Effects of carbon source on N₂O production in the process of simultaneous nitrification and denitrification via nitrite by aerobic granular sludge

Hong Liang, Xue Li, Shanshan Wang and Dawen Gao*

School of Forestry, Northeast Forestry University, Harbin 150040, China

Abstract: A sequencing batch reactor (SBR) was used to study the effect of carbon source (C₆H₁₂O₆ and CH₃COONa) and C/N ratio (C/N=4:1 and C/N=7:1) on the production of nitrous oxide (N₂O) in the process of simultaneous nitrification and denitrification via nitrite (short-cut SND) by aerobic granular sludge and the removal efficiency of nitrogen under low dissolved oxygen (DO). The results showed that short-cut SND occurred in this system, and the removal efficiency of total nitrogen (TN) at C₆H₁₂O₆ and CH₃COONa were 28.93 % and 41.19 %, respectively. However, the production of N₂O significantly increased when CH₃COONa was used as a carbon source. In addition, the rate of N₂O release when CH₃COONa was a carbon source was 8.34 times the rate when C₆H₁₂O₆ was the carbon source. With the increase of C/N, removal rate of TN and the efficiency of the short-cut SND were increased. The removal efficiency of TN at C/N=7:1 was 90.33 %, which was 2.19 times at C/N=4:1. The percentage of short-cut SND at C/N=4:1 and C/N=7:1 were 87.47% and 95.97%, respectively. The release rate of N₂O from the original 1.14 mg/(g • min) decreased to 0.10 mg/(g • min) after increased the C/N from 4:1 to 7:1.

Keywords: carbon source, C/N ratio; N₂O, nitrogen removal; SND, aerobic granular sludge

*Correspondence to: Dawen Gao, School of Forestry, Northeast Forestry University, Harbin 150040, People’s Republic of China; E-mail: dawengao@gmail.com

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1. Introduction

Nitrous oxide can cause greenhouse effect, ozone depletion and other environmental issues[1]. In wastewater treatment processes, 0.10%~0.13% of the TN emissions is N₂O[2,3]. The Intergovernmental Panel on Climate Change has reported that over 100 years, the global warming effect of N₂O is 278 times higher than that of CO₂ on a molar basis[4]. The emissions of nitrous oxide are usually accompanied by nitrification and denitrification in conventional wastewater treatment processes[5,6]. Large amounts of researches were conducted on the effect of dissolved oxygen, temperature, solid retention time (SRT) and salinity on N₂O accumulation in nitrogen removal process[7–10]. A carbon source is another factor that has an important influence on the nitrogen removal efficiency. Therefore, the addition of an external carbon source is required to improve nitrogen removal in wastewater treatment systems[11]. Previous research has shown that carbon had a significant effect on N₂O production in the process of denitrification by
aerobic granular sludge. Schalk-Otte et al. revealed the superiority of acetate, butyrate and malate as carbon source of N₂O release in the biological denitrification process[12]. The C/N ratio is a factor that influences the release of N₂O in the denitrification process[13]. Due to the deficiency of electron donors in denitrification, the SND is not proper when the C/N ratio is low[14]. Avrahami et al. studied the complete and short-cut nitrification and denitrification process and compared the sludge in different phases of the complete and short-cut SND and found that the quantity of N₂O released in short-cut nitrification and denitrification is higher than that in complete nitrification and denitrification[15].

Aerobic granular sludge will be used as a new technology in waste water treatment. Ammonia oxidizing bacteria (AOB) and denitrification bacteria can survive in aerobic granular sludge due to the particular spatial structure, thus causing SND[16,17]. Many studies have paid attention to N₂O production by conventional flocculent sludge; however, little information is available regarding the effect of carbon source on N₂O production in aerobic granular sludge process, which has lower energy, good settling performance and shorter reaction time consumption in the short-cut nitrification process.

In this research, a SBR reactor was used to study aerobic granular sludge for different carbon sources and C/N ratios, and the removal efficiencies of TN and production of N₂O during the short-cut SND process were analysed. The present study will provide a theoretical basis for studying the process that reduces the release of N₂O and improves the efficiency of nitrogen.

2. Materials and Methods

2.1 Reactor Configuration and Conditions

A laboratory-scale SBR was made of plexiglas, and the working volume was 3.2 L. The operational temperature was at 31±0.5°C (Figure 1). The DO was maintained at 1.0 mg/L. The experiment adopted the traditional time-control mode. The aerobic nitrifying system included the influent, aeration, precipitation, water drainage and idle stages. The reactor was run for two cycles every day. The side face of the reactor was twisted with heating wire and then covered with asbestos cloth to maintain the water temperature in the reactor.

