Nitrous oxide emissions from one-step partial nitritation/anammox processes

Jingjing Yang, Jozef Trela and Elzbieta Plaza

ABSTRACT

Measurements of nitrous oxide were made at pilot- and full-scale plants to evaluate greenhouse gas emissions from one-step partial nitritation/anammox processes applied in moving bed biofilm reactors treating reject water. It was found that 0.51–1.29% and 0.35–1.33% of the total nitrogen loads in the pilot- and full-scale reactor, respectively, were emitted as nitrous oxide. Between 80 and 90% of nitrous oxide emissions were in gaseous form and the rest amount was found in the reactor effluent; over 90% of nitrous oxide emissions occurred in the aerated period and less than 8% in the non-aerated period in the full-scale study. Nitrous oxide productions/consumptions were closely related to aeration and the nitrogen loads applied in the system.

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Key words | gas phase, liquid phase, moving bed biofilm reactor (MBBR), nitrous oxide emission, partial nitritation/anammox process

INTRODUCTION

Greenhouse gas emissions have attracted much attention due to the great concerns on the global warming. Among the greenhouse gases, nitrous oxide (N₂O) has a global warming potential of 265–298 CO₂-equivalents in a 100-year timescale (IPCC 2014) and therefore can play a significant role in the global warming. N₂O emissions can occur as direct emissions in gaseous form from a wastewater treatment plant (WWTP) or as indirect emissions in liquid phase in WWTP discharge. IPCC guidelines require policy makers to estimate the N₂O emissions from WWTPs. Therefore, more and more research studies carried out to investigate the N₂O formations and emissions from the wastewater treatment process.

In the biological nitrogen removal system, nitrification and heterotrophic denitrification process are involved to produce N₂O. It is widely considered that nitrite oxidizing bacteria (NOB) has no contribution on N₂O production (Law et al. 2012a). The key metabolic pathways of N₂O production are (1) nitrifier denitrification under anoxic condition; (2) autotrophic ammonium oxidation under changing from anoxic to aerobic condition (Kim et al. 2010; Chandran et al. 2011; Law et al. 2012b); (3) heterotrophic denitrification. There are several process conditions which can trigger N₂O emission and formation. The key factor for N₂O emission is stripping due to aeration since N₂O can be dissolved in water with 24 mM atm⁻¹ (at 25 °C and 0% salinity). For example, N₂O produced in the non-aerated period can be accumulated and emitted under aeration phase. Regarding to N₂O production, ammonium oxidizing bacteria (AOB) can have a high N₂O production under frequent changes between anoxic and aerobic conditions (Chandran et al. 2011). Moreover, pH over 8 will significantly increase N₂O production by AOB (Hynes & Knowles 1984). Heterotrophic denitrifying bacteria can both produce and consume N₂O. It is reported that the concentration of NO₂ up to 10 mg/L caused N₂O production in heterotrophic denitrifying biomass (Itokawa et al. 2001). Moreover, availability and types of carbon sources are also crucial for heterotrophic denitrifying bacteria producing N₂O (Pan et al. 2012; Ni et al. 2013).

This study examined emissions from the partial nitritation/anammox process applied in moving bed biofilm reactors (MBBRs). In this process, ammonium is first oxidised to nitrite by AOB under aerobic conditions and then anammox bacteria convert the remaining ammonium and nitrite to nitrogen gas under anaerobic conditions (Mulder et al. 1995). This process can be applied either in two separate reactors or one single reactor. In the one-stage partial
nitritation/anammox process, a biofilm system is used to allow AOB and anammox bacteria to grow together in layer structures (Odegaard 2006). AOB grow under aerobic conditions in the outer layer of the biofilm and anammox bacteria grow under anaerobic conditions in the inner layer (Rosenwinkel & Cornelius 2005; Joss et al. 2009). However, it is very difficult to avoid the growth of NOB and heterotrophic denitrifiers in the partial nitritation/anammox process because of over-aeration and the presence of organic matter. Therefore, the production of N₂O can originate from both AOB and denitrifying bacteria in such systems (Figure 1). So far, there is no evidence that anammox bacteria can produce/consume nitrous oxide (Van der star 2008).

