Effect of nitrite on the N₂O and NO production on the nitrification of low-strength ammonium wastewater

C.M. Castro-Barros a, A. Rodríguez-Caballero b, E.I.P. Volcke a, M. Pijuan b,*

a Department of Biosystems Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium
b Catalan Institute for Water Research (ICRA), Emili Grahit Street, 101, H₂O Building, Scientific and Technological Park of the University of Girona, 17003 Girona, Spain

HIGHLIGHTS

• Nitrite effect on N₂O–NO emission in a low strength nitrifying system was studied.
• Nitrite presence in the reactor increases N₂O and NO emissions.
• High nitrite concentrations affect more NO emissions than N₂O emissions.
• The intermediate NO cannot be neglected during autotrophic denitrification.
• High nitrite concentrations inhibit the ammonium oxidising activity.

ABSTRACT

The effect of nitrite on N₂O and NO emission was assessed in a nitrifying lab-scale reactor fed with low strength ammonium wastewater. The effect of nitrite pulse additions (5–50 mg NO₂⁻N L⁻¹) and of step-wisely increasing nitrite concentrations (0–150 mg NO₂⁻N L⁻¹) was studied. For the pulse addition experiments, N₂O and NO emissions increased upon pulse addition of nitrite, decreasing to the original concentration once nitrite was consumed. The highest peak emissions were detected at nitrite concentrations of 50 mg NO₂⁻N L⁻¹. For the step-wisely increasing nitrite tests, N₂O-N emission per NH₄⁺-N converted increased linearly from 0.16% to 1.5%. NO emissions were substantially affected at nitrite concentrations higher than 50 mg NO₂⁻N L⁻¹, reaching 3.8% NO-N per NH₃-N converted at 150 mg NO₂⁻N L⁻¹. The results provide one of the first evidences of the combined effect of nitrite on N₂O and NO emissions, showing a stronger effect on NO emissions at high nitrite concentrations.

1. Introduction

Nitrogen removal during wastewater treatment emits nitrous oxide (N₂O) and nitric oxide (NO) [1,2]. N₂O is an important greenhouse gas with a global warming potential of 298-CO₂ equivalents [3], which may contribute significantly to the carbon footprint of wastewater treatment plants (WWTPs) [4,5]. NO is a very reactive gas that is involved in different physiological reactions in living organisms and becomes toxic at certain concentrations [6]. Both N₂O and NO contribute significantly to the ozone layer depletion [7,8]. The importance of studying the emissions of these gases from wastewater treatment lies on their environmental impact and their potential function as useful indicators of process disturbances [9]. Understanding the responsible mechanisms involved during the production of N₂O and NO, as well as knowing the factors that influence these emissions is essential to minimise their emissions and to reach a better process operation.

There is increasing evidence that the first step of nitrification (the conversion of ammonia to nitrite) conducted by ammonium
oxidising bacteria (AOB), is the major contributor to N₂O and NO emissions in the biologically mediated wastewater treatment processes [10,11]. AOB can produce N₂O and NO via two known pathways: the nitrifier denitrification and the hydroxylamine oxidation pathway. The first one is favored by limited oxygen conditions [12,13] and the presence of nitrite [1]. The second is enhanced at high ammonium oxidation rates [14]. Moreover, other factors such as pH [15], transient anoxic-aerobic conditions [16], salinity [17] or aeration intensity [18] have been reported to affect N₂O and NO emissions from nitrifying systems.

A lot of studies focused on the emissions of N₂O and NO for high strength ammonium wastewater, typically the reject water from the anaerobic digester [19–21]. The removal of nitrogen from such wastewater via nitrite has been gaining importance over the last years since it allows substantial energy (less aeration) and chemical savings compared to conventional processes over nitrate. However, partial nitrification systems used for the treatment of high strength wastewater have been shown to produce higher N₂O and NO emissions compared to full nitrification reactors [10,11] probably because of the high accumulation of nitrite in those systems and also the high ammonium conversion rates.

Relatively little research has been devoted to N₂O and NO emissions from systems treating low strength wastewater. Some studies focused on N₂O emissions from nitrification or combined nitrification–denitrification systems, without measuring NO [13,22]. Kampschreur et al. [23] is one of the few studies where NO was measured together with N₂O in a nitrifying culture. They found that N₂O and NO emissions are linearly proportional to the nitrite increase. In systems dealing with low-strength wastewater, the accumulation of nitrite may happen depending on the operating conditions and loading rates of the plant at certain moments. Occasional nitrite accumulation was also put forward as the cause during municipal wastewater treatment with punctual nitrite shocks as well as step-wisely increasing nitrite concentrations in the reactor on the emissions of both gases was assessed to understand the mechanisms producing N₂O and NO and to fully capture the overall emission dynamics of both gases. The results thus contribute to the potential reduction of their emissions during municipal wastewater treatment with punctual nitrite accumulation.

