RESEARCH ARTICLE

Effects of carbon source on N_2O production in the process of simultaneous nitrification and denitrification via nitrite by aerobic granular sludge

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Abstract: A sequencing batch reactor (SBR) was used to study the effect of carbon source ($C_6H_{12}O_6$ and CH_3COONa) and C/N ratio (C/N=4:1 and C/N=7:1) on the production of nitrous oxide (N_2O) 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 $C_6H_{12}O_6$ and CH_3COONa were 28.93 % and 41.19 %, respectively. However, the production of N_2O significantly increased when CH_3COONa was used as a carbon source. In addition, the rate of N_2O release when CH_3COONa was a carbon source was 8.34 times the rate when $C_6H_{12}O_6$ 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 N_2O 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; N_2O, nitrogen removal; SND, aerobic granular sludge

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Received: November 26, 2015; **Accepted:** May 27, 2016; **Published Online:** November 24, 2016

Citation: Liang H, Li X, Wang S, Gao D, 2016, Effects of carbon source on N_2O production in the process of simultaneous nitrifica-tion and denitrification via nitrite by aerobic granular sludge. *Applied Environmental Biotechnology*, vol.1(2): 10–17. http://dx.doi.org/10.18063/AEB.2016.02.002.

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 N_2O[2,3]. The Intergovernmental Panel on Climate Change has reported that over 100 years, the global warming effect of N_2O is 278 times higher than that of CO_2 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 N_2O 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 N_2O 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 N_2O release in the biological denitrification process[12]. The C/N ratio is a factor that influences the release of N_2O 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 N_2O 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 N_2O production by conventional flocculent sludge; however, little information is available regarding the effect of carbon source on N_2O 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 N_2O 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 N_2O 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

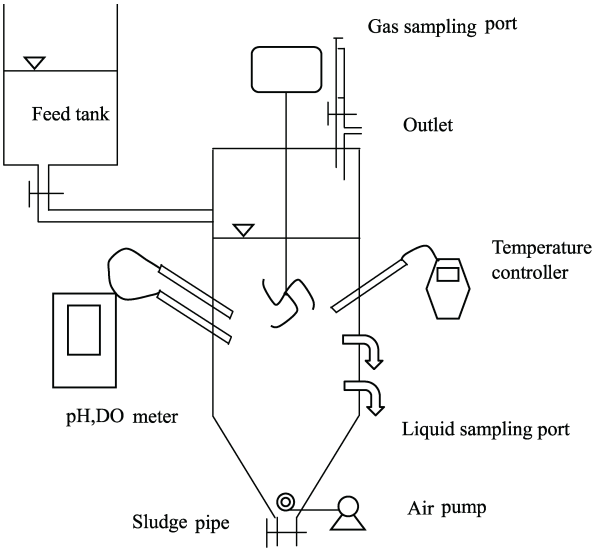

Figure 1. Schematic diagram of the experimental system.

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 PO_4^{3-}-P and 40±2 mg/L NH_4^+-N. The COD concentration control was based on the conditions set. And 1 mL/L nutrient solution was used[19]. $NaHCO_3$ was added with the pH maintained at 7.3~7.8.

The study used $C_6H_{12}O_6$ and CH_3COONa as the carbon sources at C/N=4:1. The reactor was operated at both C/N=4:1 and 7:1 when CH_3COONa 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). NO_2^--N, NH_4^+-N, NO_3^--N, MLSS and COD were measured using standard methods[20]. N_2O production during biological nitrogen removal was composed of gaseous N_2O and dissolved N_2O. The concentration of N_2O was analyzed using a gas chromatograph (GC) equipped with an electron-capture detector (ECD) (GC-14B, Shimadzu, Japan) at 345°C[19]. Dissolved N_2O was sampled and measured according to the study conducted by Chen *et al*.[19]. The analysis of N_2O

production was conducted in triplicate, and the average value was calculated.

