N₂O emissions from a one stage partial nitrification/anammox process in moving bed biofilm reactors

Jingjing Yang, Jozef Trela, Elzbieta Plaza and Kåre Tjus

ABSTRACT

Nitrous oxide (N₂O) emissions from wastewater treatment are getting increased attention because their global warming potential is around 300 times that of carbon dioxide. The aim of the study was to measure nitrous oxide emissions from one stage partial nitrification/anammox (Anaerobic Ammonium Oxidation) reactors, where nitrogen is removed in a biological way. The first part of the experimental study was focused on the measurements of nitrous oxide emissions from two pilot scale reactors in the long term; one reactor with intermittent aeration at 25 °C and the other reactor with continuous aeration at 22–23 °C. The second part of the experiment was done to evaluate the influence of different nitrogen loads and aeration strategies, described by the ratio between the non-aerated and aerated phase and the dissolved oxygen concentrations, on nitrous oxide emissions from the process. The study showed that 0.4–2% of the nitrogen load was converted into nitrous oxide from two reactors. With higher nitrogen load, the amount of nitrous oxide emission was also higher. A larger fraction of nitrous oxide was emitted to the gas phase while less was emitted with the liquid effluent. It was also found that nitrous oxide emissions were similar under intermittent and continuous aeration.

Key words | gas phase, liquid phase, moving bed biofilm reactor (MBBR), nitrous oxide emissions, one stage partial nitrification/anammox process

INTRODUCTION

The driving force of wastewater treatment plant development has changed from pollutant removal to energy and nutrient recovery. In wastewater treatment emissions of greenhouse gases (GHGs), especially carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), should be minimized. Among these GHGs, emissions of nitrous oxide from biological nutrient removal processes should be paid special attention because their global warming potential is around 300 times that of carbon dioxide. Mackenzie (1998) pointed out that there was a large amount of nitrous oxide emissions from nitrogen contained in wastewater due to microbial transformation.

The traditional way to remove nitrogen from wastewater is the combination of nitrification/denitrification reactions. New technologies have been developed based on the anammox (Anaerobic Ammonium Oxidation) reaction, which is considered as an effective and low cost process. To remove ammonium from wastewater by the anammox process, one part of ammonium is firstly required to be oxidized to nitrite by ammonium oxidizing bacteria (AOB) (1); the proper ratio between the produced nitrite and remaining ammonium is around 1.32. Then the remaining ammonium together with the formed nitrite are converted into dinitrogen gas under anaerobic conditions by anammox bacteria and a small amount of nitrate is produced simultaneously (2).

\[
\text{NH}_4^+ + 1.5O_2 + 2HCO_3^- = NO_2^- + 2CO_2 + 3H_2O \quad (1)
\]

\[
\text{NH}_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ = \\
1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (2)
\]

In a one stage partial nitrification/anammox process these two reactions occur in one single reactor. Biofilm technology, such as granular sludge and moving bed biofilm reactor (MBBR), allows the two groups of microorganism to grow in a layered structure. AOB and anammox bacteria...
stay in the outer and inner layer in the biofilm, respectively, because the outer layer is aerobic and the inner layer is under anaerobic conditions due to oxygen penetration. The MBBR system was successfully applied in the one stage partial nitrification/anammox process both in the laboratory and at full scale (Örnmark 2011; Winkler et al. 2012). In this study, MBBR was used due to its compactness, no need for sludge recirculation and flexibility (Odegaard 2006). Dissolved oxygen (DO) is a significant parameter influencing the nitrogen removal rate in the system. DO concentration should stay at a minimum level to allow AOB; a limited amount of heterotrophic bacteria was formed to identify the groups of microorganisms. The main groups of microorganisms were anammox bacteria and the anammox reactor, respectively. Desloover et al. (2011) showed that 5.1–6.6% of the nitrogen loads were converted into nitrous oxide in a full scale partial nitrification process.

The aim of the study was to measure and compare the nitrous oxide emissions in both liquid and gas phases from one stage partial nitrification/anammox processes treating reject water from sludge dewatering. The first part of the experimental work was focused on the measurements of nitrous oxide emissions from two pilot scale reactors, which were operated under different conditions; the second part aimed to investigate the influence of different nitrogen loads and aeration strategies, which was described by the ratio between the non-aerated and aerated phase (Ra) and the DO concentrations, on nitrous oxide emissions.

