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Impact of gas-water ratios on N_2O emissions in biological aerated filters and analysis of N_2O emissions pathways

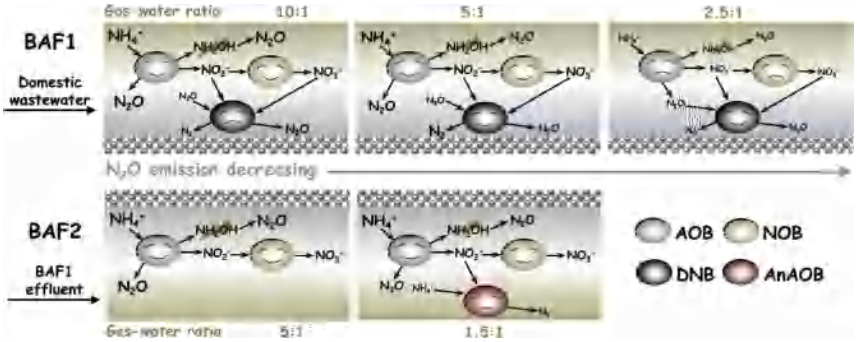
Qing Yang [a,*], Bin Cui [a], Yao Zhou [b], Jianmin Li [a], Zhibin Liu [a], Xiuhong Liu [a]

[a] National Engineering Laboratory for Advanced Municipal Wastewater Treatment and Reuse Technology, Beijing University of Technology, Beijing 100124, PR China
[b] Beijing Drainage Group Water Design & Research Institute Co., Ltd, Beijing 100022, PR China

HIGHLIGHTS

- Impact of gas-water ratios on N_2O emissions in BAF was rarely studied.
- N_2O emissions in BAFs treating domestic wastewater were analyzed.
- Batch test and isotopic measurement were used to analyze N_2O emission pathways.
- Different pathways involving N_2O emission were observed in the studied tandem BAFs setup.

GRAPHICAL ABSTRACT



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ABSTRACT

Biological aerated filter (BAF) is a widely applied biofilm process for wastewater treatment. However, characteristics of nitrous oxide (N_2O) production in BAF are rarely reported. In this study, two tandem BAFs treating domestic wastewater were built up, and different gas-water ratios were controlled to explore N_2O production pathway. Results showed that N_2O production increased with increasing gas-water ratio in both BAFs; higher gas-water ratio promoted more N_2O releasing from hydroxylamine oxidation process. To improve nitrogen removal performance and reduce N_2O emission, the optimal gas-water ratios for BAF1 and BAF2 were 5:1 and 1.5:1, respectively. Most of N_2O was produced from ammonia oxidizing bacteria (AOB) denitrification and hydroxylamine oxidation in BAF1, and heterotrophic denitrification contributed to relieve N_2O emission. In BAF2, N_2O was emitted from AOB denitrification and hydroxylamine oxidation by 87.8% and 12.2%, respectively. Heterotrophic denitrification is a N_2O sink in BAF, causing BAF1 produced less N_2O than BAF2 with the same gas-water ratio. Enhancing heterotrophic denitrification and anaerobic ammonium oxidation (Anammox) activity could reduce the release of N_2O in BAFs.

1. Introduction

N_2O is one of long living greenhouse gases that cause global warming and ozone depletion(Frutos et al., 2018). N_2O emission from wastewater treatment is a growing concern and accounts for 3.4% of the global N_2O budget (Duan et al., 2017). Thus it is necessary to explore

* Corresponding author at: No. 100 Ping Leyuan, Chao Yang District, Beijing 100124, PR China.
E-mail address: yangqing@bjut.edu.cn (Q. Yang).

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N_2O production pathways in wastewater treatment plants (WWTPs) to reduce its release. There are three pathways in wastewater biological nitrogen removal process for N_2O production: (1) aerobic oxidation of the intermediate NH_2OH during nitrification, (2) aerobic denitrification NO_2^--N by AOB, and (3) insufficient denitrification of N_2O by heterotrophic denitrifiers just lacking N_2O reductase genes or having a lower N_2O reductase expression activity (Ahn et al., 2010; Hu et al., 2012; Yang et al., 2009).

Extensive studies about N_2O emission from wastewater treatment have been conducted in the past decades years, including N_2O release amount, impact factors and production pathways (Blum et al., 2018; Jones et al., 2013; Peng et al., 2014; Sanford et al., 2012). However, most of the investigations focused on activated sludge (suspend sludge) system. Different from activated sludge, biofilm refers to a group of organized microorganism whose surface is attached by extracellular macromolecules of microorganism (Flemming and Wingender, 2010). Due to the advantages of intercepting and enriching functional microorganism, high treatment efficiency, and less sludge yield, biofilm process has been widely applied to wastewater treatment recently (Han et al., 2020; Xu et al., 2018).

Due to the heterogeneity of substrate distribution in biofilm, dominant bacteria are different at different depth of biofilm (Sabba et al., 2018). As a result, N_2O production in biofilm process is more complex than that in activated sludge. Characteristics and mechanisms of N_2O production in autotrophic nitrifying or heterotrophic denitrifying biofilm have been widely investigated (Conthe et al., 2019; Peng et al., 2015a; Sabba et al., 2017). However, there were few reports on N_2O release in complex biofilm systems in which heterotrophic and autotrophic bacteria coexisted. Mathematical modeling has been applied to explore N_2O emitting process within stratified biofilm, but most of present models are different from existing biofilm (Todt and Dörsch, 2016). Hence it is necessary to reveal the characteristics of N_2O production in biofilm system via experimental research.

BAF is a typical fixed bed biofilm reactor that combines the functions of physical filtration and biological treatment. Because of the advantages of small footprint, large treatment volume and strong shock resistance capacity (Abou-Elela et al., 2015), BAF is widely applied to municipal wastewater treatment. In BAF, substrate concentrations gradually decrease along filter height, which influences N_2O production (Sabba et al., 2018). Furthermore, gas-water ratio is an essential parameter to optimize the performance of BAF (Leopoldo Mendoza-Espinosa, 1999). Therefore, in this paper two BAFs fed with domestic wastewater with/without biodegradable chemical oxygen demand (COD) were set up to study N_2O production. The purpose of this study is to: (1) reveal the effect of gas-water ratios on N_2O emissions in BAFs; (2) analyze N_2O generation pathways in different BAFs; (3) determine the better gas-water ratios for improving nitrogen removal and reducing N_2O emission in BAFs.

2. Materials and methods

2.1. Configuration and operation of reactors

In this study two identical lab-scale BAFs named BAF1 and BAF2 were used, and BAF1 was in series with BAF2. Each BAF was consisted of a supporting layer, a filter layer and a clarification layer, and working volume was 18.4 L. Volcanic with diameter of 3–5 mm was used as filter media. The surface of volcanic is rough and with high porosity, which is benefit for enriching and intercepting biomass. The top of BAF was sealed and connected with a gas sampling bag for gas collection. In addition, some sampling valves were distributed along filter height of the biofilter.

Domestic wastewater was treated in this study, which was taken from a septic tank in the residential area of Beijing. The concentration of COD, NH_4^+-N, NO_2^--N, and NO_3^--N in the domestic wastewater were in the range of 63–222 mg/L, 35–51 mg/L, 0.31–0.02 mg/L, and 1.21–0.48 mg/L, respectively. Domestic wastewater was first treated by BAF1, and its effluent was further treated by BAF2. Treatment volumes of two BAFs were both 41.2 L/d, and hydraulic retention times were both 2.2 h. The gas-water ratios of BAF1 were controlled at 10:1, 5:1, and 2.5:1, while the ones of BAF2 were controlled at 5:1 and 1.5:1. Both BAFs were operated with temperature of 20.3–26.8 °C.