2. Materials and methods

2.1. Reactor operation

A sequencing batch reactor (SBR) of 8 L was inoculated with activated sludge from the municipal WWTP of Girona (Spain) and was operated during 5 months, obtaining a nitrifying mixed bacterial culture. The SBR was operated in 6-h cycles: 240 min of continuous feeding and aeration, settling during 100 min and 20 min for decanting. Four litres of synthetic wastewater were fed in each cycle, resulting in a hydraulic retention time (HRT) of 12 h. The influent ammonium concentration was set low (50 mg NH₄-N L⁻¹) and no temperature control was established in the reactor. Dissolved oxygen (DO) was controlled with a programmable logic controller (PLC) between 2.0 and 2.5 mg O₂ L⁻¹ by adjusting the amount of air and nitrogen in the added gas stream, of which the total flow was kept constant at 3.5 L min⁻¹. The pH was controlled between 6.7 and 7.0 by adding 1 M NaHCO₃.

The composition of the synthetic wastewater used as influent consisted of: 282.1 mg NH₄HCO₃ L⁻¹, 87.8 mg KH₂PO₄ L⁻¹, 110.0 mg K₂HPO₄ L⁻¹ and 2 mL L⁻¹ of a trace element solution that included (g L⁻¹): 1.25 EDTA, 0.55 ZnSO₄·7H₂O, 0.40 CoCl₂·6H₂O, 1.27 MnCl₂·4H₂O, 0.40 CuSO₄·5H₂O, 0.05 Na₂MoO₄·2H₂O, 1.37 CaCl₂·2H₂O, 1.25 FeCl₃·6H₂O, 44.4 MgSO₄·7H₂O.

The batch tests were conducted when the reactor had been operating for 4 months and it showed stable operation, with all the ammonium from the influent converted to nitrate without accumulation of nitrite (detailed cycle analysis and nitrogen balance presented in Section S1 from Supplementary information).

2.2. Analysis and measurements

Cycle studies were carried out on a weekly basis to assess the reactor performance. Samples were taken along the cycle and immediately filtered through disposable Millipore filter units (0.22 µm pore size) and then analysed to determine the NH₄-N, NO₂-N and NO₃-N concentrations by ion chromatography (ICSS5000, Dionex). Mixed liquor suspended solids (MLSS) and volatile MLSS (MLVSS) were determined according to the standard methods [27]. During the batch tests the averaged MLSS and MLVSS in the reactor were 0.63 ± 0.09 g MLSS L⁻¹ and 0.50 ± 0.05 g MLVSS L⁻¹, respectively.

N₂O and NO were monitored online in the gas phase every 5 s during the batch tests. N₂O was analysed with the infrared gas analyser Servomex 4900 (Servomex Group Ltd. East Sussex, UK) and NO was measured via the chemiluminescence gas analyser CLD64 (Eco Physics. Dürnten, Switzerland).

2.3. Batch tests

All batch tests were conducted in the SBR. After the decanting phase of the cycle previous to the batch test, the feeding pump was disconnected and the desired addition of ammonium and/or nitrite started. The pH and DO were kept at the same levels as normal reactor operation (described in Section 2.1). After each test, the SBR operation was kept normal at least for 24 h before another test was conducted.

2.3.1. Intermittent ammonium feeding with nitrite pulse additions

An experiment was designed to assess the effect of nitrite on N₂O–NO emissions in the absence of ammonium oxidation. A pulse of nitrite of 10 mg NO₂-N L⁻¹ (reactor concentration) was added without ammonium feeding. Once nitrite was completely oxidised to nitrate, ammonium feeding was re-established at a rate of 0.833 mg NH₄-N min⁻¹. This test was repeated twice.