A wide range of N₂O emissions from treatment plants have been measured in different studies. For example, Kong et al. (2015) reported 5% of the nitrogen load converted into N₂O in a partial nitrification process, de Graaff et al. (2010) showed that N₂O emissions corresponded to 0.6–2.6% (mean 1.9%) of the total nitrogen load in a partial nitrification process. In a study of full-scale reject water treatment, the N₂O emissions were 1.7% and 0.6% of the total nitrogen load for a partial nitrification reactor and anammox reactor, respectively (Kampschreur et al. 2008). Mampaey et al. (2016) reported that N₂O emission was 3.7% of the incoming ammonium load in a full-scale partial nitrification reactor. Xiao et al. (2014) showed 0.14–7.25% nitrogen load emitted as N₂O in CANON process.

In the present study, N₂O emissions from a full-scale, one-stage partial nitritation/anammox process were measured and the production/consumption of N₂O from a pilot-scale and a full-scale plant were compared.

MATERIAL AND METHODS

Operation of a partial nitritation/anammox process in a pilot-scale plant

Measurements of N₂O emissions in a pilot-scale operated one-step partial nitritation/anammox process were carried out in a reactor located at the Hammarby Sjöstadsverket research facility. The measurement is not part of this study but the results are used to compare with the data from full-scale treatment plant (Yang et al. 2015). The reactor had a working volume of 200 L and was filled with 80 L of biofilm carrier (K1) with a specific surface area of 500 m²/m³. The reactor was equipped with online dissolved oxygen (DO), pH, redox and conductivity meters and temperature was kept at 25 °C. The reactor was continuously filled with the reject water from digested sludge dewatering, which contained an average ammonium (NH₄-N) concentration of 977 mg/L, chemical oxygen demand (COD) concentration of 681 mg/L, alkalinity 2,900 mg CaCO₃/L and pH between 8 and 8.5. The oxygen was supplied intermittently (Table 1) with DO 1.5 mg/L in the aerated period.

Operation of a partial nitritation/anammox process in a full-scale plant

N₂O emissions in a full-scale operated one-stage partial nitritation/anammox process were measured at Himmerfjärden WWTP, Stockholm. The plant consisted of two parallel lines (L1 and L2), each with a volume of 700 m³ divided into three zones (Z1, Z2 and Z3). Each zone was filled with 32% of biofilm carriers (K1). DO, pH, conductivity and

Figure 1 | Nitrous oxide (N₂O) production in the nitrogen cycle.
temperature were monitored online. N\textsubscript{2}O measurements were only carried out in the second treatment line (L2).

During the N\textsubscript{2}O measurements, influent supernatant from dewatering of digested sludge contained an average NH\textsubscript{4}-N concentration of 1,476 mg/L, DOC 380 mg/L, alkalinity 4,705 mg CaCO\textsubscript{3}/L and pH 7.8. The mean temperature was 28°C. Air was intermittently supplied into all the zones, with a 50 min aerated period and 10 min non-aerated period. DO level was on average 3 mg/L during the aerated period. The hydraulic retention time was 5.5 days and the nitrogen load was 188 kg N/d.

**Measurements of nitrous oxide emissions in the full-scale plant**

The set-up used for the N\textsubscript{2}O emissions measurements in the full-scale plant is shown in Figure 2. The N\textsubscript{2}O in the liquid phase was measured by online Unisense microelectrode. To measure N\textsubscript{2}O in the gas phase, a flux chamber with the size of 0.9 m \times 0.9 m, floating on the water surface, was used and acted as gas sampler. Gas samples and dilution air were collected by a sample pump and transferred through a cooling system and water trap unit to the online Teledyne T320 N\textsubscript{2}O analyzer based on infrared spectroscopy. Concentrations of different nitrogen compounds (nitrate and ammonium) were measured continuously with

![Figure 2](https://iwaponline.com/wst/article-pdf/74/12/2870/456028/wst074122870.pdf)

**Figure 2** | Experimental set-up used for measurement of nitrous oxide emissions in the full-scale one-stage partial nitritation/anammox reactor.

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**Table 1** | Summary of nitrous oxide (N\textsubscript{2}O) production/consumption in the partial nitritation/anammox process in the pilot-scale and full-scale reactor

<table>
<thead>
<tr>
<th></th>
<th>Pilot-scale</th>
<th>Full-scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen load (gN/m\textsuperscript{2}/d)</td>
<td>1.7</td>
<td>2.5</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Intermittent aeration aerated (min)/non-aerated period (min)</td>
<td>45/15</td>
<td>45/15</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerated</td>
<td>+/a</td>
<td>+</td>
</tr>
<tr>
<td>Non-aerated</td>
<td>+/−</td>
<td>−</td>
</tr>
</tbody>
</table>

\textsuperscript{a}N\textsubscript{2}O production and −N\textsubscript{2}O consumption.
online sensors connected to the floating chamber. The N2O emission in both the liquid and gas phases were measured in all zones one after another. Based on those measured values, the total N2O emissions and production/consumption were calculated (supplemental material, available with the online version of this paper).