2.2. Chemical analysis

Influent and effluent samples were daily collected and filtered through a 0.45 um acetate fiber filter, and the concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were determined by a Lachat Quik Chem8500 Flow Injection Analyzer (Lachat Instruments, Milwaukee, USA). COD concentration was measured by a COD quick-analysis apparatus (Lianhua Tech. Co., Ltd., 5B-1, China). Dissolved oxygen (DO) concentration and pH value were detected by a DO probe and a pH probe (WTW 3420, German), respectively. Mixed liquor volatile suspended solid (MLVSS) was analyzed according to the standard method (APHA, 1998).

2.3. NO and N_2O measurement

The concentration of gaseous N_2O was determined by gas chromatograph (Agilent 7890A, USA) in triplicate as previous report (Yang et al., 2009). A N_2O microsensor (Unisense A/S, Aarhus, Denmark) connected to a high-sensitivity picoammeter (PA 2000, Unisense A/S, Aarhus, Denmark) was used for measuring dissolved N_2O and recording N_2O on-line in batch tests. A NO microsensor (Unisense A/S, Aarhus, Denmark) was also used in this study to monitor NO on-line. Before each measurement the standard curves of NO and N_2O were calibrated according to operation manual.

2.4. Isotopic measurement

A continuous flow - isotope ratio mass spectrometry (Thermo Fisher Scientific, Dellta V Plus, USA) was used in this study to identify the sources of N_2O. The first step in the isolation and purification procedure involves the removal of water and CO_2 through a chemical trap filled with Ascarite-II and Mg $(ClO_4)_2$. Nitrous oxide, together with trace amounts of water and CO_2 which have not been retained by the chemical trap are condensed in a cryogenic trap maintained at −196 °C by immersion in liquid N_2. O_2 and N_2 are not trapped by this process and are vented to atmosphere. The trapped species are released on warming and the contents transferred using a reduced He flow (1.0 mL/min) to a small cryofocus unit adjacent to the Poraplot column (25 m × 0.32 mm i.d.). The column is maintained at 40 °C and the carrier gas flow at 1.0 mL/min. Downstream of the column, the effluent passes through an Open Split where one portion is drawn towards the mass spectrometer. A trap maintaining at −100 °C removes any water present, while the remaining GC effluent is diluted by additional He and flows through to a Thermal Conductivity Detector (TCD). Analysis was performed with the mass spectrometer tuned for masses 44, 45 and 46.

2.5. Batch tests

When gas-water ratios were controlled at 5:1 in BAF1, and 1.5:1 in BAF2, batch tests were carried out to analyze N_2O release pathways under aerobic and anoxic conditions. A sealed 600 mL reactor equipped with pH, DO probes and NO, N_2O microsensors was used. Before each test, constant DO concentration or anaerobic condition of 500 mL reaction medium was kept by aerating high purity nitrogen or the mixture of oxygen and argon. The pH value of reaction medium was adjusted to 7.0–7.5 by dosing 1 M $NaHCO_3$ or 1 M HCl. 50 mL filter media taken out from BAF was washed three times by distilled water to remove the background concentrations of COD, NH_4^+-N, NO_2^--N, and NO_3^--N, and then transferred to filter media basket. Constant reaction temperature (30 °C) and mixed state were kept by magnetic stirring apparatus.

The values of DO, pH, NO and N_2O were logged in 60 s intervals. Liquid samples were collected at intervals to analyze medium compositions (COD, NH_4^+-N, NO_2^--N and NO_3^--N). After each test MLVSS of the used filter medium was measured.

2.6. DNA extraction and real-time quantitative PCR (QPCR)

Genomic DNA was extracted from freeze-dried biofilm using the Soil DNA Extraction Kit (MP Biomedical, Solon, OH, USA) according to the manufacturer's instructions. Extracted DNA samples were kept at −20 °C. DNA concentrations were measured by a Nano Drop ND-1000 (Nano Drop Technologies, Wilmington, DE, USA). Functional gene abundances including ammonia monooxygenase subunit A of ammonia-oxidizing archaea (*amoA*-AOA), ammonia monooxygenase subunit A of ammonia-oxidizing bacteria (*amoA*-AOB), nitrite oxidoreductase beta subunit (*nxrB*), nitrate reductase alpha subunit (*narG*), cd-cytochrome nitrite reductase (*nirS*), Cu-containing nitrite reductase (*nirK*), nitric oxide reductase subunit B (*cnorB*), nitrous-oxide reductase (*nosZ*), and hydrazine synthase subunit (*hzsB*) were quantified via QPCR. The operation procedure of QPCR was same to our previous study (Cui et al., 2019).

2.7. Calculations

The ammonia removal efficiency (ARE) and total inorganic nitrogen removal efficiency (TINRE) were calculated by Eqs. (1) and (4), respectively.

$$\mathrm{ARE} = \left(NH_4^+ - N_{inf} - NH_4^+ - N_{eff}\right)/NH_4^+ - N_{inf} \times 100 \quad (1)$$

$$TIN_{inf} = NH_4^+ - N_{inf} + NO_2^- - N_{inf} + NO_3^- - N_{inf} \quad (2)$$

$$TIN_{eff} = NH_4^+ - N_{eff} + NO_2^- - N_{eff} + NO_3^- - N_{eff} \quad (3)$$

$$\mathrm{TINRE} = \left(TIN_{inf} - TIN_{eff}\right)/TIN_{inf} \times 100\% \quad (4)$$

where NH_4^+-N_{inf}, NO_3^--N_{inf} and NO_2^--N_{inf} are the ammonia, nitrate and nitrite concentration of the influent, respectively, and NH_4^+-N_{eff}, NO_3^--N_{eff} and NO_2^--N_{eff} are the ammonia, nitrate and nitrite concentration of the effluent, respectively, TIN_{inf} and TIN_{eff} are the concentrations of influent total inorganic nitrogen and effluent total inorganic nitrogen, respectively.

The site preference (SP) was calculated by Equation. (5). The contributions of NH_2OH oxidation and AOB denitrification to the total N_2O production can be obtained from Equation. (6):

$$\mathrm{SP} = \delta^{15}N_\alpha - \delta^{15}N_\beta \quad (5)$$

$$F_{ND} = (1 - F_{NN}) = (SP - SP_{NN})/(SP_{ND} - SP_{NN}) \times 100 \quad (6)$$

where $\delta^{15}N_\alpha$ is the value of central position of N atom, $\delta^{15}N_\beta$ is the value of outer position of N atom. F_{ND} is the relative contribution to total N_2O from AOB denitrifiction pathway, F_{NN} is the relative contribution to total N_2O from NH_2OH oxidation pathway. SP_{NN}, the SP signature value for