The seeding sludge was a well-cultivated granular sludge with a high nitrogen removal efficiency. The mixed liquor suspended solids (MLSS) were maintained at approximately 2500 mg/L. The size of the mature aerobic granular sludge is substantially equal to the average particle (round or oval) diameter of 2 mm; the colour of the sludge is golden yellow, and it has a compact structure[18]. The composition of the synthetic wastewater included 4±0.5 mg/L PO₄³⁻-P and 40±2 mg/L NH₄⁺-N. The COD concentration control was based on the conditions set. And 1 mL/L nutrient solution was used[19]. NaHCO₃ was added with the pH maintained at 7.3~7.8.

The study used C₆H₁₂O₆ and CH₃COONa as the carbon sources at C/N=4:1. The reactor was operated at both C/N=4:1 and 7:1 when CH₃COONa was the carbon source.

2.2 Analytical Methods

During the nitrogen removal process, DO, pH, oxidation-reduction potential (ORP), and temperature were measured using a WTW Handheld Multi-parameter Instrument (WTW 340i, WTW Company, Germany). NO₂⁻-N, NH₄⁺-N, NO₃⁻-N, MLSS and COD were measured using standard methods[20]. N₂O production during biological nitrogen removal was composed of gaseous N₂O and dissolved N₂O. The concentration of N₂O was analyzed using a gas chromatograph (GC) equipped with an electron-capture detector (ECD) (GC-14B, Shimadzu, Japan) at 345°C[19]. Dissolved N₂O was sampled and measured according to the study conducted by Chen et al.[19]. The analysis of N₂O
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production was conducted in triplicate, and the average value was calculated.

2.3 Date Analysis

$E_{\text{SND}}$ (%) is the efficiency of the short-cut SND process. The $E_{\text{SND}}$ value is calculated according to the following formula (Equation 1).

$$E_{\text{SND}} = \frac{\Delta \text{TN}}{\Delta (\text{NH}_4^+ - \text{N})} \times 100 \quad (1)$$

In the formula, $\Delta \text{TN}$ is the reduced TN concentration (mg/L), and $\Delta (\text{NH}_4^+ - \text{N})$ is the reduced NH$_4^+$-N concentration (mg/L).

The dosing of nitrogen is from NH$_4$Cl and does not contain organic nitrogen. TN is therefore determined by theoretical calculations, including NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N. The removal efficiency of TN is calculated according to the following formula (Equation 2).

$$\text{TN removal} = \frac{TN_{\text{(influent)}} - TN_{\text{(effluent)}}}{TN_{\text{(influent)}}} \times 100 \quad (2)$$

In the formula, $TN_{\text{(influent)}} = NO_2^- - N_{\text{(influent)}} + NO_3^- - N_{\text{(influent)}} + NH_4^+ - N_{\text{(influent)}} \cdot TN_{\text{(effluent)}} = NO_2^- - N_{\text{(effluent)}} + NO_3^- - N_{\text{(effluent)}} + NH_4^+ - N_{\text{(effluent)}}$

$Re \ (\text{mg/m}^3 \cdot \text{min})$ is the N$_2$O release rate. $Re$ is calculated according to the following formula (Equation 3).

$$Re = \frac{\Delta C_{\text{N}_2\text{O}-\text{N}(\text{emission})}}{\Delta t} \quad (3)$$

In the formula, $\Delta C_{\text{N}_2\text{O}-\text{N}(\text{emission})}$ is the emitted N$_2$O (mg/m$^3$); $\Delta t$ is the reaction time (min).

3. Results and Discussion

3.1 Effects of Carbon Source on Short-cut SND Nitrogen Removal and N$_2$O Emissions

Effects of Carbon Source on the Short-cut SND Nitrogen Removal

The experiment used the C$_6$H$_{12}$O$_6$ as the sole carbon source to start the SBR reactor (C/N = 4:1), the system remained stable through 120 d. The SBR reactor used CH$_3$COONa as the carbon source until 195 d, and keeping the other conditions unchanged. The mean TN removal efficiency was not significant when C$_6$H$_{12}$O$_6$ was the carbon source (Figure 2). The effluent concentration of NH$_4^+$-N was kept below 3 mg/L. The NO$_3^-$-N concentration in the effluent was low. But the NO$_2^-$-N concentration was approximately 18.86 mg/L, which is similar to the results of the SND of aerobic granular sludge [21]. After changing the carbon source (CH$_3$COOH), the effluent concentration of NH$_4^+$-N reached up to 19 mg/L, and the NO$_2^-$-N concentration and NO$_3^-$-N concentration in the effluent were kept below 5 mg/L. At 195 days, the TN removal reached to 40.19% which is higher than using C$_6$H$_{12}$O$_6$ as the carbon source.

