Aerobic N₂O emission for activated sludge acclimated under different aeration rates in the multiple anoxic and aerobic process

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ABSTRACT

Nitrous oxide (N₂O) is a potent greenhouse gas that can be emitted during biological nitrogen removal. N₂O emission was examined in a multiple anoxic and aerobic process at the aeration rates of 600 mL/min sequencing batch reactor (SBR₁) and 1200 mL/min (SBR₂). The nitrogen removal percentage was 89% in SBR₁ and 71% in SBR₂, respectively. N₂O emission mainly occurred during the aerobic phase, and the N₂O emission factor was 10.1% in SBR₁ and 2.3% in SBR₂, respectively. In all batch experiments, the N₂O emission potential was high in SBR₁ compared with SBR₂. In SBR₂, with increasing aeration rates, the N₂O emission factor during nitrification, denitrification and simultaneous nitrification and denitrification (SND) was relatively low and changed little with increasing aeration rates. The microbial competition affected the N₂O emission during biological nitrogen removal.

Introduction

Biological nitrogen removal has been applied widely to remove nitrogen from wastewater to protect water bodies from eutrophication. In conventional biological nitrogen removal processes, ammonia is oxidized to nitrite and nitrate under aerobic conditions, and then nitrate is reduced sequentially to nitrite, nitric oxide, nitrous oxide (N₂O) and dinitrogen (N₂) under anoxic conditions with organic carbon as the electron donor. Pre-denitrification is usually applied in conventional biological nitrogen removal processes. Therefore, the nitrogen removal efficiency is mainly dependent on the ratio of recycling nitrified mixed liquor from the aerobic reactor to the anoxic reactor and the influent organic carbon to nitrogen ratio. In particular, the nitrogen removal percentage is relatively low for wastewater with limited organic carbon sources. Therefore, some new processes, such as partial nitrification/denitrification and anaerobic ammonia oxidation, have been developed and applied (Hellinga et al., 1998). Among these processes, the multiple anoxic/aerobic (AO) or intermittently aerated process, as a new enhanced nitrogen removal process, has also been examined for the treatment of various types of wastewater, such as domestic wastewater, pig slurry and slaughterhouse wastewater (Beline and Martinez, 2002; Zeng et al., 2003; Pan et al., 2015). The conventional AO process is modified to the multiple AO process through intermittent aeration in time (for a single reaction tank) or in space (for multiple reaction tanks). With alternative anoxic and aerobic conditions applied in the...
During biological nitrogen removal, the greenhouse gas N$_2$O may be produced and emitted. The global warming potential of N$_2$O is about 300 times that of carbon dioxide (CO$_2$); N$_2$O can last about 114 years in the atmosphere and is one of the most important substances depleting the ozone layer in the 21st century (Ravishankara et al., 2009). The main processes contributing to N$_2$O emission during biological nitrogen removal include nitrifier denitrification and hydroxylamine oxidation by AOB under aerobic conditions, and heterotrophic denitrification by denitrifiers under anoxic conditions (Kampschreur et al., 2009; Wunderlin et al., 2012). In the multiple AO biological nitrogen removal process, intermittent aeration may enhance N$_2$O emission, which would limit its sustainable application. De Mello et al. (2013) found that in a full-scale intermittently aerated urban wastewater treatment plant (WWTP), the N$_2$O emission factor was 0.10% to the influent total nitrogen and N$_2$O emission mainly occurred through air stripping under aerobic conditions. By using the intermittently aerated system, Zhang et al. (2012) found that the N$_2$O emission factor to the total nitrogen removed was 15.6% when treating effluent from anaerobically digested pig manure and 10.1% when treating synthetic wastewater, showing the highest N$_2$O emission potential of the system; aerobic emission was the major source and contributed to 92% of the total N$_2$O emission. Therefore, most studies have demonstrated that in intermittently aerated systems, N$_2$O is mainly emitted under aerobic conditions. In addition, dissolved oxygen (DO), nitrite and the ratio of anoxic/aerobic phase are also important factors influencing N$_2$O emission in intermittently aerated processes. Zhang et al. (2006) investigated swine manure treatment by a two-step fed sequencing batch reactor (SBR) and found that N$_2$O was mainly emitted during the secondary feeding and anoxic/aerobic phases, in which the accumulation of nitrite and low concentration of DO and carbon sources enhanced N$_2$O emission from both nitrification and denitrification. Kong et al. (2013) investigated the N$_2$O emission in an intermittently aerated sequencing batch biofilm reactor with a controlled DO concentration of 1.5 mg/L, and the results showed that partial nitrification occurred in the system and 1.5% of the influent nitrogen was emitted as N$_2$O. Zeng et al. (2003) adopted a low DO (0.5 mg/L) intermittently aerated system to achieve simultaneous nitrogen and phosphorus removal, and discovered that the end product of denitrification was N$_2$O instead of N$_2$. Jia et al. (2012) achieved simultaneous nitrification and denitrification (SND) successfully through low DO intermittent aeration, and the N$_2$O emission in the SND process was almost four times higher than that of the sequential nitrification and denitrification process with continuous aeration. Therefore, it is also necessary to examine the effect of aeration rate on aerobic N$_2$O emissions from different pathways in the multiple AO process.