RESULTS AND DISCUSSION

The N2O emissions from the pilot-scale reactor are reported in Yang et al. (2013) and those from the full-scale process are presented below. Examples of N2O dynamic changes in the pilot-scale and full-scale plants are then given. This is followed by a comparison of N2O production/consumption in order to investigate the relationship between nitrogen loads and aeration conditions.

Nitrous oxide emissions from the full-scale process

During the measurements, the nitrogen load was 188 ± 8 kgN/m²/d and the average nitrogen removal efficiency was 81%. N2O emissions measured included N2O released in the gas phase and that in the liquid phase, dissolved in the discharge. The N2O in the discharge can be later stripped out and emitted to the atmosphere. The average N2O emissions was 0.95 kg N2O-N/d, which represented 0.51% of the total nitrogen load and 0.62% of the removed nitrogen (Figure 3(a)). This corresponds to 342.1 kgN2O-N/y and a global warming potential of almost 101 t CO2/y. This value is much less than that reported for the full-scale Dokhaven-Sluisjesdijk treatment plant, which uses a two-stage process (Kampschreur et al. 2009). Moreover, most of the N2O emissions occurred in the gas phase during the aerated period. These emissions were almost nine-fold those dissolved in the discharge (Figure 3(b)).

The nitrogen load in the supernatant treatment line was almost constant when N2O was measured. The N2O emission varied between 0.7 and 2.3 kgN2O-N/d (Figure 3(b)). On days 14 and 26, the aeration was stopped for a certain period of time. Because of this, N2O emissions were much lower than the values obtained on the other days. When there was no aeration, most of the N2O produced was in the liquid phase and some was probably consumed. Therefore, very little N2O was stripped out in the gas phase. Figure 3(b) presents the relative distribution of N2O emissions in the gas and liquid phases. Over 80% of the emissions happened in the gas form and rest in liquid form.

Production/consumption of nitrous oxide in one-stage partial nitritation/anammox reactors

Pilot-scale reactor

When the nitrogen load changed from 1.7 to 2.5 and to 3.3 gN/m²/d, the nitrogen removal efficiency was 88.3%, 80.2% and 76.8%, respectively. N2O emissions were 0.51%, 1.29% and 1.12% of the nitrogen loads, respectively. Figure 4 shows 6-h results from the pilot-scale reactor operated under different nitrogen loads. Within Figure 4, parts A2, B2 and C2 show the production and consumption of N2O in 6-h cycles. As it can be seen, there were no N2O production peaks (Figure 4, A2, B2 and C2) in the liquid phase at the beginning of the aerated periods under any operating conditions tested, which means that the measured peak concentrations of N2O in the gas phase (Figure 4, A1, B1 and C1) were mainly due to physical stripping.

When the nitrogen load was 1.7 gN/m²/d (Figure 4(a)) with DO 1.5 mg/L, N2O could be produced by ammonium oxidation during the aerated period. However, it also showed that N2O concentrations both in the gas and liquid phase were lower in the aerated period. At the
Beginning of the non-aerated period, N$_2$O formation is most likely due to nitrifier denitrification and incomplete heterotrophic denitrification because excess DO at end of aerated period causing low DO conditions at beginning of non-aerated period (Figure 5(a)). Heterotrophic denitrifying bacteria can produce N$_2$O due to oxygen inhibition (Park et al. 2005; Dundee & Hopkins 2001; Beaumont et al. 2004). DO became zero after a while in the non-aerated period and heterotrophic denitrifier started to consume N$_2$O. Pan et al. (2013) reported that when the pH increased from 7.0 to 8.0, the N$_2$O reduction rate by denitrifying bacteria increased from 270 to 350 mgN/gVSS/h, which would decrease N$_2$O accumulation. In the present study, the average pH was 7.1, 7.5 and 7.6 when the nitrogen load was 1.7, 2.5 and 3.3 gN/m$^2$/d, respectively (Figure 5(a)–(c)). N$_2$O reduction was observed in the non-aerated period when the nitrogen load was 2.5 and 3.3 gN/m$^2$/d. Moreover, the higher ammonium oxidation rate at higher nitrogen loading conditions decreases much quicker the DO level during non-aerated period thus favoring N$_2$O reduction by heterotrophic denitrifier. Similar results has been obtained in Law et al. (2022a).
Full-scale reactor