Comparison of the two types of carbon sources shows that different carbon sources have a significant impact on the short-cut SND (Figure 3). The $E_{\text{SND}}$ increased from 32.17% to 87.47% when the carbon source was changed from C$_6$H$_{12}$O$_6$ to CH$_3$COONa. The reason for this phenomenon may be the promotion of the denitrification process when the carbon source was changed from C$_6$H$_{12}$O$_6$ to CH$_3$COONa. The reason for this phenomenon may be the promotion of the denitrification process when the carbon source was changed from C$_6$H$_{12}$O$_6$ to CH$_3$COONa.
source was CH₃COONa (C/N=4:1), leading to the increase of TN removal efficiency and the reduction of the final effluent NO₂⁻-N concentration. Therefore, the use of CH₃COONa as the carbon source in the de-
nitrification process can result in a higher specific de-
nitrification rate[22].

**Effect of Carbon Source on N₂O Production**

The release of N₂O was significantly different in different carbon source under the same conditions (Figure 4). When the carbon source was C₆H₁₂O₆ (Figure 4A), the release of N₂O reaches the maximum (175.16 mg/m³) in 120 min. The dissolved N₂O increased up to 59.31 mg/m³. The dissolved N₂O increased when the carbon source was CH₃COONa (Figure 4B), and it increased to the maximum (198.86 mg/m³) when the typical reaction is over. The release of N₂O is apparently higher when the carbon source was CH₃COONa than when the carbon source was C₆H₁₂O₆. The results proved that CH₃COONa is a superior carbon source to denitrifying bacteria in the removal of NO₂⁻-N compared to C₆H₁₂O₆.

When the carbon source was CH₃COONa the release of N₂O is 11.51 times that when the carbon source was C₆H₁₂O₆ (Figure 5). The efficiency of the two carbon sources is different for denitrifying bacteria in the short-cut SND process. CH₃COONa was superior for denitrifying bacteria in the reduction in the concentration of NO₂⁻-N, while it increases the concentration of N₂O. The short-cut denitrification process is the major procedure to produce and release N₂O.

**3.2 Effects of the C/N ratio on the Short-cut SND Nitrogen Removal and N₂O Emissions**

**Effects of the C/N ratio on the Short-cut SND Nitrogen Removal**

The effect of different conditions of the C/N ratios of 4:1 and 7:1 for a typical cycle with CH₃COONa as the carbon source was studied, considering the following: NH₄⁺-N, NO₂⁻-N, NO₃⁻-N concentrations and removal efficiency of TN (Figure 6). The NO₃⁻-N and NO₂⁻-N concentrations were 1.96 mg/L and 3.56 mg/L at

![Figure 5. Effects of different types of carbon sources on the production of N₂O.](image)
The typical cycle of NO$_2^-$-N, NO$_3^-$-N, and NH$_4^+$-N concentrations and the TN removal efficiency at different C/N ratios; C/N=4:1 (A), C/N=7:1 (B).

C/N=4:1, respectively (Figure 6A). In addition, the NH$_4^+$-N concentration at the beginning of the cycle decreased from 42.45 mg/L to 21.12 mg/L. The NO$_3^-$-N, NO$_2^-$-N and NH$_4^+$-N concentrations were 0.51 mg/L, 1.12 mg/L and 2.71 mg/L at C/N=7:1, respectively (Figure 6B). The higher C/N ratio will be better for the TN removal, and the short-cut SND efficiency improved with increasing C/N.

The two C/N ratio operating conditions have a significant impact on the effect of short-cut SND by aerobic granular sludge. After the C/N ratio increased from 4:1 to 7:1, the removal efficiency of TN increased from 41.19% to 90.33%. In addition, for the different C/N ratios, the NO$_2^-$-N concentration did not change. The effect of the C/N ratio on denitrification by aerobic granular sludge was remarkable. The amount of NO$_2^-$-N in the nitrification process is determined by the C/N ratio. The C/N ratio increased from 4:1 to 7:1, and the system of E$_{SN}$D increased from 87.47% to 95.97% (Figure 7). Other research also showed that the C/N ratio can control the SND efficiency throughout the SND system$^{[14]}$. Providing inadequate electronics at the denitrification process and inhibiting the denitrification process at C/N=4:1, results in a low E$_{SN}$D. After the C/N ratio increased from 4:1 to 7:1, the growth of the denitrifying bacteria was promoted, thereby reducing the effluent NO$_2^-$-N concentration.