Nutrient removal from wastewater derives from functions of various microbial communities. The interaction of different microbial communities not only affects the performance of nutrient removal, but also the N$_2$O emission during biological nitrogen removal. Shen et al. (2015) found that heterotrophic activity had a significant effect on N$_2$O emission in a biological nitrogen removal process, and high heterotrophic activity induced a high N$_2$O emission during nitrification. Park et al. (2000) examined N$_2$O emission in two intermittently aerated (aeration on/off of 60 min/30 min) reactors with and without biofilm, and the results showed that the N$_2$O emission factor to the influent total nitrogen was 4.57% in the suspension reactor and 3% in the biofilm reactor. Moreover, addition of sufficient methanol for denitrification could reduce the N$_2$O emission factor to less than 0.2%; since the cooperation among microbial communities reduced the emission of N$_2$O, and a biofilm consisting of different microbial communities is beneficial for the control of N$_2$O emission (Park et al., 2000). Therefore, it is important to investigate N$_2$O production and emission mechanisms during biological nitrogen removal process from the viewpoint of microbial ecology (competition or synergism), and by this means, effective strategies for mitigating N$_2$O emission may be brought forward. However, to date, only a few related studies have been carried out in this field.

In this study, characteristics of N$_2$O emission from multiple AO SBRs under different aeration rates were investigated through simulated reaction cycle and batch experiments for nitrification, denitrification and SND. The aerobic N$_2$O emission was analyzed from the viewpoint of microbial ecology, so as to provide guidelines for the mitigation of N$_2$O emission during biological nitrogen removal from wastewater.

### 1. Materials and methods

#### 1.1. SBRs

Two parallel 6-L SBRs were operated under aeration rates of 600 mL/min (SBR$_1$) and 2400 mL/min (SBR$_2$), respectively. Both SBRs included four reaction cycles per day and each reaction cycle consisted of 120 min anaerobic phase (including 10 min filling), 120 min intermittent aeration (aeration on/off of 30 min/30 min), 60 min extended aeration and 60 min of settlement, decanting and idle phases. The reactors were inoculated with activated sludge taken from a WWTP in Shenzhen, China. Once a day, 0.4 L of mixed liquor from both reactors was discharged just before the settlement phase, resulting in a sludge retention time (SRT) of 15 days. In each reaction cycle, 3 L of treated supernatant (in the sludge removal reaction cycle of 2.6 L) was discharged and 3 L of synthetic wastewater was pumped into the reactor by peristaltic pumps, resulting in a hydraulic retention time (HRT) of 12 hr. The reactors were mixed by mechanical stirrers except during the phases of settlement, decanting and idle. During the aerobic phase, aeration was achieved by micro-pore stones placed at the bottom of the reactors. The filling, withdrawal, aeration and mixing of the reactors were controlled by timers. Temperature inside the reactors was controlled at 25°C.
The synthetic wastewater consisted of 510 mg/L sodium acetate, 153 mg/L NH4Cl, 14 mg/L CaCl2·2H2O, 90 mg/L MgSO4·7H2O, 46 mg/L Na2HPO4, 10 mg/L yeast extract, 200 mg/L NaHCO3 and 0.4 mg/L trace elements. The composition of trace elements was according to Smolders et al. (1994). The synthetic wastewater contained 400 mg/L of chemical oxygen demand (COD), 40 mg/L of ammonium nitrogen (NH4-N) and 10 mg/L of orthophosphate (PO4-P).