**MATERIAL AND METHODS**

**Description and operation of one stage partial nitrification/anammox reactors**

Two MBBRs, with a working volume of 200 L each, have been operated at the research facility Hammarby Sjöstadsverk, Stockholm, Sweden. Each reactor was filled with 80 L of partial nitrification/anammox biofilm carriers (K1), which have a specific surface area of 500 m² m⁻³. The total specific surface area of the biofilm carrier was 40 m² in each reactor. The biofilm carriers were taken from a full scale one stage partial nitrification/anammox plant in Himmerfjärden wastewater treatment plant (Örnmark 2011). FISH (fluorescence in situ hybridization) analysis was performed to identify the groups of microorganisms. The main groups of microorganisms were anammox bacteria and AOB; a limited amount of heterotrophic bacteria was observed in the biofilm (Winkler et al. 2012). Inflow of the reactor was the reject water from a dewatering digested sludge process. The reject water was taken and transported from Bromma wastewater treatment plant, Stockholm, Sweden. The characteristics of the reject water are shown in Table 1. On-line instruments were installed in the reactors

**Table 1** | Characteristics of influent reject water

<table>
<thead>
<tr>
<th></th>
<th>NH₄-N (mg L⁻¹)</th>
<th>NO₂-N (mg L⁻¹)</th>
<th>NO₃-N (mg L⁻¹)</th>
<th>Alkalinity (mmol/L)</th>
<th>COD° (mg O₂ L⁻¹)</th>
<th>Conductivity° (µS cm⁻¹)</th>
<th>pH°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av.</td>
<td>977.1</td>
<td>0.04</td>
<td>1.84</td>
<td>77.0</td>
<td>681</td>
<td>9.48</td>
<td>8.09</td>
</tr>
<tr>
<td>Min.</td>
<td>817.5</td>
<td>0.00</td>
<td>1.32</td>
<td>65.4</td>
<td>390</td>
<td>6.12</td>
<td>7.60</td>
</tr>
<tr>
<td>Max.</td>
<td>1,110</td>
<td>0.11</td>
<td>2.66</td>
<td>112.0</td>
<td>1,280</td>
<td>11.36</td>
<td>8.46</td>
</tr>
</tbody>
</table>

°Chemical oxygen demand (COD) concentration in the filtrated sample (0.45 μm).
°Daily average value from on-line measurements.
to measure conductivity, pH, DO and redox potential values. The duration of nitrous oxide measurements was around 6 months. The first part of the experiment took 3 months and the rest of the time was used for the second part.

Experimental studies (part I)

In the first part of the experimental study (part I), the first reactor (R1) was operated using intermittent aeration in a 1 hour cycle (45 min aerated phase/15 min non-aerated phase). DO concentration in aerated phase was maintained in the range of 0.5–1 mg L\(^{-1}\). The temperature inside the reactor was 25 \(\pm\) 1°C. The second reactor 2 (R2) was operated with continuous aeration at 22–23 \(\pm\) 1°C with DO values between 1 and 2.5 mg L\(^{-1}\). The hydraulic retention time for both reactors was 1.5–2 d.

Experimental studies (part II)

The second part of the experimental work aimed to evaluate the influence of different aeration strategies on N\(_2\)O concentrations both in the liquid and gas phases. The experiments were carried out in R1. The temperature inside the reactor was maintained at 25 °C. Shown in Table 2 are the different periods of operation when nitrous oxide was measured in R1.

Nitrous oxide measurement

When nitrous oxide was measured, the reactor was covered with a plastic board to achieve a closed system during measurement. Aeration was supplied from the bottom of the reactor (\(Q_2\)) and an external pump, which had a constant air flow \(Q_1\) (flow meter 1), was used to collect the air above the water surface. Considering there would be periods without aeration during intermittent aeration, an extra inlet of air (\(Q_3\) for dilution and constant air flow) and flow meter 3, were added. In this way, \(Q_1 = Q_2 + Q_3\). A Unisense nitrous oxide microelectrode, and Teledyne analytical instruments (Model GFC-7002E) were used to measure the nitrous oxide concentration in the liquid and gas phase, respectively (Figure 1).

Chemical analysis

In the experimental part I, chemical analysis were carried out by Dr. Lange Kits to measure concentrations of ammonium in the influent as well as different forms of nitrogen compounds in the effluent. In part II of this study, an on-line VARION ammonium electrode and nitrate electrode (WTW) were installed to measure ammonium and nitrate concentrations. Concentration of nitrite and COD were measured spectrophotometrically both in the influent and the effluent by Dr. Lange Kits.