2.3 Date Analysis

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

$$E_{SND} = \frac{\Delta TN}{\Delta(NH_4^+ - N)} \times 100 \quad (1)$$

In the formula, ΔTN is the reduced TN concentration (mg/L), and $\Delta(NH_4^+$-N) is the reduced NH_4^+-N concentration (mg/L).

The dosing of nitrogen is from NH_4Cl 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).

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

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

Re (mg/m^3·min) is the N_2O release rate. Re is calculated according to the following formula (Equation 3).

$$Re = \frac{\Delta C_{N_2O\text{-}N(emission)}}{\Delta t} \quad (3)$$

In the formula, $\Delta C_{N_2O\text{-}N(emission)}$ is the emitted N_2O (mg/m^3); Δt is the reaction time (min).

3. Results and Discussion

3.1 Effects of Carbon Source on Short-cut SND Nitrogen Removal and N_2O Emissions

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

The experiment used the $C_6H_{12}O_6$ 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_3COONa as the carbon source until 195 d, and keeping the other conditions unchanged. The mean TN removal efficiency was not significant when $C_6H_{12}O_6$ 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_3COOH), 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 $C_6H_{12}O_6$ 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_{SND} increased from 32.17% to 87.47% when the carbon source was changed from $C_6H_{12}O_6$ to CH_3COONa. The reason for this phenomenon may be the promotion of the denitrification process when the carbon

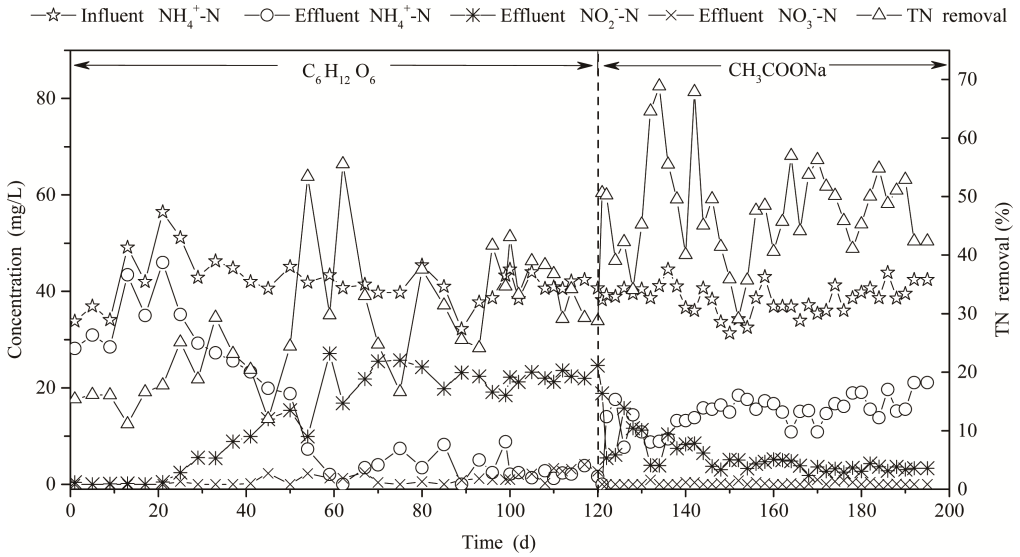

Figure 2. NO_2^--N, NO_3^--N and NH_4^+-N concentrations and TN removal with different carbon sources.