Under steady state, the N2O emission characteristics during a typical reaction cycle for both SBRs were investigated. As the parent SBRs were open to the atmosphere, the cycle experiment was carried out in 2 L gastight reactors to stimulate the reaction cycle of the SBRs. 2 L of mixed liquor was taken from each reactor just before settlement and transferred to two gastight bottles. After settlement, 1 L of supernatant in each reactor was decanted and exchanged with 1 L of synthetic wastewater. Then the reactors were sealed using rubber stoppers with three pipelines, one for aeration, one for liquid sampling, and one for gas flow or sampling or balancing. During the non-aerated period, the three pipelines were all blocked except during the sampling period, and pressure inside the headspace of each reactor was balanced with N2 after sampling. During the aerobic phase, the pipelines for aeration and gas out were open. The reactors were mixed by magnetic stirrers during the simulated cycle study. Gas and liquid samples were taken at intervals of 10-30 min to analyze the concentrations of N2O, NH4-N, nitrite nitrogen (NO2-N), nitrate nitrogen (NO3-N), PO4-P, suspended solids (SS) and volatile suspended solids (VSS), and dynamics of DO and pH were also measured.

1.2. Aerobic N2O emission batch experiments

From the simulated cycle study, N2O emission mainly occurred under aerobic conditions rather than anaerobic or anoxic conditions. Therefore, batch experiments were carried out in 1000 mL bottled reactors to examine the characteristics of aerobic N2O emission under conditions of nitrification, denitrification and SND. During all batch experiments, the batch reactors were aerated at the same aeration rate of 300 mL/min but with different air to N2 ratios (75:225, 150:150 and 300:0) to examine the effect of aeration rates on N2O emission. During batch experiments, 2400 mL of mixed liquor was taken from both SBRs, and then divided into three 1 L gastight bottles equally.

For batch nitrification experiments, mixed liquor was taken from both SBRs at the end of the final aerobic phase, NH4-N was added with an initial concentration of 20 mg/L and then the experiment was started at the three aeration rates.

For batch denitrification experiments, mixed liquor was taken from both SBRs at the end of the final aerobic phase, sodium acetate was added with an initial concentration of 250 mg/L, reacted under anaerobic conditions for 120 min to accumulate internal organic carbon, and then NO3-N was added with an initial concentration of 20 mg/L before the experiment was started.

For batch SND experiments, mixed liquor was taken from both SBRs at the end of the anaerobic phase and then the experiment was started directly.

During all batch experiments, gas and liquid samples were taken at intervals of 10 min to analyze the concentration of gas N2O and concentrations of NH4-N, NO2-N, NO3-N, PO4-P, SS and VSS in the liquid; meanwhile, the dynamics of DO concentration and pH were also measured.

1.3. Analytical methods

COD, NH4-N, NO2-N, NO3-N, PO4-P, SS and VSS were measured according to standard methods (APHA, 1995). DO and pH were measured by a portable DO meter (Flexi, HACH, USA) and a pH meter (pH 30d, HACH, USA), respectively. N2O was tested by a gas chromatograph (GC-6820, Agilent Technologies, USA) with an electron capture detector and a HP-PLOT/Q column (J&W GC Columns, Agilent Technologies, USA), with the protocol according to Shen et al. (2015). The emission factor of N2O was calculated according to Kimochi et al. (1998).