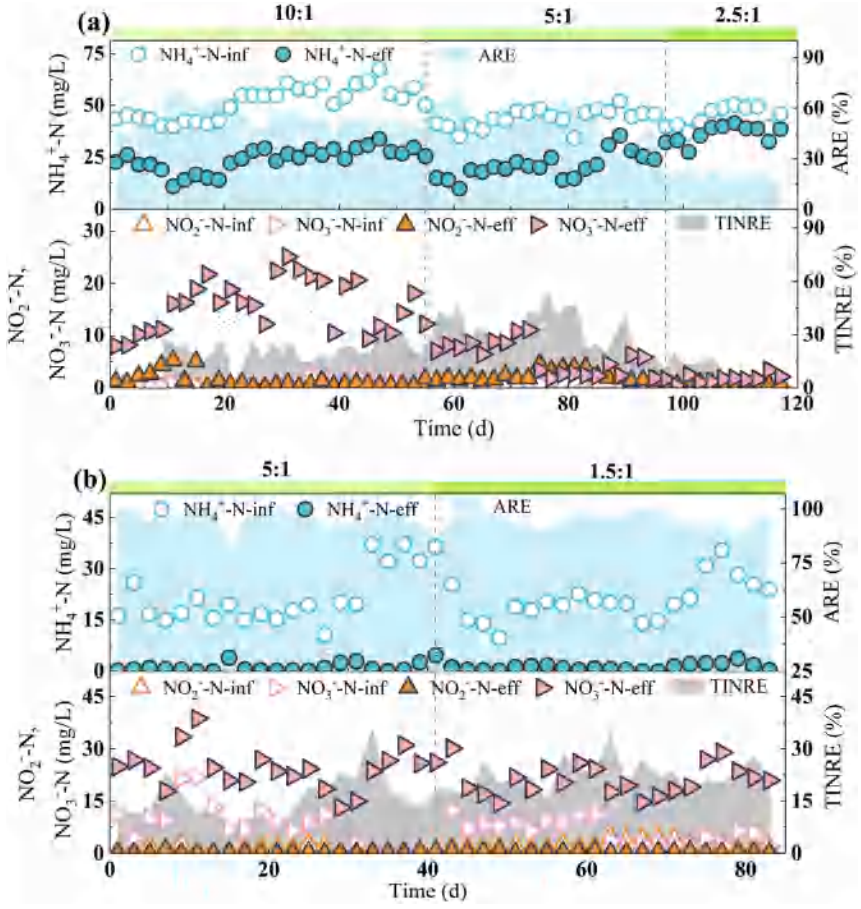

Fig. 1. Variations of NH_4^+-N, NO_2^--N, NO_3^--N, ARE and TINRE in the long-term operation of BAF1 and BAF2 with different gas-water ratios ((a): BAF1, (b): BAF2). Blue hollow circle represents influent NH_4^+-N, blue solid circle represents effluent NH_4^+-N, blue area represents ARE, orange hollow triangle represents influent NO_2^--N, orange solid triangle represents effluent NO_2^--N, pink hollow triangle represents influent NO_3^--N, pink solid triangle represents effluent NO_3^--N, gray area represents TINRE.

NH_2OH oxidation pathway, is 28.5‰. SP_{ND}, the SP signature value of AOB denitrification, is −2‰.

3. Results and discussions

3.1. The performance of BAFs under different gas and water ratios

Performance of BAF1 under gas-water ratio values of 10:1, 5:1 and 2.5:1 is shown in Fig. 1(a). COD oxidation, nitrification and denitrification occurred to BAF1. AREs stabilized at 53.0% when gas-water ratios were 10:1 and 5:1, while it dropped to 19.4% with gas-water ratio decreasing to 2.5:1. TINRE was 21.5% at gas-water ratio of 10:1, while it enhanced to 35.7% with gas-water ratio decreasing to 5:1, and little NO_3^--N remained in effluent. When gas-water ratio declined to 2.5:1, decreasing nitrification activity caused TINRE to be only 14.1%, and NO_3^--N was undetectable in effluent. Therefore, gas-water ratio influenced nitrification and denitrification activity of BAF1; gas-water ratio of 5:1 could achieve the better nitrogen removal performance.

BAF1 effluent was further treated by BAF2 with gas-water ratios of 5:1 and 1.5:1 (Fig. 1(b)). ARE over 90% was found in the effluent at both conditions, and almost no NH_4^+-N was found in effluent. Although little biodegradable COD was remained in BAF1 effluent, TINRE of BAF2 reached to 15.3% and 23.2% with gas-water ratios of 5:1 and 1.5:1, respectively, which indicated that other reactions occurred to BAF2 for

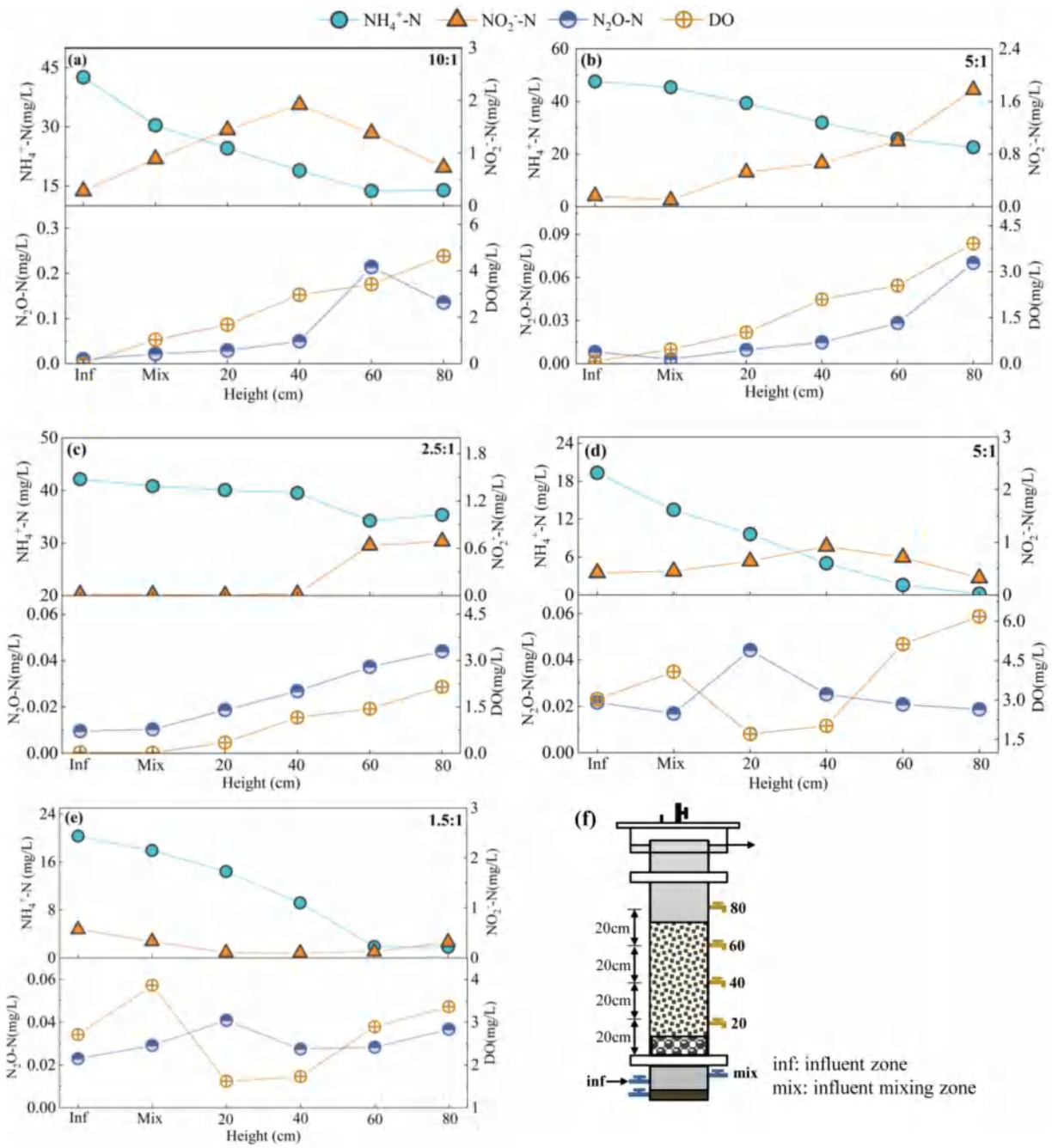

Fig. 2. Variations of effluent NH_4^+-N, NO_2^--N, DO and dissolved N_2O at different positions with different gas-water ratios. (a), (b) and (c) are BAF1, (d) and (e) are BAF2, and X axis represents the position of BAF, which is illustrated in (f). Blue solid circle represents effluent NH_4^+-N, orange solid triangle represents effluent NO_2^--N, dark blue half solid circle represents dissolved N_2O, orange circle represents DO.

nitrogen removal. Therefore, gas-water ratio of 1.5:1 was favorable for complete nitrification and nitrogen removal in BAF2.