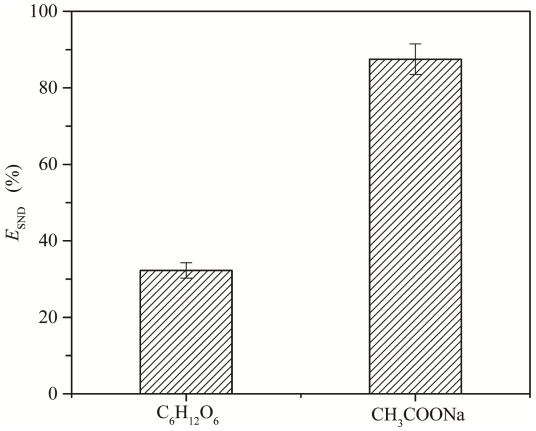

Figure 3. E_{SND} with different carbon source.

source was CH_3COONa (C/N=4:1), leading to the increase of TN removal efficiency and the reduction of the final effluent NO_2^--N concentration. Therefore, the use of CH_3COONa as the carbon source in the denitrification process can result in a higher specific denitrification rate[22].

Effect of Carbon Source on N_2O Production

The release of N_2O was significantly different in different carbon source under the same conditions (Figure 4). When the carbon source was $C_6H_{12}O_6$ (Figure 4A), the release of N_2O reaches the maximum (175.16 mg/m^3) in 120 min. The dissolved N_2O increased up to 59.31 mg/m^3. The dissolved N_2O increased when the carbon source was CH_3COONa (Figure 4B), and it increased to the maximum (198.86 mg/m^3) when the typical reaction is over. The release of N_2O is apparently higher when the carbon source was CH_3COONa than when the carbon source was $C_6H_{12}O_6$. The results proved that CH_3COONa is a superior carbon source to denitrifying bacteria in the removal of NO_2^--N compared to $C_6H_{12}O_6$.

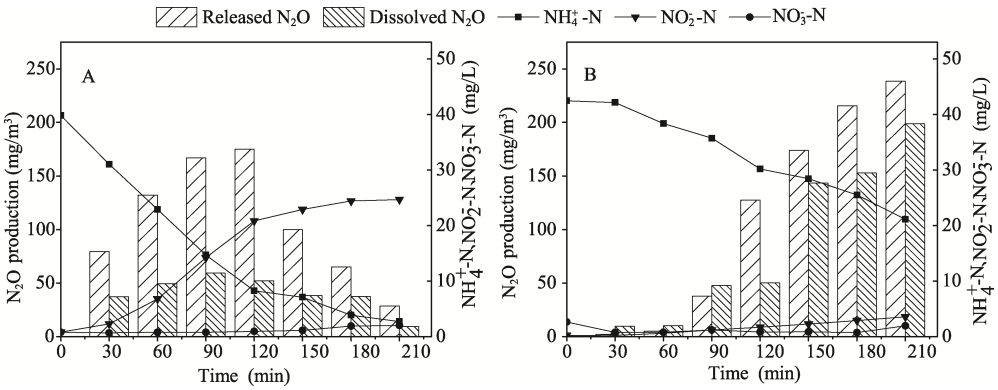

Figure 4. The typical cycle of NH_4^+-N, NO_2^--N, and NO_3^--N concentrations and N_2O production with different carbon sources, with $C_6H_{12}O_6$ as the carbon source **(A)** and CH_3COONa as the carbon source **(B)**.

When the carbon source was CH_3COONa, the release of N_2O is 11.51 times that when the carbon source was $C_6H_{12}O_6$ (Figure 5). The efficiency of the two carbon sources is different for denitrifying bacteria in the short-cut SND process. CH_3COONa was superior for denitrifying bacteria in the reduction in the concentration of NO_2^--N, while it increases the concentration of N_2O. The short-cut denitrification process is the major procedure to produce and release N_2O.

3.2 Effects of the C/N ratio on the Short-cut SND Nitrogen Removal and N_2O 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 CH_3COONa as the carbon source was studied, considering the following: NH_4^+-N, NO_2^--N, NO_3^--N concentrations and removal efficiency of TN (Figure 6). The NO_3^--N and NO_2^--N concentrations were 1.96 mg/L and 3.56 mg/L at

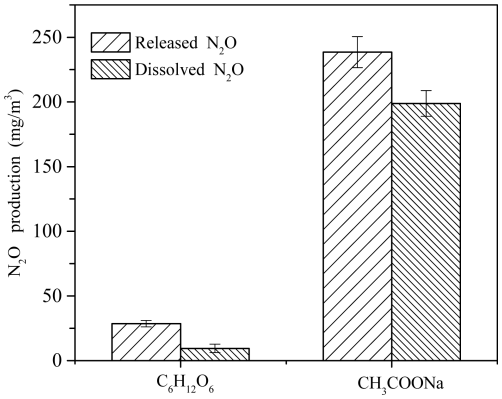

Figure 5. Effects of different types of carbon sources on the production of N_2O.

