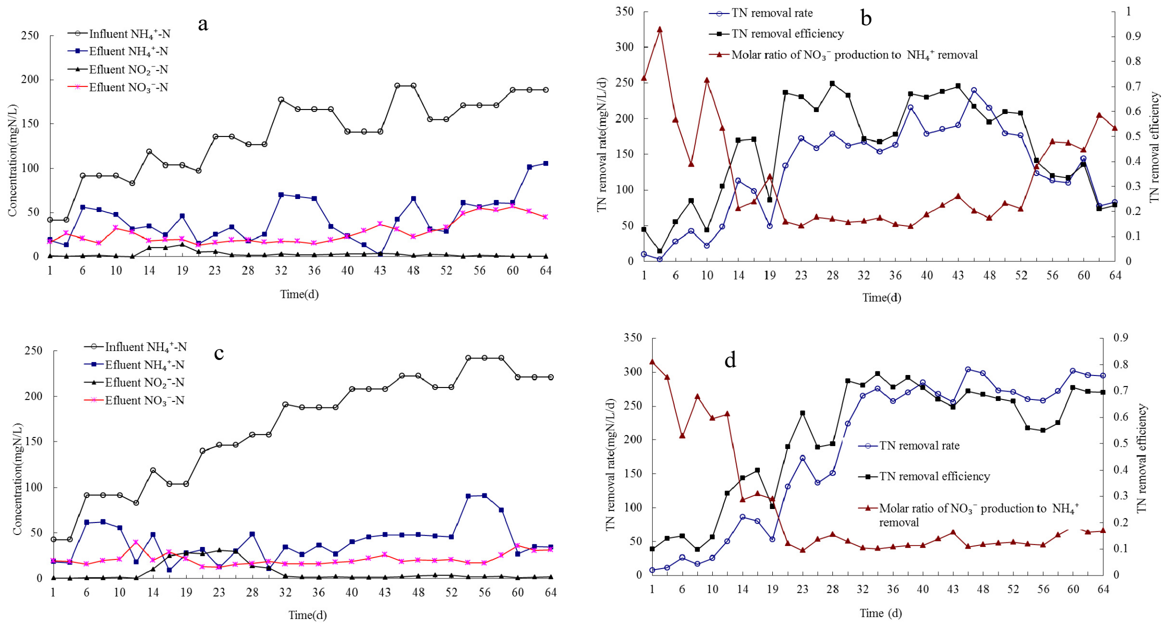
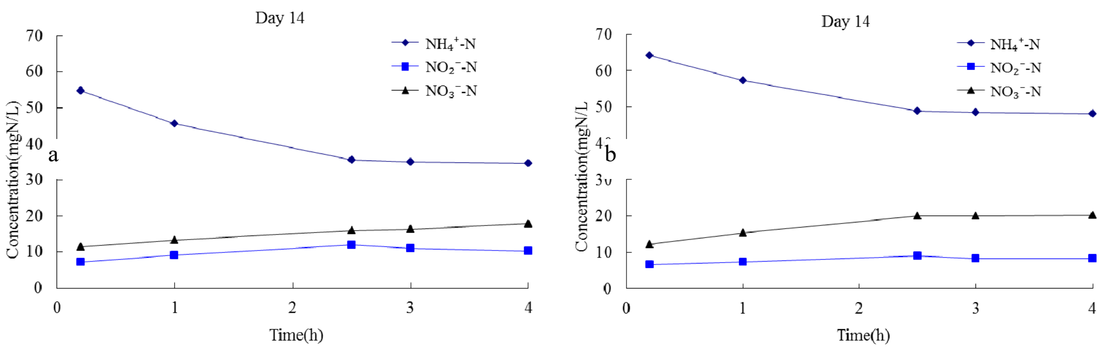



**Fig 2** Performance of CANON SBR with/without trace $NO_2$ addition

To further study the influence of trace $NO_2$ on autotrophic nitrogen removal, a typical operation cycle of 14th day (no trace $NO_2$ was added to both reactors) and 48th day (trace $NO_2$ was added to SBR2) was selected to investigate changes in the concentration of ammonia nitrogen, nitrite nitrogen and nitrate nitrogen in SBR1 and SBR2. The results are shown in Figure 3. On the 14th day of operation before trace $NO_2$ addition (see Figure 3a and 3b for operation on the 14th day), nitrite accumulation was found in both reactors, indicating both SBR reactors were in the initial stage of CANON dominated by nitrosation. SBR1 had a removal rate of total nitrogen of 0.113 kgN/m$^3$/d and an efficiency rate of 48.4%, while the number was 0.086kgN/m$^3$/d and 37.0% for SBR2 respectively. The total nitrogen removal rate and efficiency as well as effluent nitrite accumulation concentration of SBR1 were higher than those of SBR2, while the effluent nitrate nitrogen concentration was lower than that of the latter, indicating that SBR1 had a slightly higher efficiency than that of SBR2. The operation of both reactors on day 48 with trace $NO_2$ addition to SBR2 (67ppm) are shown in Figures 3c and 3d, and no nitrite accumulation was found in the two reactors. The ammonia nitrogen degradation rate of SBR2 was obviously higher than that of SBR1, while the effluent nitrate nitrogen concentration was lower than that of the latter. The removal rate of total nitrogen in reactor SBR1 was 0.216 kgN/m$^3$/d, with a removal efficiency of total nitrogen of 55.8%. The number for SBR2 was 0.299kgN/m$^3$/d and 68.6% respectively. The removal rate and efficiency of total nitrogen in SBR1 were lower than those of SBR2, indicating that long-term addition of trace $NO_2$ can effectively enhance autotrophic nitrogen removal.