3.2. N_2O production in BAFs

3.2.1. Dissolved N_2O

Dissolved and gaseous N_2O were measured at intervals during this study. Variations of effluent compositions and dissolved N_2O concentration along filter height were shown in Fig. 2, which were measured on the 13th, 71th, and 103th day of BAF1, and on the 25th and 55th day of BAF2. DO concentrations gradually increased along filter height of BAF1 under different gas-water ratios. In contrast, NH_4^+-N and COD concentrations both gradually decreased. Generally, NO_2^--N was regarded as the main inducing factor for N_2O production. NO_2^--N reached the peak of 1.92 mg/L at filter height of 40 cm when gas-water ratio was 10:1, and it kept increasing along filter height at gas-water ratio both of 5:1 and 2.5:1. It should be note that dissolved N_2O-N concentration gradually increased with the increasing NO_2^--N concentration along filter height. When gas-water ratio was 10:1, dissolved N_2O-N reached to 0.214 mg/L at filter height of 60 cm, and 0.136 mg/L N_2O-N was remained in effluent. Whereas dissolved N_2O-N concentrations were only 0.070 mg/L and 0.044 mg/L in effluent with gas-water ratios of 5:1 and 2.5:1, respectively.

Performance of BAF2 along filter height was presented in Fig. 2 (d) and (e). Along filter height, DO concentration quickly decreased with the oxidation of NH_4^+-N, and then it gradually increased at biofilter top. There was no obvious nitrite accumulation phenomenon under both gas-water ratio conditions. Dissolved N_2O-N reached peak at the height of 20 cm filter media, and it was only 0.041 mg/L with gas-water ratio of 1.5:1, which was less than that of gas-water ratio of 5:1.

3.2.2. Gaseous N_2O

Due to air stripping, part of gaseous product released to air, thus gaseous product was also analyzed. As shown in Fig. 3(a), higher gas-water ratio led to more N_2O emitting in BAF1. When gas-water ratio was 10:1, gaseous N_2O concentration was 32.1–45.7 ppm, average 0.441 mg N_2O-N was emitted from per liter wastewater correspondingly. However, when gas-water ratios decreased to 5:1 and 2.5:1, gaseous N_2O concentrations were only 0.151 mg/L and 0.065 mg/L, respectively. N_2O emission factors including $N_2O/\Delta NH_4^+$-N and $N_2O/\Delta TIN$ were calculated. At gas-water ratios of 10:1 and 5:1, BAF1 displayed the similar ammonium removal capacity, but the higher TINRE was attained with gas-water ratio of 5:1. However, both N_2O emission factors were much higher at gas-water ratio of 10:1, which indicated that gas-water ratio effected reaction activity and N_2O emission process. Furthermore, both N_2O production and reduction can be due to heterotrophic denitrification process; enhanced denitrification activity might reduce N_2O releasing at limited DO concentration. When gas-water ratio declined from 5:1 to 2.5:1, ARE and TINRE both decreased, while N_2O emission factors were similar.

In BAF2, averaged N_2O emission concentrations were 33.9 and 16.9 ppm at gas-water ratios of 5:1 and 1.5:1, respectively. Complete nitrification process was achieved at both gas-water ratios, while the higher nitrogen removal performance was attained at gas-water ratio of 1.5:1. When gas-water ratio decreased from 5:1 to 1.5:1, $N_2O/\Delta NH_4^+$-N declined from 1.22% to 0.35%, and $N_2O/\Delta TIN$ declined from 4.78% to 1.16%. Additionally, no denitrification activity was detected under aerobic conditions. Therefore, gas-water ratio influenced N_2O emission from AOB denitrification and NH_2OH oxidation process. Previous study showed that in nitrifying sludge system, N_2O production rate increased with increasing DO concentration within 0–3 mg/L (Peng et al., 2014). Biofilm model prediction found that $N_2O/\Delta NH_4^+$-N varied from 1.6% to 7.2% with DO concentration in the range of 0–8 mg/L (Sabba et al., 2015), which was obviously higher than that measured in BAF. Thus, it is necessary to reveal the effect of gas-water ratio on N_2O production via experimental study.

3.3. N_2O production pathway in BAF1

N_2O production pathway in BAF1 was analyzed by using the combination of batch test and N_2O isotope analysis. As Fig. 4 shown N_2O-N concentrations were the highest with NO_2^--N and $NH_2OH{\cdot}HCl$ as substrates under aerobic condition among seven batch tests (Fig. 4(b) and (c)), which indicated that both AOB denitrification and hydroxylamine oxidation contributed to N_2O production in BAF1. The SP values (Table 1) of N_2O emitted from batch test (b) and (c) further confirmed that N_2O produced from AOB denitrification and hydroxylamine oxidation pathway (Wunderlin et al., 2013). In batch test (d) and (e), values of pH slowly increased, and SP value of N_2O in batch test (e) was −0.604, indicating that heterotrophic denitrification was prevalent. Furthermore, dissolved N_2O performed a first increasing first and then decreasing tendency, suggesting the capacity of producing and reducing N_2O via heterotrophic denitrification in BAF1. Although initial nitrogen concentrations were different among the batch tests, the $\Delta N_2O/\Delta N$ of (b) that was calculated on the 120th min was the highest than that of (d), (e) and (g) (Table 2), indicating that AOB denitrification produced more N_2O than heterotrophic denitrification. Comparing Fig. 4(b) and (f), at the early stage of batch tests, N_2O concentration of Fig. 4(b) was lower than that of Fig. 4(f). While at later stage, N_2O concentration of Fig. 4(b) was higher than that of Fig. 4(f), implying that denitrifiers can reduce N_2O even under aerobic condition (Sabba et al., 2017). Therefore, AOB denitrification, hydroxylamine oxidation and heterotrophic denitrification all contributed to N_2O production in BAF1.

Functional gene copy numbers were quantified via QPCR to reveal nitrogen transformation process (Fig. 5). Hydroxylamine oxidation has been described for AOB and AOA (Mark Poth, 1985; Stieglmeier et al., 2014), while nitrifier denitrification has only been reported for AOB (Kozlowski et al., 2016b; Stieglmeier et al., 2014). *AmoA* gene of AOA was in a low abundance in BAF1. Copy number of *amoA* of AOB reached to 10^7 copies/mg dry sludge, and kept constant at different phases. Gene

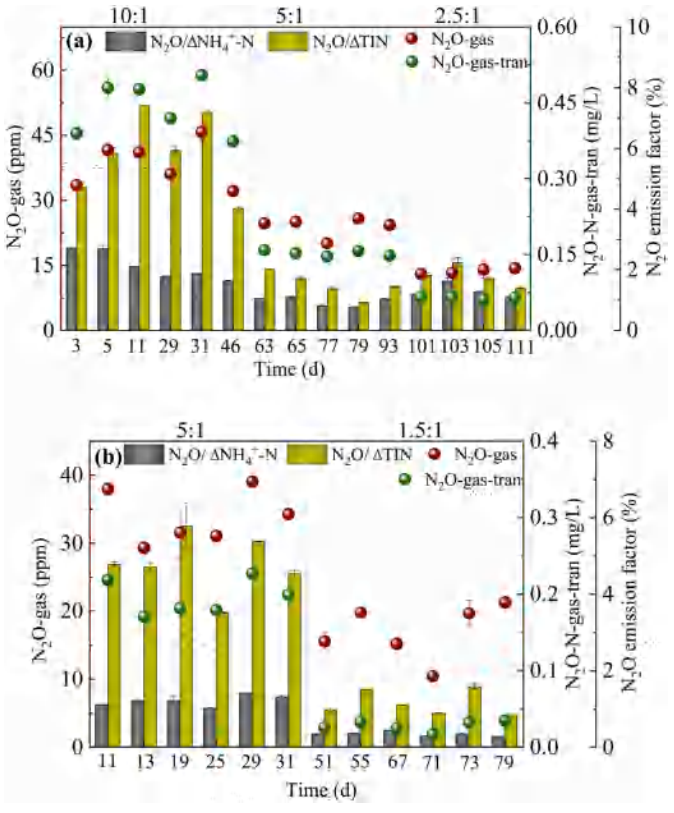

Fig. 3. Variations of gaseous N_2O and N_2O emission factors of (a) BAF1 and (b) BAF2 under different gas and water ratios.