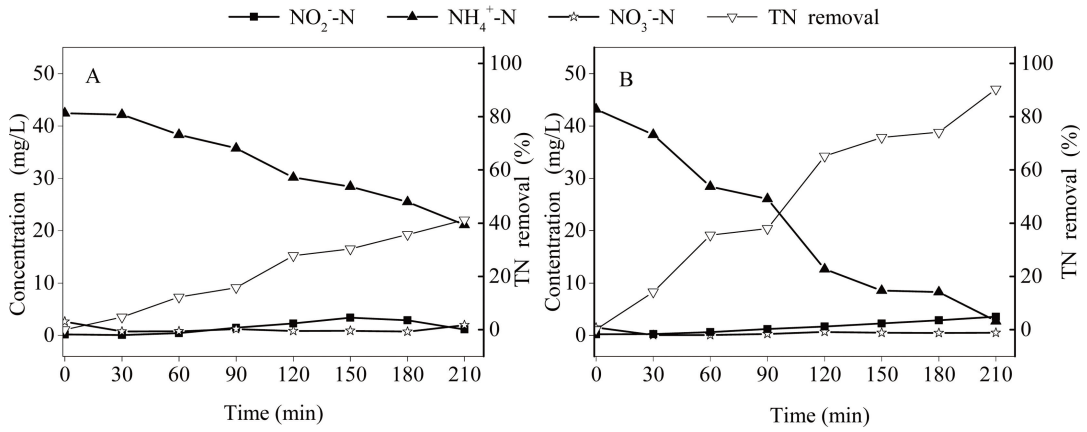

Figure 6. The typical cycle of NO_2^--N, NO_3^--N, and NH_4^+-N concentrations and the TN removal efficiency at different C/N ratios; C/N=4:1 **(A)**, C/N=7:1 **(B)**.

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_{SND} 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_{SND}. 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.

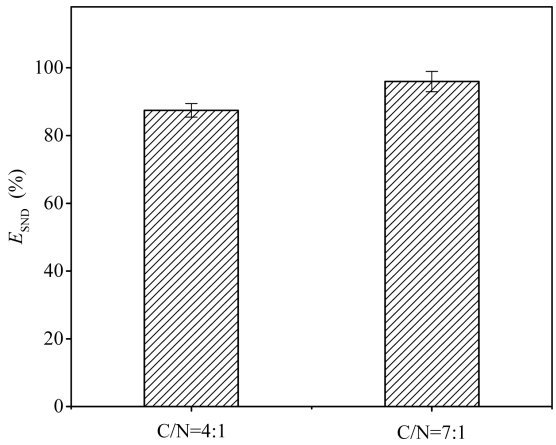

Figure 7. The effect of different C/N ratios on E_{SND}.

Effect of C/N Ratio on N_2O Production

At different C/N ratios (C/N=4:1 and C/N=7:1), the N_2O 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_2O and dissolved N_2O generation showed a gradual upward trend at C/N=7:1, and it was found that there was more released N_2O produced than dissolved N_2O. 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_2O 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_2O and dissolved N_2O in a typical cycle system are studied (Figure 8B). The released N_2O and dissolved N_2O 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_2O emission at C/N=7:1. During the process of short-cut SND by aerobic granular sludge, N_2O 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