RESULTS AND DISCUSSION

The results of the experimental study part I aimed to show the nitrous oxide emissions both in gas and liquid phases in the long term processes performance under different operation conditions, for instance, intermittent and continuous aeration and different temperatures. The purpose of the sub-section ‘Dynamic behavior of nitrous oxide concentration under intermittent/continuous aeration’ was to present the representative patterns of nitrous oxide concentration under intermittent and continuous aeration obtained in the experimental study part I. The results from the experimental study part II were demonstrated and discussed in the sub-section ‘Influence of different factors on nitrous oxide emissions’.

<table>
<thead>
<tr>
<th>Period</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeration strategies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO (mg L(^{-1}))^a</td>
<td>1.5</td>
<td>2.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Intermittent aeration (Ra)</td>
<td>1/3b</td>
<td>1/3b</td>
<td>0^c</td>
<td></td>
</tr>
<tr>
<td>Nitrogen load (gN m(^{-2}) d(^{-1}))</td>
<td>1.7</td>
<td>2.5</td>
<td>3.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Duration (d)</td>
<td>7</td>
<td>7</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

*aDissolved oxygen concentration during aerated phase.

^b45 min of aerated phase in 1 hour operation.

^cContinuous aeration.
Nitrous oxide emissions from two pilot scale reactors (part I)

The measurements of nitrous oxide in R1 were performed for 85 d (Figure 2(a)). During this period, the nitrogen load was 2.3 gN m$^{-2}$ d$^{-1}$ at the beginning and after day 25 it was increased to 3.0 gN m$^{-2}$ d$^{-1}$. The average value of the nitrogen load was 2.8 gN m$^{-2}$ d$^{-1}$ from day 25 to 70. From day 80, it decreased to 2.3 gN m$^{-2}$ d$^{-1}$. The average nitrogen removal efficiency was 82%. Nitrous oxide emission to the gas phase was 0.015 gN m$^{-2}$ d$^{-1}$ when the nitrogen load was 2.3 gN m$^{-2}$ d$^{-1}$, which means that around 0.65% of the nitrogen load was converted into nitrous oxide and emitted into the gas phase. Nitrous oxide production rate increased to 0.047 gN m$^{-2}$ d$^{-1}$ when the nitrogen load applied to the reactor was 2.8 gN m$^{-2}$ d$^{-1}$, which indicates that 1.7% of the nitrogen load was emitted into the gas phase as nitrous oxide. Based on the results obtained in part I, only 0.012 gN m$^{-2}$ d$^{-1}$ nitrous oxide was present in the liquid phase, which was around 0.043% of the nitrogen load.

Nitrogen load in R2 (Figure 2(b)) was around 2.1 gN m$^{-2}$ d$^{-1}$ during the 45 d of nitrous oxide measurements.
Nitrous oxide emitted into the air was 0.01–0.03 gN m⁻² d⁻¹, which indicates that 0.4–1.6% of the nitrogen load was converted to nitrous oxide and emitted to the gas phase. The highest emission was observed at DO concentration of 2.5 mg L⁻¹. Nitrous oxide emitted with outgoing water accounted for only 0.02% of the nitrogen load.

The results from both R1 and R2 showed that 0.4–2% of the nitrogen load was emitted as nitrous oxide to the air and less than 0.05% was released as nitrous oxide dissolved in the outgoing water, which is comparable with the results obtained by Kampschreur et al. (2008) and Okabe et al. (2011). The range of nitrous oxide emissions from R1 and R2 was 0.09–2.34 g N₂O d⁻¹ and 0.4–1.4 g N₂O d⁻¹, respectively, which were lower compared with the results obtained when measuring emission from the full scale plant (Kampschreur et al. 2008).

Dynamic behavior of N₂O concentration under intermittent/continuous aeration (part I)

Concentrations of nitrous oxide in both the gas and the liquid phase changed with oxygen supply (Figure 3). With intermittent aeration, nitrous oxide concentrations in the liquid and gas phases followed the oxygen supply pattern (Figure 3(a)). When aeration stopped, concentration of nitrous oxide in the gas phase decreased. However, the concentration of nitrous oxide in the gas phase never reached zero, even though it was continuously flushed with the air. It is probable that during the non-aerated phase, a small amount of nitrous oxide, produced in the liquid phase, could transfer into the gas phase on the surface of liquid–gas contact. Meanwhile, most of the produced nitrous oxide stayed in the liquid phase during the period with no aeration and an increase of nitrous oxide level in the liquid could be observed. When aeration started, a peak value of nitrous oxide concentration in the gas phase was detected. The reason was that a large amount of nitrous oxide was stripped out by the air from the liquid causing an increase of nitrous oxide concentration in the gas phase. After the peak, nitrous oxide in the gas and liquid phase reached a stable level.