The efficiency of SND was calculated by the following equation:

\[ \mu_{\text{SND}} = \frac{t_{\text{NH}_4-N} - t_{\text{NO}_2-N} - t_{\text{NO}_3-N}}{t_{\text{NH}_4-N}} \times 100\% \]  

where, \( \mu_{\text{SND}} \) (% is the efficiency of SND; \( t_{\text{NH}_4-N} \) (mg/(g VSS·hr)), \( t_{\text{NO}_2-N} \) (mg/(g VSS·hr)) and \( t_{\text{NO}_3-N} \) (mg/(g VSS·hr)) are the oxidation rate of ammonia, and the generation rates of NO2-N and NO3-N, respectively.

2. Results and discussion

2.1. System performance and N2O emission in the simulated SBR cycle

Under steady state, nitrogen and phosphate were removed efficiently in the two SBRs. Concentrations of NH4-N, NO2-N and PO4-P in the effluent were all quite low. The effluent NO3-N concentration was 4.5 ± 1.4 mg/L in SBRL and 12 ± 2.1 mg/L in SBRH, respectively. Accordingly, the inorganic nitrogen removal percentages were 89% in SBRL and 71% in SBRH, indicating that an increase of 18% in the removal percentage was achieved at the low aeration rate. The increased nitrogen removal under low aeration rate could be due to the following reasons. Firstly, the low aeration rate benefited the efficient utilization of the influent organic carbon source by slow utilization of stored intracellular organic carbons, which promoted denitrification and nitrogen removal. Secondly, the low DO concentration under low aeration rate promoted SND during the initial aerobic phase so as to improve nitrogen removal efficiency. Finally, under low DO concentrations, activities of NOB would be inhibited, resulting in nitrite accumulation, and this induced partial nitrification and denitrification, improving the utilization efficiency of organic carbon sources for denitrification (Zheng et al., 1994; Bernet et al., 2001; Chuang et al., 2007). Therefore, for nitrogen removal, low aeration benefited the removal percentage.

The simulated SBR cycle results are shown in Fig. 1. During the initial 120 min anoxic/anaerobic period, denitrification and phosphorus release occurred in both SBRs. During the following whole intermittent aeration period in SBRL and the first hour in SBRH, nitrite was accumulated with little nitrate produced, indicating that low aeration was beneficial for
N2O might be dissolved under anaerobic and anoxic phases. These results are consistent with some previous studies (Zhang et al., 2010) found that N2O produced under anaerobic or anoxic conditions could be reduced to N2 before it was transferred to the aerobic phase, while N2O produced in the aerobic period could be quickly stripped to the atmosphere. In addition, the organic carbon source was sufficient for denitrification during the anaerobic/anoxic period following the filling phase, which might promote complete denitrification. Wicht (1996) and Itokawa et al. (2001) reported that the reduction rate of N2O was higher than those of nitrite and nitrate, confirming that N2O produced under aerobic or anoxic conditions could be reduced to N2 without being accumulated. The N2O emission factor to the influent ammonia was 10.1% in SBRr and 2.3% in SBRh, respectively. Therefore, the N2O emission factor was relatively high in both SBRs with the intermittent aeration, and N2O emission should be investigated and controlled. By comparing the N2O emission factor under the examined two aeration rates, the N2O emission could be decreased by increasing the aeration rate. In the present study, when the aeration rate was increased from 600 to 2400 mL/min, the N2O emission factor decreased by 80%. Therefore, the aeration rate was a key factor affecting aerobic N2O emission in the multiple AO process. In addition, in the SBRr, N2O emission mainly occurred during the last aerobic phase, indicating that the multiple AO operation mode could reduce N2O emission, which should be considered in future studies for mitigating N2O emission during biological nitrogen removal.

2.2. Characterization of aerobic N2O emission

According to the simulated SBR cycle result, N2O emission mainly occurred under aerobic conditions in both SBRs, but its emission characteristics and factors were different. In order to further clarify the N2O emission mechanism and its control strategy under aerobic conditions, N2O emission characteristics during nitrification, denitrification and SND were examined at the same aeration rate but with different air and N2 ratios.