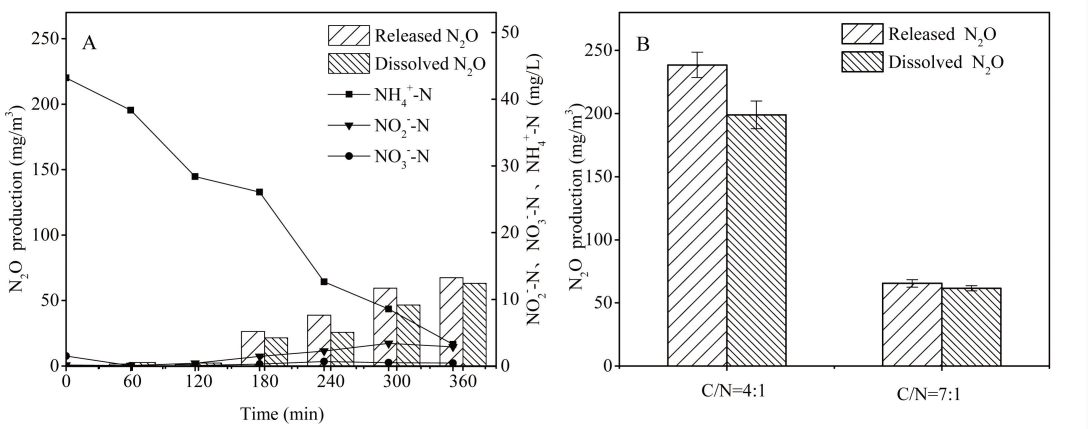

Figure 8. A typical cycle of NO_2^--N, NO_3^--N, and NH_4^+-N concentrations and N_2O production **(A)**. The influence of different C/N ratios on N_2O production **(B)**.

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 N_2O[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 NH_4^+-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_2O produced was 437.37 mg/m^3, and it was accounting for 1.03% of the total nitrogen. In addition, the percentage of the nitrogen loss was 36.2% in the system (Figure 9A). When C/N was 7:1, the residual NH_4^+-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_2O 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_{SND} also increased to 95.82%, and the rate of N_2O release was significantly reduced from 1.14 mg/(m^3 • min) to 0.10 mg/(m^3 • min). The difference of N_2O 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_2O release can be reduced.

4. Conclusion

(1) The effect of the carbon source on E_{SND} by aerobic granular sludge was remarkable. In a typical cycle, the

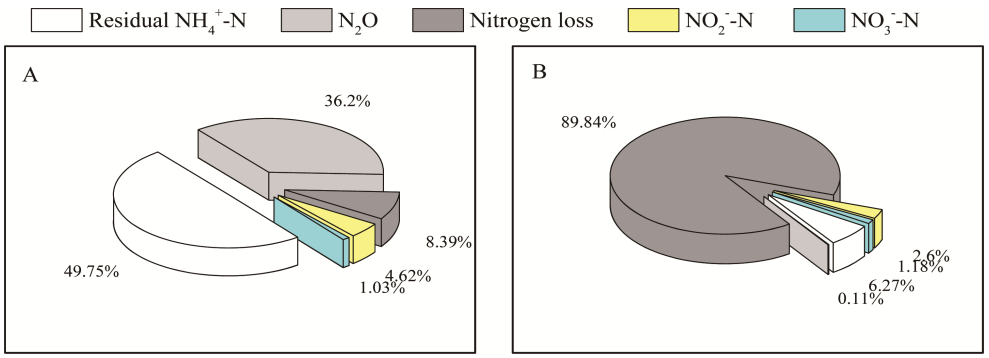

Figure 9. The analysis of the nitrogen balance at different C/N ratios, C/N=4:1**(A)** and C/N=7:1**(B)**.