The concentrations of N\textsubscript{2}O in the gas and liquid phases of the full-scale reactor under stable conditions are shown in Figure 6(a). The level of N\textsubscript{2}O in the liquid phase was built up in the non-aerated period. When aeration started, a large amount of N\textsubscript{2}O in the liquid was stripped out into the gas phase. Figure 6(b) shows the N\textsubscript{2}O production/consumption in 6 h periods. N\textsubscript{2}O production took place in the non-aerated period probably by heterotrophic bacteria and nitrifier denitrification due to excess DO from aerated period. At aerated period, N\textsubscript{2}O reduction occurred in the first 20 min due to stripping. As Figure 6(c) indicates, N\textsubscript{2}O production occurred mostly in the non-aerated period.
Comparison between pilot- and full-scale reactors

Based on the results presented above, a summarising table was drawn up to compare N\textsubscript{2}O production/consumption under different operating conditions (Table 1). When the DO concentration was fixed at 1.5 mg/L in the pilot-scale reactor, N\textsubscript{2}O was produced during the aerated period with the nitrogen load of 2.5 and 3.3 gN/m\textsuperscript{2}/d. When the DO was 3.0 mg/L and the nitrogen load was 1.5 gN/m\textsuperscript{2}/d, N\textsubscript{2}O was mainly produced in the non-aerated period in the full-scale reactor. This suggests that when the one-stage partial nitritation/anammox process is enough and over-aerated, there is a trend for N\textsubscript{2}O production to decrease in the aerated period and occur in the non-aerated period, where most of the N\textsubscript{2}O produced stayed in the liquid phase. When the N\textsubscript{2}O was produced in the aerated period, it could be emitted to the atmosphere directly.

Factors influencing nitrous oxide production/consumption

The operational factors which can lead to N\textsubscript{2}O emissions from partial nitritation/anammox MBBR process are discussed below.

Aeration, nitrogen and organic loads

In the present study, N\textsubscript{2}O production and consumption in one stage partial nitritation/anammox process under conditions of different nitrogen loads and aeration were investigated. When the nitrogen loads were getting higher, the N\textsubscript{2}O emissions were also higher, which were 0.51%, 1.29% and 1.12% when the nitrogen loads were 1.7, 2.5, 3.3 gN/m\textsuperscript{2}/d in pilot-scale study. Applying a high nitrogen load in the system means that a large amount of ammonium has to be oxidized to nitrite in the partial nitritation step (However, we did not detect the high NO\textsubscript{2} concentration in the bulk liquid. The concentration of NO\textsubscript{2} was less than 10 mg/L when we took the grab sample both in the pilot and full scale). The more nitrogen there is to be converted, the greater the risk of N\textsubscript{2}O production. Law et al. (2012a) has also reported that with the increased specific ammonia oxidation rate, specific N\textsubscript{2}O production rate increased. However, in this study, there is no production and emission difference when the nitrogen loads were 2.5 and 3.3 gN/m\textsuperscript{2}/d due to intermittent aeration (Yang et al. 2013). The reason was that a part of the produced N\textsubscript{2}O was consumed during the non-aerated period and might give less N\textsubscript{2}O emission.

In the pilot-scale study, the DO was fixed at 1.5 mg/L with different nitrogen loads. When the oxidation rate of nitrifiers is lower, oxygen can diffuse deeper into the biofilm, which could cause N\textsubscript{2}O production by heterotrophic denitrifying bacteria. Moreover, if the COD load is low during over-aerated conditions, heterotrophic denitrifying bacteria produce more N\textsubscript{2}O due to organic matter limitation (Pan et al. 2013). Itokawa et al. (2003) found that a high N\textsubscript{2}O emission rate occurred at a COD/N ratio of less than 3.5. In this research, both in pilot- and full-scale process, COD/N ratio was less than 1, which could trigger N\textsubscript{2}O production by heterotrophic denitrification. When the system was under insufficient aeration, a high ammonium concentration would be observed in the effluent and AOBs would produce N\textsubscript{2}O due to the limited oxygen conditions (Table 2). Moreover, pH will increase when the system is lack of oxygen, which would encourage N\textsubscript{2}O production also by AOB (Law et al. 2011). A summary of the conditions which trigger N\textsubscript{2}O production and consumption is presented in Table 2.