When continuous aeration was applied (Figure 3(b)), nitrous oxide concentration in both liquid and gas phases showed negligible variations. N₂O concentration in the liquid phase was much lower than its solubility in the water at the same temperature, which means that a large fraction of the produced nitrous oxide in the liquid phase was transferred into the gas phase due to stripping.

Influence of different factors on nitrous oxide emissions (part II)

The summary of nitrous oxide measurements under different aeration strategies and nitrogen loads is shown in Table 3. During the nitrous oxide measurements, nitrogen removal efficiency varied from 70 to 90% while COD removal was around 30%. When the same aeration strategies were applied in the system, an increased level of pH and ammonium together with a decreased concentration of nitrate in the effluent were obtained when nitrogen loads increased. Figure 4 gives examples of dynamic changes of nitrous oxide (mg N₂O min⁻¹) in the different phases; the reported data were obtained when the system was stable.

Nitrous oxide levels both in the gas and liquid phases increased when higher nitrogen loads were applied to the reactor under the same aeration strategy (Table 3). It was probably because with higher nitrogen loads, a larger amount of nitrogen was converted between different nitrogen forms, which increased the chances for AOB and denitrifiers to produce nitrous oxide. Several studies...
confirmed that high nitrite concentrations led to high levels of nitrous oxide emissions (Kampschreur et al. 2008, 2009). Moreover, increased pH value can also be a reason for higher level of nitrous oxide production. Similar results were obtained by Law et al. (2011) who observed a positive linear relationship between N2O productions and the pH value within the pH range of 6.0–8.5. With a similar nitrogen load (3.5 gN m⁻² d⁻¹), 0.8 and 1.12% of the nitrogen loads transferred into nitrous oxide when the DO was 2.5 and 1.5 mg L⁻¹, respectively (Table 3). Increased DO concentration led to a drop of pH value and decreased level of nitrous oxide.

Shown in Figure 4(a) are the examples of the dynamic behavior of nitrous oxide emissions with the same DO level and Ra. It was found that when the nitrogen load was low (1.7 gN m⁻² d⁻¹), a peak of nitrous oxide in the liquid phase occurred in the non-aerated phase. A possible explanation is that AOB or denitrifiers used free oxygen and nitrite/nitrate in the bulk liquid which was left from produced during the aerated phase. AOB can produce nitrous oxide from nitrite under low oxygen conditions. Moreover, denitrifiers can also produce nitrous oxide due to oxygen inhibition (Park et al. 2000; Dundee & Hopkins 2001; Beaumont et al. 2004). By contrast, under nitrogen loads of 2.5 and 3.3 gN m⁻² d⁻¹, nitrous oxide in the liquid phase reached the peak at the end of the aerated phase and decreased in the non-aerated phase. In the aerated phase, it was most likely that AOB produced nitrous oxide. Nitrous oxide in the liquid phase increased even though most of the produced nitrous oxide was stripped out into the gas phase. The drop of nitrous oxide level in the non-aerated phase both in the gas and liquid phase suggested that nitrous oxide was consumed during this phase, most likely by denitrifiers.

The results showed that when nitrogen load was low (1.7 gN m⁻² d⁻¹) and DO = 1.5 mg L⁻¹, nitrous oxide production rate was higher than nitrous oxide consumption rate during the whole operation period. However, with the same DO concentration (1.5 mg L⁻¹), higher nitrogen load (2.5 and 3.3 gN m⁻² d⁻¹) led to a higher rate of nitrous oxide consumption in the non-aerated phase (Figure 4(a)).

Joss et al. (2009) studied nitrous oxide emissions from the one-step granular partial nitrification-anammox process under different aeration strategies and showed that lower emission (0.4% comparing to 0.6% of the nitrogen load) was observed when air was supplied continuously. However, nitrous oxide emissions as a percentage (%) of the nitrogen load in the intermittent and continuous aeration in our study were very close. It is reasonable to say that intermittent aeration would stimulate more nitrous oxide production than continuous aeration if all the other operation parameters are the same. However, in this study, the source of nitrous oxide production was not only AOB; denitrifiers also contributed to nitrous oxide emission.