Table 1. The specific nitrification rate and the N_2O release rate at different C/N ratio

Carbon source	C/N ratio	TN removal (%)	Specific nitrification rate mg/(g·min)	N_2O release rate mg/(m^3·min)	E_{SND} (%)
CH_3COONa	4:1	41.19	0.041	1.14	87.47
CH_3COONa	7:1	90.33	0.045	0.10	95.82

effluent NO_2^--N concentration is always high when $C_6H_{12}O_6$ 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_3COONa was the carbon source. The rate of N_2O release for CH_3COONa as the carbon source is 8.34 times as high as the rate for $C_6H_{12}O_6$ as the carbon source. CH_3COONa is conducive to achieving short-cut SND but will dramatically increase N_2O production at the same time.

(2) The effect of the C/N ratio on N_2O 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_2O 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_2O as well.

Conflict of Interest and Funding

No conflict of interest was reported by the authors.

References

1. Søvik A K and Kløve B, 2007, Emission of N_2O and CH_4 from a constructed wetland in southeastern Norway. *Science of the Total Environment*, vol.380(1–3): 28–37. http://dx.doi.org/10.1016/j.scitotenv.2006.10.007.
2. Takaya N, Catalan-Sakairi M A B, Sakaguchi Y, *et al*. 2003, Aerobic denitrifying bacteria that produce low levels of nitrous oxide. *Applied and Environmental Microbiology*, vol.69(6): 3152–3157. http://dx.doi.org/10.1128/aem.69.6.3152-3157.2003.
3. Wang J, Zhang J, Wang J, *et al*. 2011, Nitrous oxide emissions from a typical northern Chinese municipal wastewater treatment plant. *Desalination and Water Treatment*, vol.32(1–3): 145–152. http://dx.doi.org/10.5004/dwt.2011.2691.
4. Intergovernmental Panel on Climate Change, 2013, *Climate Change 2013: The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, viewed March 15, 2013, <http://www.climatechange2013.org>
5. Okabe S, Oshiki M, Takahashi Y, *et al*. 2011, N_2O emission from a partial nitrification-anammox process and identification of a key biological process of N_2O emission from anammox granules. *Water Research*, vol.45(19): 6461–6470. http://dx.doi.org/10.1016/j.watres.2011.09.040.
6. Wunderlin P, Mohn J, Joss A, *et al*. 2012, Mechanisms of N_2O production in biological wastewater treatment under nitrifying and denitrifying conditions. *Water Research*, vol.46(4): 1027–1037. http://dx.doi.org/10.1016/j.watres.2011.11.080.
7. Schulthess R v, Kühni M and Gujer W, 1995, Release of nitric and nitrous oxides from denitrifying activated sludge. *Water Research*, vol.29(1): 215–226. http://dx.doi.org/10.1016/0043-1354(94)e0108-i.
8. Von Schulthess R, Wild D and Gujer W, 1994, Nitric and nitrous oxides from denitrifying activated sludge at low oxygen concentration. *Water Science and Technology*, vol.30(6): 123–132.
9. Inubushi K, Barahona M A and Yamakawa K, 1999, Effects of salts and moisture content on N_2O emission and nitrogen dynamics in Yellow soil and Andosol in model experiments. *Biology and Fertility of Soils*, vol.29(4): 401–407. http://dx.doi.org/10.1007/s003740050571.
10. Thörn M and Sörensson F, 1996, Variation of nitrous oxide formation in the denitrification basin in a wastewater treatment plant with nitrogen removal. *Water Research*, vol.30(6): 1543–1547. http://dx.doi.org/10.1016/0043-1354(95)00327-4.
11. Isaacs SH, Henze M, Søeberg H, *et al*. 1994, External carbon source addition as a means to control an activated sludge nutrient removal process. *Water Research*, vol.28(3): 511–520. http://dx.doi.org/10.1016/0043-1354(94)90002-7.
12. Schalk-Otte S, Seviour R J, Kuenen J, *et al*. 2000, Nitrous oxide (N_2O) production by *Alcaligenes faecalis* during feast and famine regimes. *Water Research*, vol.34(7): 2080–2088. http://dx.doi.org/doi:10.1016/S0043-1354(99)00374-7.
13. Tallec G, Garnier J, Billen G, *et al*. 2008, Nitrous oxide emissions from denitrifying activated sludge of urban wastewater treatment plants, under anoxia and low

oxygenation. *Bioresource Technology*, vol.99(7): 2200–2209. http://dx.doi.org/10.1016/j.biortech.2007.05.025.