It is considered that a frequent switch between aerobic and anoxic condition would trigger AOB produce more N\textsubscript{2}O (Chandran et al. 2011). However, since in one stage of the partial nitritation/anammox process, heterotrophic denitrifying bacteria is very hard to avoid, intermittent aeration gives a chance for heterotrophic denitrification to reduce N\textsubscript{2}O production. In this study, there was no significant high N\textsubscript{2}O emission compared with other studies (Kampschreur et al. 2008).

Table 2 | Possible nitrous oxide (N\textsubscript{2}O) production/consumption pathway in a one-stage partial nitritation/anammox biofilm system under intermittent aeration

<table>
<thead>
<tr>
<th>Biofilm system</th>
<th>Intermittent aeration</th>
<th>Aerated period</th>
<th>Non-aerated period</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOB (outer layer)</td>
<td>Insufficient aeration</td>
<td>N\textsubscript{2}O formation by nitrifier denitrification</td>
<td>N\textsubscript{2}O production by nitrifier denitrification</td>
</tr>
<tr>
<td></td>
<td>Over-aeration</td>
<td>N\textsubscript{2}O production by hydroxylamine oxidation</td>
<td></td>
</tr>
<tr>
<td>Heterotrophic denitrifiers (inner layer)</td>
<td>Insufficient aeration</td>
<td>N\textsubscript{2}O consumption as long as COD is not limiting and NO\textsubscript{2} is not inhibiting</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Over-aeration</td>
<td>DO diffusion into inner layer of the biofilm causes N\textsubscript{2}O production</td>
<td></td>
</tr>
</tbody>
</table>
Biofilm system/activated sludge system

In the one-stage partial nitritation/anammox process, the layered structure of the biofilm system allows anaerobic and aerobic bacteria to grow together and co-exist in the same reactor. Intermittent aeration creates aerobic and anaerobic periods. Since biological N₂O production can occur in both aerobic and anaerobic conditions, it is very difficult to avoid N₂O production (Table 2). When the system is properly operated, heterotrophic denitrifiers in the inner layer of the biofilm avoid producing N₂O caused by oxygen inhibition but consume N₂O which is produced by AOB in the outer layer. However, it is possible that DO cannot diffuse deep enough in the biofilm and N₂O can be produced by nitrifier denitrification. In the activated sludge system, heterotrophic denitrifiers could be easily inhibited by DO during the aerated time and produce N₂O. Therefore, biofilm system would produce less N₂O compared with activated sludge system. Similar results have been reported by Panwivia et al. (2014) where anammox process in an attached biomass biofilm system showed less N₂O production compared to a suspended growth biomass system. Lo et al. (2010) studied simultaneous nitrification, denitrification and phosphorus removal process in a hybrid, pure biofilm and suspended sludge system. The results showed that the hybrid system could make a higher contribution of N₂O emissions compared with the pure biofilm system and suspended sludge system. In a two-step partial nitritation/anammox process, the partial nitritation can be performed in a suspended sludge reactor. It has been shown that 1.6% of the nitrogen load is converted into N₂O in this type of reactor (Kampschreur et al. 2009). In that case, the high DO and the air flow system strip out large amounts of N₂O in the gas phase.

CONCLUSIONS

- The average nitrous oxide emission rate from the studied full-scale partial nitritation/anammox process was 0.95 kg N₂O-N/d, which represented 0.51% of total nitrogen load and 0.62% of removed nitrogen.
- Between 80 and 90% of nitrous oxide emissions occurred in gaseous form in full-scale study and the rest amount was released in the liquid discharge.
- In the full-scale reactor operated with intermittent aeration, N₂O was mainly produced during the non-aerated periods, probably by nitrifiers and heterotrophic denitrification affected by low DO concentration at the beginning of the non-aerated intervals.
- In the pilot-scale reactor, nitrous oxide was mainly produced in the aerated period and consumed in the non-aerated period, when the nitrogen load was 2.5 and 3.3 gN/m²/d at DO 1.5mg/L. However, when the nitrogen load was 1.7 gN/m²/d, the N₂O production and consumption did not show clear relation with the different operational periods.
- Both dissolved oxygen and nitrogen load influenced nitrous oxide production. Air stripping was the main factor for nitrous oxide emissions.
- It is important to find the right balance between DO and the nitrogen load to minimize N₂O production during the aerated period (from ammonium oxidation and nitrifier denitrification) and not cause excess DO at the beginning of anoxic cycle.

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