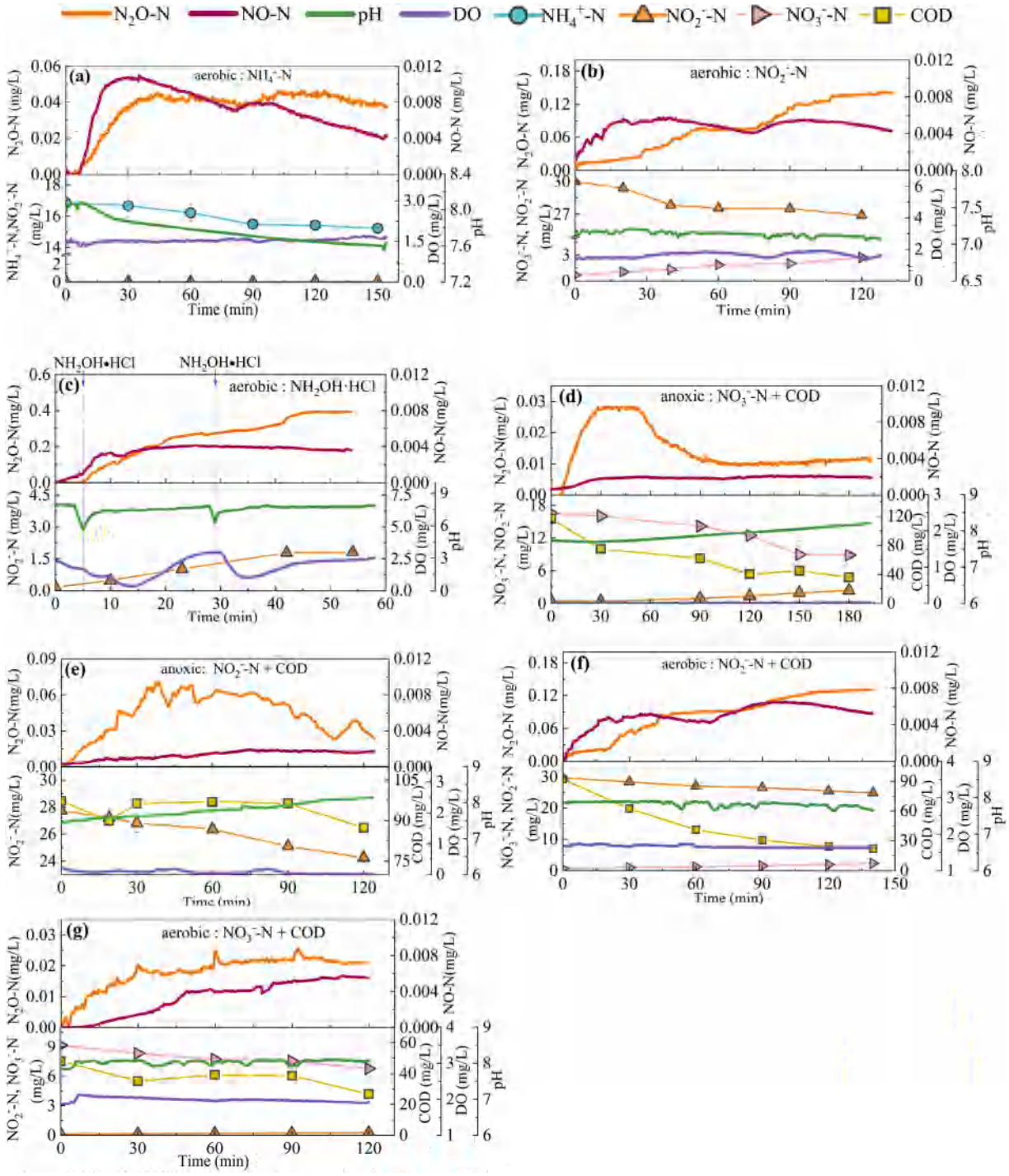

Fig. 4. Variations of NH_4^+-N, NO_2^--N, NO_3^--N, COD, DO, pH and dissolved N_2O-N, NO-N concentration in different batch tests with BAF1biofilm sample.

abundances involved in denitrification were much higher than other functional gene abundances. Previous study showed that the copper nitrite reductase in AOB has been found to be more oxygen tolerant and less pH sensitive than the equivalent enzyme in heterotrophic denitrifiers (Lawton et al., 2013). Therefore, AOB might reduce more NO_2^--N than heterotrophic denitrifiers. Generally, N_2O is regarded as the end-product of AOB denitrification, because conventional N_2O reductase gene, *nosZ*, has not been detected in nitrifiers (Kozlowski et al., 2016a). However, nitrosocyanin, a copper protein would enable AOB to denitrify completely, was detected in AOB (Editorial, 2016; Todt and Dörsch, 2016). Compared with nitrous oxide reductase of denitrifying bacteria, nitrous oxide reductase of AOB may be less sensitive to nitrous oxide, causing that N_2O concentration kept increasing under aerobic condition. Moreover, the abundance of *nosZ* in denitrifiers was obviously higher than *cnorB*, indicating the potentials of reducing N_2O by denitrifiers, which was proved via batch test. Thus, compared with heterotrophic denitrification, AOB denitrification was much easier to induce N_2O release in BAF1.

Microbial activities were varied with different gas-water ratios, causing disparate amount of N_2O emission. When gas-water ratio was controlled at 10:1, DO and NO_2^--N concentration were the highest in BAF1 (Fig. 2). High DO concentration seriously inhibited heterotrophic denitrification activity, causing nitrogen removal performance to decline. Previous studies showed that N_2O generated from heterotrophic

Table 1
SP values of N_2O isotopic measurement.

Reaction type	SP value (‰)[a]	F_{ND} (%)[b]	F_{NN} (%)[c]
BAF2-(5:1)[d]	12.125	53.7	46.3
BAF2-(1.5:1)	1.719	87.8	12.2
BAF1-(b)[e]	7.214	69.8	30.2
BAF1-(c)	20.371	26.7	73.3
BAF1-(e)	−0.604	–	–
BAF2-(b)	3.337	82.5	17.5
BAF2-(c)	21.659	22.4	77.6

[a] SP value (‰): site preference value.
[b] F_{ND} (%): relative contribution to total N_2O from AOB denitrifiction pathway.
[c] F_{NN} (%): relative contribution to total N_2O from NH_2OH oxidation pathway.
[d] BAF2-(5:1):BAF2 was operated with gas-water ratio value of 5:1.
[e] BAF1-(b): Batch test (b) with BAF1 biofilm.

denitrification increased with increasing DO concentration and NO_2^--N concentration (Alinsafi et al., 2008; R. Von Schulthess, 1994). On the other hand, higher DO concentration could also lead to more N_2O producing via hydroxylamine oxidation pathway (Peng et al., 2014; Peng et al., 2015b). Consequently, N_2O emission concentration was the highest at gas-water ratio of 10:1. When gas-water ratio was 5:1, DO concentration declined, the increasing heterotrophic denitrification activity led to a higher nitrogen removal performance. Heterotrophic denitrification produced less N_2O than AOB denitrification. Besides, N_2O could be further reduced by heterotrophic denitrifiers when N_2O accumulated to a certain concentration. Hence, at gas-water ratio of 5:1 N_2O concentration was significantly less than that of gas-water ratio of 10:1. When gas-water ratio further decreased to 2.5:1, reaction activity of nitrifiers and denitrifiers significantly declined. Consequently, the least amount of N_2O was detected in BAF1. Previous research found that N_2O concentration increased with increasing DO concentration in the range of 2–6 mg/L, because NH_4^+-N could be immediately oxidized into NO_3^--N and heterotrophic denitrification was inhibited with high DO concentration (He et al., 2017). However, in this study DO concentration was below 2 mg/L in BAF1. Nitrification activity obviously declined and heterotrophic denitrification activity improved, causing N_2O emission declined with decreasing gas-water ratio. Thus, controlling appropriate gas-water ratio is benefit to reduce N_2O release by enhancing heterotrophic denitrification activity.