2.3.2. Continuous ammonium feeding with nitrite pulse addition

Four sets of tests were conducted to check the effect of 4 different nitrite concentrations (5, 10, 25 and 50 mg NO₂-N L⁻¹), added as pulses, on N₂O–NO emissions. During all the tests, ammonium was fed continuously at a rate of 0.833 mg NH₄-N min⁻¹ and 3–4 pulses of nitrite (to provide the desired concentration of NO₂-N L⁻¹ in the reactor, see Table 1) were added.
and the different nitrite concentrations present inside the reactor. Continuous nitrite additions (described in Sections 2.3.2 and 2.3.3) maintained in the reactor for a period of 30–60 min. During all the trials, the community. Each concentration of nitrite tested was main-

same rate as it was being consumed by the nitrite oxidising bacte-

rion. The desired constant nitrite concentration was achieved by adding nitrite to the SBR until the desired concentration and keeping this concentration by adding nitrite continuously to the reactor at the concentration by adding nitrite to the reactor with a loading rate of 0.10 g NH₄-N L⁻¹ was conducted. Firstly, the nitrite consumption rate was determined from the tests with nitrite pulse addition (Section 2.3.2) (see Section S2 from Supplementary information). The desired constant nitrite concentration was achieved by adding nitrite to the reactor until the desired concentration and keeping this concentration by adding nitrite continuously to the reactor at the same rate as it was being consumed by the nitrite oxidising bacterial community. Each concentration of nitrite tested was maintained in the reactor for a period of 30–60 min. During all the tests, ammonium was fed continuously at a rate of 0.833 mg NH₄-N min⁻¹.

Table 1 summarises the sets of tests performed with pulse and continuous nitrite additions (described in Sections 2.3.2 and 2.3.3) and the different nitrite concentrations present inside the reactor.

2.4. Calculations

The specific N₂O and NO production rates were calculated as:

\[ \text{r}_{\text{N}_2\text{O} \text{ or NO}} = \frac{\text{C}_{\text{N}_2\text{O} \text{ or NO}} \cdot \text{Q}_\text{G}}{\text{M}_{\text{biomass}}} \text{ g N MLVSS}^{-1} \text{ h}^{-1} \]

where:

\[ \text{C}_{\text{N}_2\text{O} \text{ or NO}} \text{ N}_2\text{O-N or NO-N concentration in the gas phase (g N L}^{-1} \text{) \]

\[ \text{M}_{\text{biomass}} \text{ total biomass in the reactor (gMLVSS)} \]

The N₂O-N and NO-N emissions factors per ammonium converted during an interval \( t_n - t_0 \) (h) with constant ammonium addition were obtained as:

\[ \text{EF}_{\text{N}_2\text{O} \text{ or NO}} = \frac{\sum_{i=0}^{n-1} \text{C}_{\text{N}_2\text{O} \text{ or NO}}(t_0) \cdot \text{Q}_\text{G} \cdot \left( t_i - t_{i-1} \right)}{\text{C}_{\text{NH}_4} \cdot \text{V}_{\text{L}} + \text{C}_{\text{NH}_4} \cdot \text{Q}_\text{G} \cdot (t_n - t_0) - \text{C}_{\text{NH}_4} \cdot \text{V}_{\text{G}} \cdot 100(\% \text{, W/W})} \]

in which the numerator denotes the N₂O or NO emitted during the interval \( t_n - t_0 \) in mg N, and the denominator corresponds to the NH₄-N consumed during the mentioned interval in mg N, and obtained from mass balances:

\[ \text{C}_{\text{NH}_4} \cdot \text{V}_{\text{G}} \cdot \text{V}_{\text{L}} \text{ : liquid volume of the reactor at } t_0 \text{ and } t_n \text{ (L) \]

\[ \text{Q}_\text{G} \text{ : incoming liquid flow rate into the reactor (L h}^{-1} \text{) \]

3. Results and discussion

3.1. N₂O and NO emissions during nitrification of low strength wastewater during normal reactor operation

During normal reactor operation, only ammonium was fed into the reactor with a loading rate of 0.10 g NH₄-N L⁻¹. All the incoming ammonium was converted to nitrate without accumulation of ammonium or nitrite. The N₂O and NO emissions during normal reactor operation were 0.16 ± 0.11% of the ammonium converted being emitted as N₂O-N and negligible NO emissions. These results are comparable to the ones found in literature for systems treating low strength ammonium wastewater through nitrification only or combined nitrification-denitrification (Table 2).