2.2.1. Aerobic N2O emissions under batch nitrification conditions

Results for aerobic N2O emissions under batch nitrification conditions are shown in Fig. 2 and the related bio-kinetics are given in Table 1.

With the ratio of air to N2 increased from 75:225 to 300:0, for activated sludge taken from both SBRs, DO concentrations gradually increased, and the oxidation rate of ammonia and nitrate production rates increased. Under the three aeration conditions, the ratio between the nitrite accumulation rate and the ammonia oxidation rate was 0.18, 0.29 and 0.35 for activated sludge taken from SBRh and 0.09, 0.15 and 0.23 for activated sludge taken from SBRr. This indicated that compared with AOB, the inhibition of NOB activities in SBRr was higher than in SBRh. The possible reason could be due to the fact that with DO concentration of less than 0.5 mg/L, nitrification was inhibited at the same aeration rate but with different air and N2 ratios.
that NOB had a lower ability to compete for DO than those of AOB and heterotrophs, and their activities would be more inhibited under low DO concentrations. In addition, the recovery of NOB activities lagged behind AOB under intermittent aeration, which might also contribute to nitrite accumulation (Kornaros et al., 2010). For the same activated sludge, the accumulation of nitrite could be reduced through increasing aeration rates. All these observations suggested that DO had an important effect on the activities of AOB and NOB.

During nitrification, with the ratio of air to N\textsubscript{2} increased from 75:225 to 150:150 and then to 300:0, the N\textsubscript{2}O emission factor decreased gradually from 6.44% to 4.35% and then to 1.62% in SBR\textsubscript{L}, which was higher than that of 1.12%, 2.28% and 1.22% in SBR\textsubscript{H}. The difference could be due to the fact that the activities of NOB in SBR\textsubscript{L} were lower than those in SBR\textsubscript{H}, and this caused nitrite accumulation, resulting in high N\textsubscript{2}O emission. With an increase in the ratio of air to N\textsubscript{2}, the N\textsubscript{2}O emission factor in SBR\textsubscript{L} decreased gradually, while that in SBR\textsubscript{H} increased initially and then decreased thereafter. Under low DO concentrations, more N\textsubscript{2}O could be emitted through nitrifier denitrification, with nitrite as the electron acceptor (Colliver and Stephenson, 2000). The difference in N\textsubscript{2}O emission from both SBRs might be also correlated with the different DO concentrations inside the reactors. The different

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**Fig. 2 – Dynamics of parameters during the batch nitrification experiment for activated sludge taken from SBR\textsubscript{L} (left) and SBR\textsubscript{H} (right). SBR: sequencing batch reactor.**
affinity for DO of microbial communities acclimated inside the reactors might also contribute to the different characteristics of N2O emission. DO concentrations in SBRH were relatively high and the N2O emission could be reduced. Therefore, the acclimation condition should be considered for its effect on microbial activities, including N2O emission characteristics.

2.2.2. Aerobic N2O emission under batch denitrification conditions

Characteristics of N2O emissions under aerobic batch denitrification conditions are shown in Fig. 3 and the related bio-kinetics are given in Table 2.

For activated sludge taken from SBRH, denitrification occurred under all aeration conditions, but the duration of denitrification decreased with increasing aeration rates. When the ratios of air to N2 were 75:225 and 150:150, the DO concentrations were all relatively low (less than 0.24 mg/L), and so were the kinetic parameters. When the ratio was increased to 300:0, denitrification occurred only in the initial 20 min, and the DO concentration was 0.43–0.73 mg/L during this period; the nitrite accumulation rate was 1.55 mg N/(g VSS-hr), lower than those of 3.95 and 3.65 mg N/(g VSS-hr) under the ratios of 75:225 and 150:150, respectively. The nitrate denitrification rate was 1.84 mg N/(g VSS-hr), lower than those of 5.41 and 5.80 mg N/(g VSS-hr) at the ratios of 75:225 and 150:150, respectively; the N2O emission rate was 1.22 mg N/(g VSS-hr), lower than those of 3.95 and 3.65 mg N/(g VSS-hr) under the ratios of 75:225 and 150:150, respectively. These were all lower than the corresponding values in SBRH. No denitrification occurred when the ratio of air to N2 was 300:0.