3.4. N_2O production pathway in BAF2

Similar to BAF1, more N_2O was produced from batch tests fed with NO_2^--N and $NH_2OH \cdot HCl$ in aerobic condition (Fig. 6). The SP values also confirmed that N_2O was produced from AOB denitrification and hydroxylamine oxidation pathway (Table 1). At aerobic condition, denitrification activity was not detected when NO_3^--N or NO_2^- was supplied, which suggested that N_2O was mainly produced from AOB denitrification and hydroxylamine oxidation in BAF2. The values of $\Delta N_2O/\Delta N$ of batch test (a) and (b) were similar. Because almost no heterotrophic denitrification occurred to BAF2, the SP values of collected gaseous N_2O from BAF2 were directly measured by isotope ratio mass spectrometry. The SP values were 12.125 and 1.719 with gas-water ratio of 5:1 and 1.5:1, respectively, proving that N_2O was mainly produced from AOB denitrification and hydroxylamine oxidation pathways.

Table 2
N_2O emission factors of batch tests with BAF1 and BAF2 biofilm.

Batch test number	BAF1						BAF2	
	(a)[a]	(b)	(d)	(e)	(f)	(g)	(a)	(b)
$\Delta N_2O/\Delta N$[b]	3.01%	4.43%	0.24%	0.88%	3.05%	0.89%	2.52%	2.16%

ΔN_2O: the concentration of generated N_2O in batch test.
ΔN: the concentration of removed nitrogen in batch test.
[a] (a): Batch test number with BAF1/BAF2 biofilm.
[b] $\Delta N_2O/\Delta N$: the ratio of generated N_2O concentration to removed nitrogen concentration.

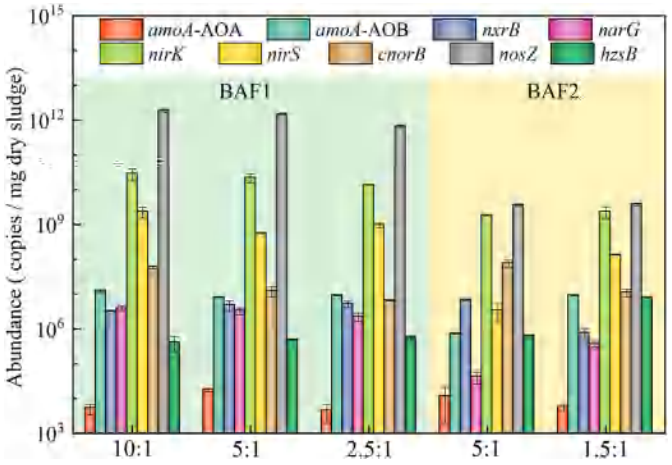

Fig. 5. Variations of nitrogen transformation functional gene abundances in BAF1 and BAF2 at different gas-water ratios.

When gas-water ratio was 5:1, the proportions of N_2O produced from AOB denitrification and hydroxylamine oxidation were 53.7% and 46.3%, respectively. When gas-water ratio was reduced to 1.5:1, proportion of N_2O released from hydroxylamine oxidation pathway decreased to 12.2%. Gas-water ratio influenced N_2O production pathway, and reducing DO concentration resulted in less N_2O releasing from hydroxylamine oxidation pathway, which was consistent with previous results attained from nitrifying sludge (Peng et al., 2014; Peng et al., 2015a; Rathnayake et al., 2015). In addition, when gas-water ratio was 1.5:1, N_2O production concentration was only 50% of that at gas-water ratio of 5:1, indicating that N_2O concentration released from AOB denitrification also declined with decreasing gas-water ratio. Previous report demonstrated that NH_4^+-N oxidation rate was higher with higher gas-water ratio, which could lead to an exponential N_2O production rate (Law et al., 2012). On the other hand, biofilm is fixed in BAF. Increasing aeration volume could promote more NH_4^+-N transferring into biofilm interior. As a result, the lower DO concentration in biofilm induced more N_2O generating from AOB denitrification (Kampschreur et al., 2008; Sabba et al., 2017; Tallec et al., 2006). Moreover, *hzsB* gene abundance increased to 10^7 copies/mg dry sludge since gas-water ratio declined to 1.5:1, suggesting that low gas-water ratio was benefit for anammox bacteria (AnAOB) growing. Consequently, AnAOB competed with AOB for NH_4^+-N, and competed with nitrite oxidizing bacteria for NO_2^--N, leading to the higher nitrogen removal performance and lower N_2O release. Previous study found that N_2O emission factor decreased with increasing aeration load in BAF, which was contradictory to this study (Bollon et al., 2016). It was mainly resulted from the growth of AnAOB in BAF2, causing part of NH_4^+-N and NO_2^--N to be removed via anammox pathway. Therefore, it is a feasible strategy to reduce N_2O emission via strengthening autotrophic nitrogen removal.

3.5. Comparison of N_2O production in BAF1 and BAF2

When gas-water ratio was 5: 1, the concentrations of NH_4^+-N removed from both BAF1 and BAF2 were about 22 mg/L. However, gaseous N_2O emission was obviously higher in BAF2. The main discrepancy between BAF1 and BAF2 was the presence/absence of biodegradable COD. Higher DO concentration led to higher NH_4^+-N oxidation rate and more N_2O emission from BAF2. On the other hand, compared with AOB denitrification, heterotrophic denitrification relieved N_2O production in BAF1. Hence, heterotrophic denitrification is a potential N_2O sink in biological nitrogen removal system as previous investigated (Conthe et al., 2019).