The variability of the N₂O emissions is notable, especially for full-scale measurements (N₂O emissions ranging from 0.01% to 25% of N removed, Table 1). Moreover, most of the studies focus on N₂O emissions, while there is a clear lack of data regarding

Table 2

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type of process</th>
<th>Influent (mg N L⁻¹)</th>
<th>N₂O emission (%)</th>
<th>NO emission (%)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>[28]</td>
<td>Separate-stage BNR</td>
<td>309 (TKN)</td>
<td>0.01–0.03 ³</td>
<td>N.M.</td>
<td>Both aerobic + anoxic tanks</td>
</tr>
<tr>
<td>[28]</td>
<td>Step-feed non-BNR</td>
<td>410 (TKN)</td>
<td>0.37–3.3 ³</td>
<td>N.M.</td>
<td>Aerated tanks</td>
</tr>
<tr>
<td>[29]</td>
<td>Activated sludge</td>
<td>47–114 (TKN)</td>
<td>0.6–25 ⁴</td>
<td>N.M.</td>
<td>7 WWTP evaluated</td>
</tr>
<tr>
<td>[22]</td>
<td>Activated sludge</td>
<td>47.9 ± 2.3 (NH₄)</td>
<td>0.127⁴</td>
<td>N.M.</td>
<td></td>
</tr>
<tr>
<td>[28]</td>
<td>Sequencing batch reactors</td>
<td>38.7 ± 2.1 (NH₄)</td>
<td>6.8⁵</td>
<td>N.M.</td>
<td></td>
</tr>
<tr>
<td>[5]</td>
<td>Sequencing batch reactors</td>
<td>61.6 ± 7.8 (TKN)</td>
<td>6.8⁵</td>
<td>N.M.</td>
<td></td>
</tr>
<tr>
<td>[30]</td>
<td>Continuous nitrifying activated sludge</td>
<td>184–244 (NH₃)</td>
<td>0.08–1.17⁶</td>
<td>N.M.</td>
<td>NH₃ shock loads</td>
</tr>
<tr>
<td>[13]</td>
<td>Nitrifying activated sludge</td>
<td>30 ± 4 (NH₄)</td>
<td>0.10–0.4⁴</td>
<td>N.M.</td>
<td></td>
</tr>
<tr>
<td>[23]</td>
<td>Continuous nitrifying SBR activated sludge</td>
<td>34 ± 0.8 (TKN)</td>
<td>2.8⁴</td>
<td>0.03⁴</td>
<td></td>
</tr>
<tr>
<td>This study</td>
<td>Nitrifying system</td>
<td>105 (NH₄)</td>
<td>0.16 ± 0.11⁴</td>
<td>Negligible</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 (NH₄)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BNR, biological nitrogen removal; TKN: total Kjeldahl nitrogen; N.M.: not measured; TN: total nitrogen.

¹ Ahn et al. [28] evaluated 12 WWTP. This table shows the results for those WWTP with the minimum and maximum N₂O emission found.

² NH₄/TN removed.

³ N₂O/N removed.

⁴ N₂O/NH₃ loaded.

⁵ N₂O/TKN removed.

⁶ N₂O/NH₃ removed.
NO emissions for nitrifying systems treating low strength wastewater (Kampschreur et al. [23] and our study only).

3.2. Effect of nitrite pulses on N₂O and NO production without ammonium feeding

The effect of nitrite pulse addition without ammonium feeding was assessed (Fig. 1).

When ammonium was not added, nitrite pulses of 10 mg NO₂⁻-N L⁻¹ did not report any emission of N₂O or NO. The small N₂O peaks shown in Fig. 1 were not taken into account since these correspond to the lower detection limit of the analyser. Interestingly, the activation of the ammonium feeding in the reactor (grey zones in Fig. 1) resulted in an immediate N₂O emission peak (≥2 ppmv) even though nitrite had not accumulated. No NO emission was detected in this test. The results demonstrate that the presence of ammonium is required for the production of N₂O.

3.3. Effect of nitrite pulses on N₂O and NO production during continuous ammonium feeding

Consecutive pulse additions of nitrite in the tests conducted with continuous ammonium feeding resulted in an increase of N₂O emissions, which decreased to the values before the pulse addition once nitrite was completely oxidised to nitrate (Fig. 2). Similar maximum N₂O emission factors with an average of 0.21 ± 0.08% N₂O-N/NH₄⁺-N converted were found among the tests conducted with nitrite pulses in the range of 5–37 mg NO₂⁻-N L⁻¹. When 50 mg of NO₂⁻-N L⁻¹ was supplied (Fig. 2D), the N₂O emission increased to a mean value of 0.90 ± 0.27% N₂O-N/NH₄⁺-N converted.