For both SBRs, the duration of denitrification decreased with an increase in the aeration rate. The reaction shifted from denitrification to nitrification gradually when the ratio of air to N2 increased from 75:225 to 300:0. For activated sludge taken from SBRH, DO increased rapidly and the transition from denitrification to nitrification was also fast. For example, in SBRH, when the ratio of air to N2 was 300:0, the concentration of DO was in the range of 0.99–6.32 mg/L, and denitrification was completely replaced by nitrification. The reason for this might be that denitrification could be significantly inhibited by DO, and denitrifier activities could be completely inhibited under high DO concentrations (Bonin et al., 1989).

During aerobic denitrification, with increasing aeration rates, the N2O emission rate decreased, while the N2O emission factor increased. This showed that denitrification was seriously inhibited with increasing aeration rates, leading to higher N2O emission. In addition, under all conditions, both the N2O emission rate and factor were higher in SBRH than in SBRL. The N2O reductases were more sensitive to DO than nitrite and nitrate reductases, and the activities of N2O reductases were inhibited immediately when DO was present, which might increase the emission of N2O (Otte et al., 1996; Law et al., 2012). Therefore, increasing the aeration rate might increase the N2O emission factor. On the other hand, denitrifying activities using intracellular stored organic carbon were higher in SBRH than in SBRL, which might also contribute to the high N2O emission potential in SBRH. Therefore, both the DO concentration and internal organic carbon affected N2O emissions during aerobic denitrification, with a high N2O emission potential for activated sludge acclimated at low aeration conditions.

### Table 1 – Bio-kinetics during the batch nitrification experiment for activated sludge taken from both SBRs.

<table>
<thead>
<tr>
<th>Air:N2</th>
<th>DO (mg/L)</th>
<th>( f_{NH_4-N} ) (mg NH4-N/g VSS-hr)</th>
<th>( f_{NO_2-N} ) (mg NO2-N/g VSS-hr)</th>
<th>( f_{NO_3-N} ) (mg NO3-N/g VSS-hr)</th>
<th>( f_{NO_2-N} ) (mg NO2-N/g VSS-hr)</th>
<th>( f_{N_2O-N} ) (mg N2O-N/g VSS-hr)</th>
<th>( f_{N_2O-N} / f_{NH_4-N} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBRH</td>
<td>75:225</td>
<td>0.28–0.29</td>
<td>–2.04</td>
<td>0.36</td>
<td>0.91</td>
<td>0.13</td>
<td>6.44%</td>
</tr>
<tr>
<td></td>
<td>150:150</td>
<td>0.37–0.46</td>
<td>–3.30</td>
<td>0.97</td>
<td>1.97</td>
<td>0.14</td>
<td>4.35%</td>
</tr>
<tr>
<td></td>
<td>300:0</td>
<td>1.68–3.38</td>
<td>–5.08</td>
<td>1.79</td>
<td>3.40</td>
<td>0.08</td>
<td>1.62%</td>
</tr>
<tr>
<td>SBRL</td>
<td>75:225</td>
<td>0.37–0.45</td>
<td>–2.0</td>
<td>0.17</td>
<td>1.69</td>
<td>0.02</td>
<td>1.12%</td>
</tr>
<tr>
<td></td>
<td>150:150</td>
<td>0.69–0.84</td>
<td>–3.31</td>
<td>0.51</td>
<td>2.73</td>
<td>0.08</td>
<td>2.28%</td>
</tr>
<tr>
<td></td>
<td>300:0</td>
<td>3.01–3.75</td>
<td>–5.57</td>
<td>1.30</td>
<td>1.30</td>
<td>0.07</td>
<td>1.22%</td>
</tr>
</tbody>
</table>

SBR: sequencing batch reactor; DO: dissolved oxygen.