Effect of C/N Ratio on N$_2$O Production

At different C/N ratios (C/N=4:1 and C/N=7:1), the N$_2$O generation and NH$_4^+$-N, NO$_2^-$-N, and NO$_3^-$-N concentrations changed in the short-cut SND system (Figure 4B and Figure 8A). As shown in Figure 8A, in a typical cycle, both released N$_2$O and dissolved N$_2$O generation showed a gradual upward trend at C/N=7:1, and it was found that there was more released N$_2$O produced than dissolved N$_2$O. The NO$_3^-$-N and NO$_2^-$-N concentrations in the effluent were 0.51 mg/L and 1.12 mg/L, respectively. Compared with C/N=4:1, the higher C/N ratio can reduce the amount of N$_2$O production. Increasing the C/N ratio can increase the proportion of heterotrophic bacteria and provide more electron donors$^{[23]}$.

The effects of the C/N ratio on the production of released N$_2$O and dissolved N$_2$O in a typical cycle system are studied (Figure 8B). The released N$_2$O and dissolved N$_2$O were 238.51 mg/m$^3$ and 198.86 mg/m$^3$ at C/N=4:1, respectively, which was greater than the amount of N$_2$O emission at C/N=7:1. During the process of short-cut SND by aerobic granular sludge, N$_2$O productions exhibited significant changes mainly due to low C/N ratios, leading to a shortage in the carbon supply. It is a disadvantage to denitrifying bacteria to use their own internal carbon sources in the
denitrification process. Therefore, the bacteria are in a state of hunger, which leads to the process of denitrification remaining incomplete, resulting in an increase in the emissions of N2O\[12,24\].

**Nitrogen Balance Analysis**

A typical cycle analysis of the nitrogen balance is conducted at C/N=4:1 and C/N= 7:1 (Figure 9). At the end of the aeration process in the system, when the C/N was 4:1, the residual NH4\(^+\)-N concentration was 49.75% of the total nitrogen dosage, and the NO\(_2\) -N and NO\(_3\) -N were 8.39% and 4.62% of the total nitrogen dosage, respectively. Simultaneously, the amount of N\(_2\)O produced was 437.37 mg/m\(^3\), and it was accounting for 1.03% of the total nitrogen. In addition, the percentage of the total nitrogen loss was 36.2% in the system (Figure 9A). When C/N was 7:1, the residual NH4\(^+\)-N concentration was 6.27% of the total nitrogen dosage, and the NO\(_2\) -N and NO\(_3\) -N concentrations were 2.60%, and 1.18% of the total nitrogen dosage, respectively.

Simultaneously, the amount of N\(_2\)O produced was 60.27 mg/m\(^3\), and it was accounting for 0.11% of the total nitrogen dosage. In addition, the percentage of nitrogen loss was 89.82% in the system (Figure 9B). We found the system have more residues when the C/N ratio was 4:1 because the removal efficiency of TN and SND were both low (Table 1). After the C/N ratio increased to 7:1, the removal efficiency of TN increased to 90.33%, which was 2.19 times at C/N= 4:1; E\(_{SNDD}\) also increased to 95.82%, and the rate of N\(_2\)O release was significantly reduced from 1.14 mg/(m\(^3\) • min) to 0.10 mg/(m\(^3\) • min). The difference of N\(_2\)O productions is mainly due to the C/N ratio, and the low C/N ratio would lead to a shortage of the carbon supply. This shortage is detrimental to denitrifying bacteria, as it causes them to use their own internal carbon sources for the denitrification process\[25\]. Thus, by increasing the C/N ratio, the rate of N\(_2\)O release can be reduced.

**4. Conclusion**

(1) The effect of the carbon source on E\(_{SNDD}\) by aerobic granular sludge was remarkable. In a typical cycle, the
effluent NO$_2^-$-N concentration is always high when C$_6$H$_{12}$O$_6$ was used as the carbon source at the short-cut SND by aerobic granular sludge in SBR. The system of TN removal rose to 41.19%, the SND percentage greatly increased to 87.47%, and the effluent NO$_2^-$-N concentration was 3.56 mg/L when CH$_3$COONa was the carbon source. The rate of N$_2$O release for CH$_3$COONa as the carbon source is 8.34 times as high as the rate for C$_6$H$_{12}$O$_6$ as the carbon source. CH$_3$COONa is conducive to achieving short-cut SND but will dramatically increase N$_2$O production at the same time.

(2) The effect of the C/N ratio on N$_2$O production and E$_{SND}$ by aerobic granular sludge was remarkable. The removal efficiency of TN at C/N=7:1 was 90.33%, which was 2.07 times the production at C/N=4:1. After the C/N ratio increased to 7:1, the specific nitrification rate did not change significantly, and the rate of N$_2$O release decreased from 1.14 mg/(m$^3$ • min) to 0.10 mg/(m$^3$ • min), whereas the E$_{SND}$ increased from 87.47% to 95.82%. In conclusion, the higher C/N ratio is good for short-cut SND and can greatly reduce the production of N$_2$O as well.

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No conflict of interest was reported by the authors.

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