14. Chiu Y C, Lee L L, Chang C N, *et al*. 2007, Control of carbon and ammonium ratio for simultaneous nitrification and denitrification in a sequencing batch bioreactor. *International Biodeterioration & Biodegradation*, vol. 59(1): 1–7. http://dx.doi.org/10.1016/j.ibiod.2006.08.001.
15. Avrahami S, Conrad R and Braker G, 2002, Effect of soil ammonium concentration on N_2O release and on the community structure of ammonia oxidizers and denitrifiers. *Applied and Environmental Microbiology*, vol.68(11): 5685–5692. http://dx.doi.org/10.1128/aem.68.11.5685-5692.2002.
16. Gao D W, Liu L, Liang H, *et al*. 2011, Aerobic granular sludge: characterization, mechanism of granulation and application to wastewater treatment. *Critical Reviews in Biotechnology*, vol.31(2): 137–152. http://dx.doi.org/10.3109/07388551.2010.497961.
17. Pochana K and Keller J, 1999, Study of factors affecting simultaneous nitrification and denitrification (SND). *Water Science and Technology*, vol.39(6): 61–68. http://dx.doi.org/10.1016/S0273-1223(99)00123-7.
18. Gao D W, Yuan X J, Liang H, *et al*. 2011, Comparison of biological removal via nitrite with real-time control using aerobic granular sludge and flocculent activated sludge. *Applied Microbiology and Biotechnology*, vol.89(5): 1645–1652. http://dx.doi.org/10.1007/s00253-010-2950-3.
19. Chen L L, Wen Z D, Wang W H, *et al*., 2016, Effects of temperature on N_2O production in the process of nitrogen removal by micro-expansion aerobic granular sludge. *Desalination and Water Treatment*, 1–6. http://dx.doi.org/10.1080/19443994.2016.1187089.
20. Apha L M, Apha A W W A, Klinkert W E F, *et al*., 2005, *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association (APHA): Washington, D.C., USA.
21. Yang S and Yang F L, 2011, Nitrogen removal via short-cut simultaneous nitrification and denitrification in an intermittently aerated moving bed membrane bioreactor. *Journal of Hazardous Materials*, vol.195: 318–323. http://dx.doi.org/10.1016/j.jhazmat.2011.08.045.
22. Elefsiniotis P, Wareham D G and Smith M O, 2004, Use of volatile fatty acids from an acid-phase digester for denitrification. *Journal of Biotechnology*, vol.114(3): 289–297. http://dx.doi.org/10.1016/j.jbiotec.2004.02.016.
23. Fu B, Liao X, Ding L, *et al*. 2010, Characterization of microbial community in an aerobic moving bed biofilm reactor applied for simultaneous nitrification and denitrification. *World Journal of Microbiology and Biotechnology*, vol.26(11): 1981–1990. http://dx.doi.org/10.1007/s11274-010-0382-y.
24. Matsumoto S, Terada A and Tsuneda S, 2007, Modeling of membrane-aerated biofilm: effects of C/N ratio, biofilm thickness and surface loading of oxygen on feasibility of simultaneous nitrification and denitrification. *Biochemical Engineering Journal*, vol.37(1): 98–107. http://dx.doi.org/10.1016/j.bej.2007.03.013.
25. Itokawa H, Hanaki K and Matsuo T, 2001, Nitrous oxide production in high-loading biological nitrogen removal process under low COD/N ratio condition. *Water Research*, vol.35(3): 657–664. http://dx.doi.org/10.1016/s0043-1354(00)00309-2.