### 2.2.3. Aerobic N2O emissions under batch SND conditions

Results of aerobic N2O emissions under batch SND conditions are shown in Fig. 4. Under the ratios of air to N2 of 75:225, 150:150 and 300:0, the ammonia oxidization rates were 0.96, 2.60 and 6.32 mg N/(g VSS-hr) for activated sludge taken from SBRH, and 1.36, 2.47 and 6.02 mg N/(g VSS-hr) for activated sludge taken from SBRL. As the ratio of air to N2 increased from 75:225 to 300:0, the DO concentration increased gradually; as a result, the ammonia oxidization rate, the accumulation rate of nitrite and the production rate of nitrate also increased gradually in both reactors. The ammonia oxidization rates under the same aeration conditions were similar in both SBRs. At the lowest air proportion for activated sludge taken from both SBRs, the ammonia oxidization rates, the NO3−-N production rates and the N2O emission were all relatively low, and the efficiency of SND was as high as 90% or more. With increasing aeration rate, the ammonia oxidization rate, the nitrite and nitrate production rates and the N2O emission rate increased, while the efficiency of SND decreased gradually. As denitrifiers were sensitive to DO, their activities would be inhibited under high DO concentrations, causing a low SND efficiency.
Under the three aeration conditions, the nitrate production rates were all lower in SBRL than in SBRH, indicating the inhibition of NOB activities as described in batch nitrification experiments. As the ratio of air to N₂ increased from 75:225 to 300:0, for sludge from SBRL, the N₂O emission rates were 0.02, 0.44 and 1.83 mg N/(g VSS·hr) and the N₂O emission factor to the oxidized ammonia were 2.3%, 17.0% and 31.0%, respectively, which were all higher than the corresponding values from SBRH. This indicated that the N₂O emission potential was high for activated sludge acclimated at low aeration rates. One of the reasons was the inhibition of NOB activities at low aeration rates as mentioned above. It was shown that during simultaneous nitrification and denitrification, partial nitrification and nitrifier denitrification by AOB with the inhibition of NOB activities could enhance the N₂O emission (Pellicer-Nacher et al., 2010; Ahn et al., 2011).

2.3. Aerobic N₂O emission mechanisms with the effect of microbial ecology

In the multiple AO process, both the SND efficiency and the nitrogen removal percentage were higher under conditions of
Table 2 – Bio-kinetics during the batch denitrification experiment for activated sludge taken from both SBRs.

<table>
<thead>
<tr>
<th></th>
<th>Air:N2</th>
<th>DO (mg/L)</th>
<th>( r_{NO_3-N} ) (mg NO(_3)-N/g VSS·hr)</th>
<th>( r_{NO_2-N} ) (mg NO(_2)-N/g VSS·hr)</th>
<th>( r_{N_2O-N} ) (mg N(_2)O-N/g VSS·hr)</th>
<th>( \frac{N_{\text{N}<em>2O-N}}{r</em>{NO_3-N}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBRL</strong></td>
<td>75:225</td>
<td>0.21-0.24</td>
<td>-5.41</td>
<td>3.95</td>
<td>1.50</td>
<td>27.62%</td>
</tr>
<tr>
<td></td>
<td>150:150</td>
<td>0.17-0.24</td>
<td>-5.80</td>
<td>3.65</td>
<td>1.63</td>
<td>27.66%</td>
</tr>
<tr>
<td></td>
<td>300:0</td>
<td>0.43-0.73</td>
<td>-1.84</td>
<td>1.55</td>
<td>1.22</td>
<td>66.48%</td>
</tr>
<tr>
<td><strong>SBRH</strong></td>
<td>75:225</td>
<td>0.28-0.29</td>
<td>-2.52</td>
<td>2.64</td>
<td>0.05</td>
<td>1.90%</td>
</tr>
<tr>
<td></td>
<td>150:150</td>
<td>0.34-0.39</td>
<td>-0.77</td>
<td>1.45</td>
<td>0.03</td>
<td>3.81%</td>
</tr>
<tr>
<td></td>
<td>300:0</td>
<td>0.99-6.32</td>
<td>-</td>
<td>-00</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