NO emissions also increased in response to nitrite pulses (Fig. 2). The emission was practically constant for the range of nitrite pulses added (0.11 ± 0.12% NO-N/NH₄⁺-N converted). In conclusion, the production of NO needs ammonium (no NO emission detected when supplying only nitrite, white zones in Fig. 1), but also nitrite in excess (Fig. 2). This clearly discards the N₂O and NO production from heterotrophic denitrification in this reactor, since ammonium is the source for the production of both gases. This is in accordance with the findings from Kampschreur et al. [23], stating that AOB are the main responsible organisms for the production of N₂O and NO in nitrifying systems treating low strength ammonium wastewater.

Remarkably, the emission of NO was substantially larger during the second nitrite pulse added compared to the emission detected during the first addition (Fig. 2A–C). This behaviour was observed for NO during all the tests with repeated addition of pulses at the same concentration, but not for N₂O. It seems that short time exposure to nitrite influences the NO emission and indicates that nitrite prompts the mechanism responsible for NO production, which suggests that the nitrifier denitrification pathway significantly contributes to the formation of NO. A possible explanation for this behaviour could be related to the expression of the gene nitrite reductase (nir), responsible for the codification of the enzyme NirK, which carries out the reduction of nitrite to NO in AOB. Nitrite has been reported to improve the expression of NirK [31], which could stimulate the production of NO after the first pulse of nitrite. On the other hand, Kwiatkowski and Shapleigh [32] reported that the presence of relatively small (non-cytotoxic) NO concentrations result in an enhanced expression of nir. Likely in the present study, the supply of nitrite during the first pulse improves the enzymatic activity for the reduction of nitrite to NO, either by the formation of NO or the addition of nitrite itself.

3.4. Effect of step-wisely increasing nitrite concentrations on N₂O and NO production

Nitrite was continuously supplied at the same rate as the nitrite consumption rate in the reactor in order to maintain a constant desired concentration (see Section S3 from Supplementary information). The nitrite consumption rate remained practically constant (at 35.9 ± 4.9 mg N gMLVSS⁻¹ h⁻¹, see Fig. S3) even with increasing nitrite concentrations, suggesting that the NOB activity was not affected by the increased nitrite concentration. This is in accordance to the review of Painter [33], where much higher nitrite concentration than in the present study (>1000 mg NO₂⁻-N L⁻¹) were necessary to partially inhibit nitrite oxidizers. Step-wisely increasing nitrite concentrations up to 100 mg NO₂⁻-N L⁻¹ resulted in increasing N₂O emissions (Fig. 3). For higher nitrite concentrations, the N₂O concentration slightly decreased and remained constant (at around 1 ppmv) (Fig. 3C). NO emissions increased with increasing nitrite concentrations in the reactor, surpassing the N₂O emissions for nitrite concentrations around 50 mg

![Fig. 1](https://example.com/fig1.png)  Fig. 1. Effect of the nitrite pulse addition without ammonium feeding (white intervals) and effect of the start of the ammonium feeding without addition of nitrite (grey intervals).
NO$_2$-N L$^{-1}$ or higher. The NO emission profile differed from the N$_2$O emission profile in the fact that NO not only increased with increasing nitrite concentration but also with increasing exposure to a given nitrite concentration (Fig. 3). Furthermore, the remarkable difference between N$_2$O and NO emissions at high nitrite concentrations (NO emission up to 8 times higher than N$_2$O emission, Fig. 3C) strengthens the strong effect of nitrite on the NO emissions already observed for the nitrite pulse addition tests (Fig. 2).