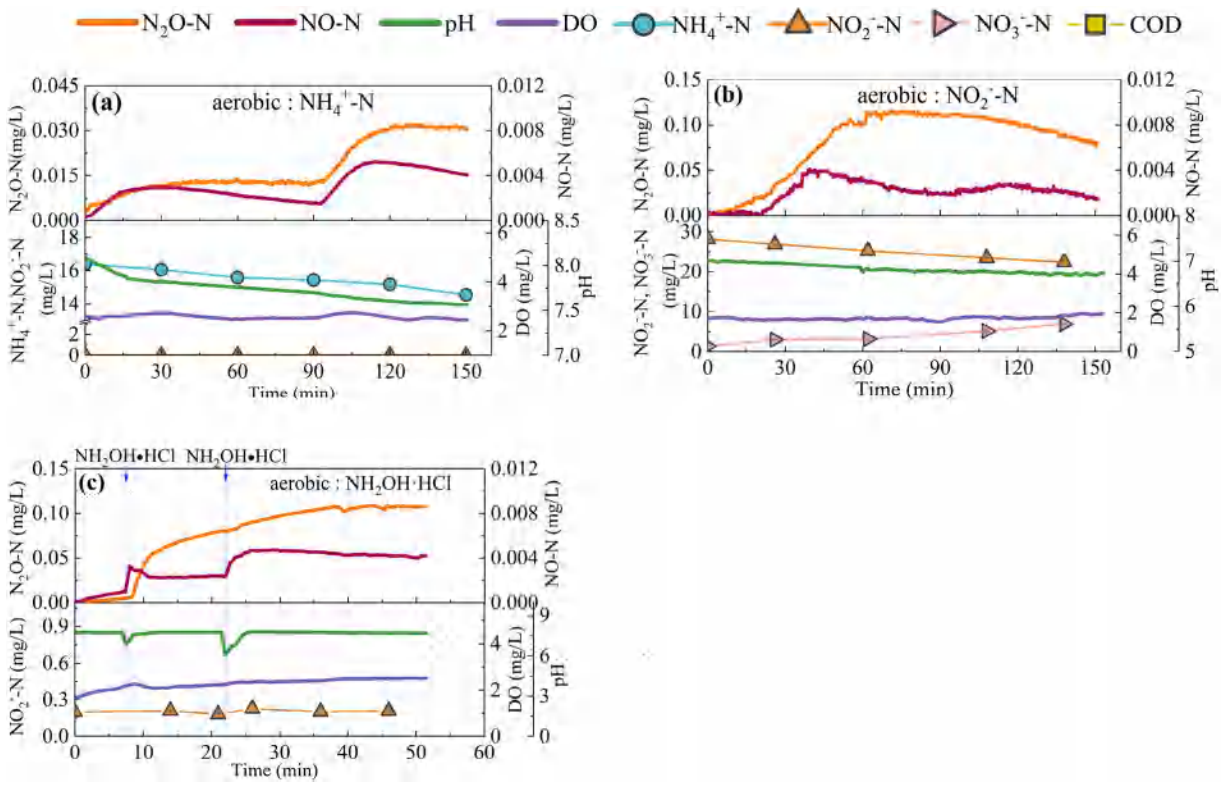

Fig. 6. Variations of NH_4^+-N, NO_2^--N, NO_3^--N, COD, DO, pH and dissolved N_2O—N, NO-N concentration in different batch tests with BAF2 biofilm sample.

For dissolved N_2O, BAF1 effluent was higher than BAF2 effluent with the same gas-water ratio. In BAF1 nitrification process occurred to the whole filter layer, which was also accompanied by N_2O production. In BAF2, NH_4^+-N was depleted within the height of 40 cm filter media, and part of dissolved N_2O diffused to atmosphere with air stripping, causing less dissolved N_2O to be detected in effluent. BAF1 effluent was supplied to BAF2, and more N_2O was produced in BAF2 bottom. Therefore, at filter bottom dissolved N_2O concentration was higher in BAF2.

Notably, obvious dissolved nitric oxide (NO) production phenomenon was detected in batch tests. However, because of the insolubility in liquid and instability in air of NO, it was not detected in BAF1 and BAF2. Generally, NO can be produced via the following three biochemical pathways: (1) denitrification, (2) anammox, and (3) NOH transforming to NO biologically (Rathnayake et al., 2018). Recently, an additional route for NO production was found from AOB. Hydroxylamine oxidoreductase (HAO) oxidizes NH_2OH by only three electrons to NO under both anaerobic and aerobic conditions in AOB (Wrage-Mönnig et al., 2018), and then NO can be oxidized into NO_2^--N in a non-enzymatic reaction or with *nirK* (Caranto and K.M.L., 2017). In this study, NO quickly increased when $NH_2OH \cdot HCl$ was added in batch test (Fig. 6(c)). Besides, NO concentration reached the highest level with NH_4^+-N as substrate (Fig. 4(a)), which verified NO as the intermediate of nitrification. Previous study also found that NO released from NH_4^+-N oxidation process in biofilm system (Schreiber et al., 2009). Comparing all the results of batch test, under anoxic condition NO concentration was obviously lower than that under aerobic condition, suggesting more NO was released from nitrification process. In addition, when NH_4^+-N and NO_2^--N were used as substrates under aerobic condition, the changing tendency of NO was identical with N_2O, implying that NO was the precursor of N_2O. However, due to the toxicity of NO to cell, it was immediately scavenged using multitude enzymes (Hu et al., 2019), leading to the lower concentration of NO than N_2O.

4. Conclusions

- N_2O generation concentration increased with increasing gas-water ratio in both BAF1 and BAF2, and the higher gas-water ratio promoted the more N_2O releasing from hydroxylamine oxidation process.
- In BAF1 N_2O was produced from AOB denitrification, hydroxylamine oxidation and heterotrophic denitrification pathways, and the optimal gas-water ratio was 5:1.
- In BAF2 N_2O was produced from AOB denitrification and hydroxylamine oxidation pathways. The optimal gas-water ratio was 1.5:1 for BAF2, and 87.8% of N_2O was produced from AOB denitrification pathway.
- AOB denitrification induced more N_2O generation than heterotrophic denitrification, causing BAF2 produced more N_2O than BAF1 with the same gas-water ratio.

CRediT authorship contribution statement

Qing Yang: Funding acquisition, Project administration, Conceptualization, Writing - review & editing, Supervision, Resources. **Bin Cui:** Investigation, Methodology, Data curation, Writing - original draft. **Yao Zhou:** Visualization, Software. **Jianmin Li:** Formal analysis. **Zhibin Liu:** Methodology. **Xiuhong Liu:** Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.137984.

References

Abou-Elela, S.I., Fawzy, M.E., El-Gendy, A.S., 2015. Potential of using biological aerated filter as a post treatment for municipal wastewater. Ecol. Eng. 84, 53–57.

Ahn, J.H., Kim, S., Park, H., Rahm, B., Pagilla, K., Chandran, K., 2010. N_2O emissions from activated sludge processes, 2008–2009: results of a national monitoring survey in the United States. Environ. Sci. Technol. 44, 4505–4511.

Alinsafi, A., Adouani, N., Béline, F., Lendormi, T., Limousy, L., Sire, O., 2008. Nitrite effect on nitrous oxide emission from denitrifying activated sludge. Process Biochem. 43, 683–689.

APHA, 1998. Standard Methods for Examination of Water and Wastewater. American Public Health Association, Washington, DC.

Blum, J., Jensen, M.M., Smets, B.F., 2018. Nitrous oxide production in intermittently aerated Partial Nitritation-Anammox reactor: oxic N_2O production dominates and relates with ammonia removal rate. Chem. Eng. J. 335, 458–466.

Bollon, J., Filali, A., Fayolle, Y., Guerin, S., Rocher, V., Gillot, S., 2016. N_2O emissions from full-scale nitrifying biofilters. Water Res. 102, 41–51.

Caranto, Jonathan D., K.M.L., 2017. Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase. Proc. Natl. Acad. Sci. 114, 8217–8222.

Conthe, M., Lycus, P., Arntzen, M.Ø., Ramos Da Silva, A., Frostegård, Å., Bakken, L.R., Kleerebezem, R., van Loosdrecht, M.C.M., 2019. Denitrification as an N_2O sink. Water Res. 151, 381–387.

Cui, B., Yang, Q., Zhang, Y., Liu, X., Wu, W., Li, J., 2019. Improving nitrogen removal in biological aeration filter for domestic sewage treatment via adjusting microbial community structure. Bioresour. Technol. 293, 122006.

Duan, H., Ye, L., Erler, D., Ni, B., Yuan, Z., 2017. Quantifying nitrous oxide production pathways in wastewater treatment systems using isotope technology – a critical review. Water Res. 122, 96–113.

Editorial, 2016. Now we NO. Nat. Chem. 8, 635.