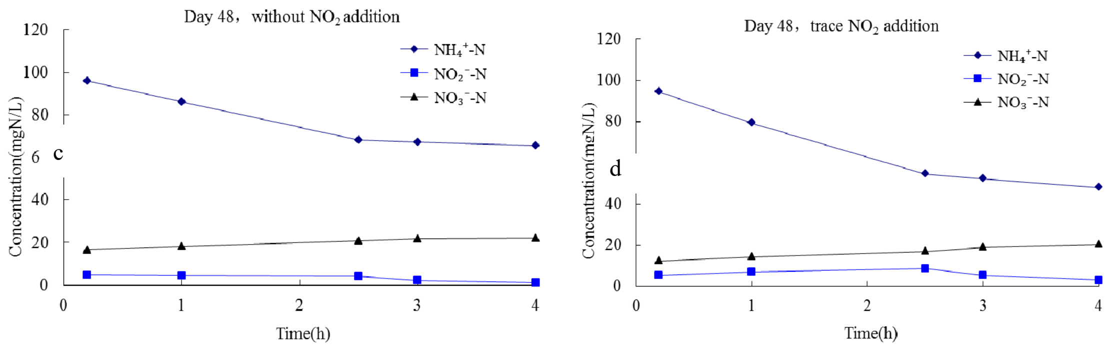



**Fig 3** Profiles of nitrogen concentration during a period with/without trace $NO_2$ addition in CANON SBR

### 3.2. Principle of enhanced autotrophic nitrogen removal by trace $NO_2$ addition

$NO_2$ can enhance aerobic ammonia oxidation. The addition of trace $NO_2$ in the aerobic ammonia oxidation allows conventional aerobic ammonia oxidation with $O_2$ as electron acceptor and $NO_2$ ammonia oxidation with $NO_2$ as electron acceptor to take place simultaneously, thus significantly improving the ammonia oxidation rate [7, 11]. Zhang et al. [11] studied the effect of trace $NO_2$ addition on aerobic ammonia-oxidizing bacteria through batch experiments, and found that the addition of 100ppm $NO_2$ into the mixed gas of 21% $O_2$ could maximize the ammonia oxidation rate (139.11mgN/COD/h) and nitrogen removal rate (34.19%). Kartal et al. [12] studied the long-term effect of high concentration $NO_2$ (4000ppm) on the expression of Nitrosomonas eutropha C91 gene and protein under aerobic and anaerobic conditions. They argued that $NO_2$ may have short-term effects on ammonia-oxidizing bacteria under anaerobic conditions. Under aerobic and anaerobic conditions, the addition of high concentration $NO_2$ resulted in a decrease in protein related to N or C assimilation storage and an increase in protein related to energy metabolism (such as ammonia monooxygenase AmoCAB), while some protein related to nitrogen oxide metabolism did not change. This explains the previously reported enhancing effect of $NO_2$ on ammonia oxidation rate [11]: in the presence of $NO_2$, the energy generated by ammonia oxidation is more used for the basic metabolism of microorganisms than the nutrients storage.

## 4 Conclusion

Two parallel CANON SBRs were quickly started by inoculating anaerobic ammonium oxidation sludge. The total nitrogen removal efficiency of SBR1 without adding trace $NO_2$ was 65.5±5.0%, and the total nitrogen removal rate was 0.198±0.023 kgN/m$^3$/d. The removal efficiency of total nitrogen in SBR2 with continuous $NO_2$ addition was 67.5±6.2%, and the removal rate of total nitrogen was 0.277±0.017 kgN/m$^3$/d. Both indicators of SBR2 were higher than those of SBR1, which means the continuous $NO_2$ addition into CANON SBR could improve the ammonia oxidation rate and enhance the autotrophic nitrogen removal performance by allowing conventional aerobic ammonia oxidation with $O_2$ as electron acceptor and $NO_2$ ammonia oxidation with $NO_2$ as electron acceptor to take place simultaneously.

## Acknowledgements

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