Note: “–” indicates no reaction occurred. SBR: sequencing batch reactor.
low aeration rates than for high aeration rates. According to the batch nitrification results, activities of NOB were inhibited under low DO concentrations. In the study of nitrogen removal by the multiple AO process under different DO concentrations, Pan et al. (2015) found that DO concentration below 4.86 mg/L was beneficial for partial nitrification and denitrification. Pellicer-Nacher et al. (2010) also showed that inhibition of NOB activities could enhance nitrogen removal. In addition, from batch denitrification experiments, for activated sludge acclimated under low aeration rates, denitrifying activities using internal organic carbon were relatively high, and also denitrifying phosphorus removal activities were induced, enhancing simultaneous nitrogen and phosphorus removal. Therefore, by controlling DO, the multiple AO process could enhance nitrogen removal efficiently (Osada et al., 1995).

Despite the fact that the high nitrogen removal efficiency was achieved under low aeration conditions, the N2O emission potential was relatively high. Under low DO concentrations, inhibition of NOB activities was achieved in the multiple AO process, resulting in partial nitrification and inducing nitrifier denitrification, therefore inducing high N2O emission during biological nitrogen removal. In addition, denitrification under low DO conditions in the multiple AO process mainly proceeded using internal organic carbon as the electron donor. Due to the slow utilization rate of internal organic carbon and the low competition capacity of N2O reductase compared with nitrite and nitrate reductases, N2O rather than N2 was the end product of the denitrification process (Schalk-Otte et al., 2000; Itokawa et al., 2001; Law et al., 2012; Pan et al., 2015).

For activated sludge from both SBRs, when the ratios of air to N2 were 75:225 and 150:150, ammonia oxidation rates in batch nitrification experiments were all higher than those of batch SND experiments, while the N2O emission rate and factor were all lower than those from the batch SND experiment. This indicated that under aerobic conditions, the N2O emission during nitrification or SND would be enhanced with the presence of heterotroph activities. Wunderlin et al. (2012) also found that the N2O emission during SND was higher than that during nitrification under the same ammonia oxidation rate, and heterotrophic denitrification was proposed to be the main source of N2O emission during SND. In activated sludge systems for biological nitrogen removal from wastewater, microorganisms relating to N2O emission mainly include AOB, NOB and heterotrophs. The relationship between microbial competition and N2O emission under aerobic conditions is shown in Fig. 5. DO is an important factor affecting the activities of different microbial communities. Low DO conditions might affect the balance among different microbial activities and result in the accumulation of nitrite, and consequently, N2O emission would be enhanced. During nitrification under low DO conditions, AOB out-competed with NOB due to the lower affinity ability for DO of NOB, which could cause nitrite accumulation, resulting in nitrifier denitrification and high N2O emission. Meanwhile, denitrification occurred under low DO conditions, which also enhanced N2O emission with nitrite as the electron acceptor. In addition, the utilization of internal organic substrates for denitrification would also contribute to high N2O emission. In the present study, NOB activities in SBR_a were inhibited during acclimation, and the N2O emission potential of activated sludge acclimated in this reactor was higher than in SBR_h, indicating that microbial competition among different microorganisms could inhibit NOB activities and enhance N2O emission. Therefore, N2O emission was significantly affected by the different microbial communities in activated sludge. In future studies, the concentrations of DO should be controlled so as to balance the activities of different microbial communities and reduce N2O emission.

3. Conclusions

(1) The nitrogen removal efficiency in the multiple AO process decreased with increasing aeration rates. (2) In the multiple AO process, N2O emission mainly occurred under aerobic conditions, and low aeration conditions induced high N2O emission. (3) Under aerobic conditions, DO was the main factor affecting N2O emission. Compared with nitrification, the N2O emission factor was relatively high with the coexistence of heterotroph activities. Investigation of N2O emission from the viewpoint of microbial competition should be further explored.

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REFERENCES

Ahn, J.H., Kwan, T., Chandran, K., 2011. Comparison of partial and full nitrification processes applied for treating