Experimental study II showed the relation between nitrous oxide emissions, aeration strategies and nitrogen loads in a one stage partial nitrification/anammox process. The relation between DO concentration and nitrous oxide emissions has been studied by many researchers. An increase of nitrous oxide emission due to decreased DO concentration is reported in different nitrogen converting systems, such as nitrifying and denitrifying reactors (Sliekers et al. 2005; Tallec et al. 2006). Poth & Focht (1985) concluded that low oxygen tension is the primary factor for nitrous

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**Table 3** | Results summary of experiment part II

<table>
<thead>
<tr>
<th>Aeration strategies</th>
<th>Nitrogen load (g N m⁻² d⁻¹)</th>
<th>NH₄-N (mg L⁻¹)</th>
<th>NO₂-N (mg L⁻¹)</th>
<th>NO₃-N (mg L⁻¹)</th>
<th>N₂O (ppm)</th>
<th>N₂O emission (%)</th>
<th>pH</th>
<th>N removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO 1.5 mg L⁻¹ Ra = 1/3</td>
<td>1.7</td>
<td>25.3</td>
<td>6.1</td>
<td>61.5</td>
<td>3.3</td>
<td>7.2</td>
<td>7.1</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>95.5</td>
<td>6.12</td>
<td>54.7</td>
<td>12.6</td>
<td>11.8</td>
<td>7.5</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>126.6</td>
<td>6.67</td>
<td>48.4</td>
<td>14.2</td>
<td>13.5</td>
<td>7.6</td>
<td>1.12</td>
</tr>
<tr>
<td>DO 1.5 mg L⁻¹ Ra = 0</td>
<td>1.5</td>
<td>27.5</td>
<td>7.6</td>
<td>130.8</td>
<td>3.7</td>
<td>6.8</td>
<td>6.5</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>74.4</td>
<td>6.5</td>
<td>86.2</td>
<td>10.3</td>
<td>13.2</td>
<td>7.3</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>87.1</td>
<td>4.4</td>
<td>72.5</td>
<td>13.3</td>
<td>11.2</td>
<td>7.4</td>
<td>1.11</td>
</tr>
<tr>
<td>DO 2.5 mg L⁻¹ Ra = 1/3</td>
<td>2.7</td>
<td>53.6</td>
<td>6.65</td>
<td>94.7</td>
<td>10.6</td>
<td>–</td>
<td>7.2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td>60.2</td>
<td>7.71</td>
<td>89.5</td>
<td>11.5</td>
<td>7.8</td>
<td>7.2</td>
<td>0.80</td>
</tr>
</tbody>
</table>

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– No data.

*The solubility of nitrous oxide in the water is 28 mmol/L at 20 °C and 21.55 mmol/L at 30 °C (Weiss & Price 1980).

*ph value inside the reactor.
oxide production by nitrifying bacteria and Park et al. (2000) showed that DO inhibition can cause denitrifying bacteria to produce nitrous oxide. However, it was also found (Kampschreur et al. 2009) that there was higher emission of nitrous oxide at higher oxygen levels. It was suspected that high DO contributes to a high nitrite/nitrate production, which can also lead to high nitrous oxide production. Our study emphasized that both nitrogen loads and DO concentration played important roles in nitrous oxide emissions. Figure 4 indicates that with the same aeration strategy, increased nitrogen load contributed to higher nitrous oxide level. Nitrous oxide emissions under intermittent and continuous aeration were similar. During intermittent aeration, higher nitrous oxide emission was observed in the aerobic phase.
Kimochi et al. (1998) obtained comparable results which showed most of the nitrous oxide was emitted into the atmosphere during the aerobic period in the nitrification process. They assumed that during the aerated phase, production of nitrous oxide was due to incomplete nitrification.

CONCLUSIONS

Two pilot scale MBBRs, performing the partial nitrification/anammox process, were operated to investigate nitrous oxide emissions both in the liquid and gas phases.

- Nitrous oxide emissions were related to nitrogen loads, DO concentrations and the ratio between the non-aerated phase and the aerated phase (Ra).
- A large fraction of nitrous oxide was emitted into the air and only a minor part of it was emitted dissolved in the effluent.
- In partial nitrification/anammox MBBR, around 0.4–2% of the nitrogen load was converted into nitrous oxide.
- Increased nitrogen loads contributed to higher nitrous oxide level under the same aeration strategy.
- Nitrous oxide emissions under intermittent aeration compared with continuous aeration were similar.
- In this study, when $DO = 1.5 \text{ mg L}^{-1}$, $Ra = 1/3$ with the nitrogen load larger than $2.5 \text{ gN m}^{-2} \text{ d}^{-1}$, nitrous oxide consumption can be observed during the non-aerated phase.

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