Flemming, H., Wingender, J., 2010. The biofilm matrix. Nat. Rev. Microbiol. 8, 623–633.

Frutos, O.D., Quijano, G., Aizpuru, A., Muñoz, R., 2018. A state-of-the-art review on nitrous oxide control from waste treatment and industrial sources. Biotechnol. Adv. 36, 1025–1037.

Han, X., Zhang, S., Yang, S., Zhang, L., Peng, Y., 2020. Full-scale partial nitritation/anammox (PN/A) process for treating sludge dewatering liquor from anaerobic digestion after thermal hydrolysis. Bioresour. Technol. 297, 122380.

He, Q., Zhu, Y., Li, G., Fan, L., Ai, H., Huang, X., Li, H., 2017. Impact of dissolved oxygen on the production of nitrous oxide in biological aerated filters. Front. Environ. Sci. Eng. 11 (6), 16.

Hu, Z., Lee, J.W., Chandran, K., Kim, S., Khanal, S.K., 2012. Nitrous oxide (N_2O) emission from aquaculture: a review. Environ. Sci. Technol. 46, 6470–6480.

Hu, Z., Wessels, H.J.C.T., van Alen, T., Jetten, M.S.M., Kartal, B., 2019. Nitric oxide-dependent anaerobic ammonium oxidation. Nat. Commun. 10.

Jones, C.M., Graf, D.R.H., Bru, D., Philippot, L., Hallin, S., Sveriges, L., 2013. The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. ISME J. 7, 417–426.

Kampschreur, M.J., Tan, N.C.G., Kleerebezem, R., Picioreanu, C., Jetten, M.S.M., Loosdrecht, M.C.M.V., 2008. Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture. Environ. Sci. Technol. 42, 429–435.

Kozlowski, J.A., Kits, K.D., Stein, L.Y., 2016a. Genome sequence of Nitrosomonas communis strain Nm2, a mesophilic ammonia-oxidizing bacterium isolated from Mediterranean soil. Genome Announcements 4, 1–2.

Kozlowski, J.A., Stieglmeier, M., Schleper, C., Klotz, M.G., Stein, L.Y., 2016b. Pathways and key intermediates required for obligate aerobic ammonia-dependent chemolithotrophy in bacteria and Thaumarchaeota. ISME J. 10, 1836–1845.

Law, Y., Ni, B., Lant, P., Yuan, Z., 2012. N_2O production rate of an enriched ammonia-oxidising bacteria culture exponentially correlates to its ammonia oxidation rate. Water Res. 46, 3409–3419.

Lawton, T.J., Bowen, K.E., Sayavedra-Soto, L.A., Arp, D.J., Rosenzweig, A.C., 2013. Characterization of a nitrite reductase involved in nitrifier denitrification. J. Biol. Chem. 288, 25575–25583.

Leopoldo Mendoza-Espinosa, T.S., 1999. A review of biological aerated filters (BAFs) for wastewater treatment. Environ. Eng. Sci. 16, 201–216.

Mark Poth, D.D.F., 1985. ^{15}N kinetic analysis of N_20 production by Nitrosomonas europaea: an examination of nitrifier denitrification. Appl. Environ. Microbiol. 49, 1134–1141.

Peng, L., Ni, B., Erler, D., Ye, L., Yuan, Z., 2014. The effect of dissolved oxygen on N_2O production by ammonia-oxidizing bacteria in an enriched nitrifying sludge. Water Res. 66, 12–21.

Peng, L., Ni, B., Ye, L., Yuan, Z., 2015a. N_2O production by ammonia oxidizing bacteria in an enriched nitrifying sludge linearly depends on inorganic carbon concentration. Water Res. 74, 58–66.

Peng, L., Ni, B., Ye, L., Yuan, Z., 2015b. The combined effect of dissolved oxygen and nitrite on N_2O production by ammonia oxidizing bacteria in an enriched nitrifying sludge. Water Res. 73, 29–36.

R. Von Schulthess, D.W.W.G., 1994. Nitric and nitrous oxides from denitrifying activated sludge at low oxygen concentration. Water Sci. Technol. 30, 123–132.

Rathnayake, R.M.L.D., Oshiki, M., Ishii, S., Segawa, T., Satoh, H., Okabe, S., 2015. Effects of dissolved oxygen and pH on nitrous oxide production rates in autotrophic partial nitrification granules. Bioresour. Technol. 197, 15–22.

Rathnayake, R.M.L.D., Oshiki, M., Ishii, S., Segawa, T., Satoh, H., Okabe, S., 2018. Experimental evidence for in situ nitric oxide production in anaerobic ammonia-oxidizing bacterial granules. Environ. Sci. Technol. 52, 5744–5752.

Sabba, F., Picioreanu, C., Pérez, J., Nerenberg, R., 2015. Hydroxylamine diffusion can enhance N_2O emissions in nitrifying biofilms: a modeling study. Environ. Sci. Technol. 49, 1486–1494.

Sabba, F., Picioreanu, C., Pérez, J., Nerenberg, R., 2017. Mechanisms of nitrous oxide (N_2O) formation and reduction in denitrifying biofilms. Biotechnol. Bioeng. 114, 2753–2761.

Sabba, F., Terada, A., Wells, G., Smets, B.F., Nerenberg, R., 2018. Nitrous oxide emissions from biofilm processes for wastewater treatment. Appl. Microbiol. Biotechnol. 102, 9815–9829.

Sanford, R.A., Wagner, D.D., Wu, Q., Chee-Sanford, J.C., Thomas, S.H., Cruz-Garcia, C., Rodriguez, G., Massol-Deya, A., Krishnani, K.K., Ritalahti, K.M., Nissen, S., Konstantinidis, K.T., Loffler, F.E., 2012. Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. Proc. Natl. Acad. Sci. 109, 19709–19714.

Schreiber, F., Loeffler, B., Polerecky, L., Kuypers, M.M.M., de Beer, D., 2009. Mechanisms of transient nitric oxide and nitrous oxide production in a complex biofilm. ISME J. 3, 1301–1313.

Stieglmeier, M., Mooshammer, M., Kitzler, B., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., Schleper, C., 2014. Aerobic nitrous oxide production through N-nitrosating hybrid formation in ammonia-oxidizing archaea. ISME J. 8, 1135–1146.

Tallec, G., Garnier, J., Billen, G., Gousailles, M., 2006. Nitrous oxide emissions from secondary activated sludge in nitrifying conditions of urban wastewater treatment plants: effect of oxygenation level. Water Res. 40, 2972–2980.

Todt, D., Dörsch, P., 2016. Mechanism leading to N_2O production in wastewater treating biofilm systems. Rev. Environ. Sci. Biotechnol. 15, 355–378.

Wrage-Mönnig, N., Horn, M.A., Well, R., Müller, C., Velthof, G., Oenema, O., 2018. The role of nitrifier denitrification in the production of nitrous oxide revisited. Soil Biol. Biochem. 123, 3–16.

Wunderlin, P., Lehmann, M.F., Siegrist, H., Tuzson, B., Joss, A., Emmenegger, L., Mohn, J., 2013. Isotope signatures of N_2O in a mixed microbial population system: constraints on N_2O producing pathways in wastewater treatment. Environ. Sci. Technol. 47, 1339–1348.

Xu, X., Wang, G., Zhou, L., Yu, H., Yang, F., 2018. Start-up of a full-scale SNAD-MBBR process for treating sludge digester liquor. Chem. Eng. J. 343, 477–483.

Yang, Q., Liu, X., Peng, C., Wang, S., Sun, H., Peng, Y., 2009. N_2O production during nitrogen removal via nitrite from domestic wastewater: main sources and control method. Environ. Sci. Technol. 43, 9400–9406.