The sharp build-up of NO in the system with increasing nitrite concentration (Fig. 3B and C) suggests that nitrite is the source for the accumulated NO. This abrupt NO formation could be attributed to biological or abiotic formation. In a medium rich in nitrite, NO could be produced chemically from the disproportionation of nitrous acid (HNO$_2$) [34] or from the reaction between nitrite and Fe (II) [35]. The highest nitrite concentration in the reactor was 150 mg NO$_2$-N L$^{-1}$ and the pH was controlled between 6.7 and 7.0. Under these conditions, HNO$_2$ inhibition of ammonium or nitrite oxidation processes is unlikely [36]. The iron source added in the medium is Fe (III) in a very low concentration (0.00925 mM). Fe (II) could be produced from the reduction of Fe (III) under anoxic conditions [37], such as during the settling period of the SBR cycle. The formation of NO from nitrite and the potential Fe (II) formed would give Fe (III), stopping the reaction when the Fe (II) is depleted [35], while the NO formed could act as an intermediate in further conversion to N$_2$O. However, in the present study the NO and N$_2$O emissions do not follow the same pattern (NO accumulates continuously and N$_2$O reaches a stable or even decreasing emission at a given nitrite concentration (Fig. 3B and C)). Besides, the formation of NO does not stop for high nitrite concentration in the medium, although the Fe (III) concentration is very low and could only yield a very low Fe (II) concentration (for the experiments of Kampschreur et al. [35] the Fe (II) concentration is more than 1000 higher), resulting in electron donor limitation for the NO and N$_2$O formation. For these reasons, chemical NO production is considered irrelevant in the present study. The NO increase with increasing nitrite concentration could be explained from a microbiological point of view. It is known that the responsible enzymes for the reduction of nitrite to NO (nitrite reductase, NirK) and for the reduction of NO to N$_2$O (nitric oxide reductase, Nor) have independent functionality [38]. Thus, the production of NO and N$_2$O can be assumed as independent, which explains the different patterns observed during the emissions of both gases (Fig. 3). Moreover, nitrite and/or NO may act as a substrate inhibitor for Nor at certain concentrations [39–41], while nitrite was observed to improve the levels of NirK [39,42,43] and nitrite and/or NO to enhance the expression of NirK [31]. Therefore, the more nitrite is in the system, the more NO is produced and the formation of N$_2$O is slowed down, resulting in NO accumulation.

3.5. Low vs. high strength wastewater

Increasing nitrite concentration in the SBR resulted in increasing N$_2$O and NO production rate per ammonium converted (Fig. 4). The NO production substantially increased when nitrite concentration was higher than 50 mg NO$_2$-N L$^{-1}$ resulting in higher emissions for NO than for N$_2$O in those tests.

These results differ from the study of Law et al. [44] where in an enriched AOB culture treating reject wastewater (1 g NH$_4$-N L$^{-1}$) and always exposed to high levels of nitrite, an increase in nitrite concentrations from 50 to 500 mg NO$_2$-N L$^{-1}$ resulted in a gradual
decrease in N₂O emissions. Additionally, Pijuan et al. [45] did not find any effect on the N₂O emissions in a granular sludge airlift reactor of 150 L performing full partial nitrification when exposed to high nitrite concentrations (368–740 mg NO₂⁻/N L⁻¹). This highlights the capability of AOB to adapt to different environments and reactor conditions (high vs. low nitrite concentrations) responding differently in terms of emissions when being exposed to the same nitrite concentration if the culture was adapted to high or low nitrite concentrations.

This adaptation is also shown by the nitrification activity. There is a significant reduction on the nitrification activity in the present study when the bacteria are exposed to concentrations higher than 50 mg NO₂⁻/N L⁻¹ (average of 12.6 ± 1.1 mg NH₄⁺-N gVSS⁻¹ h⁻¹ consumed when exposed at 0–50 mg NO₂⁻/N L⁻¹ and 5.1 ± 0.7 mg NH₄⁺-N gVSS⁻¹ h⁻¹ when exposed at 150 mg NO₂⁻/N L⁻¹) (see Fig. S3), while for those cultures adapted to high nitrite concentrations, the AOB activity was not affected [44]. Thus, high nitrite concentrations affect the AOB activity when the bacteria are not acclimated to nitrite. It is well known that nitrification is inhibited by HNO₂ [36], but minimal formation of HNO₂ is expected in the present study since the pH is controlled between 6.7 and 7.0 (mentioned in Section 3.4 already). Moreover, Law et al. [46] showed that the minimal formation of HNO₂ in a lab-scale partial nitrification reactor operated with a nitrite concentration from 3.4.

Fig. 3. Effect of the stepwise increase of the nitrite concentration on the N₂O and NO emissions: (A) test with 10 → 20 → 30 mg NO₂⁻/N L⁻¹; (B) 25 → 50 → 75 mg NO₂⁻/N L⁻¹; (C) 50 → 100 → 150 mg NO₂⁻/N L⁻¹. Note that the y axis in A is 4 times smaller than in B and C.
to nitrite, as shown by Law et al. and Pijuan et al. and for at least 24 h between consecutive tests, and the recovery of the nitrifying activity. Long term contact to nitrite may lead to permanent enzymatic changes allowing the adaptation of the bacteria to nitrite, as shown by Law et al. and Pijuan et al. Although the decrease in the nitrifying activity (Fig. S3) occurs when the NO emission is more significant, at nitrite concentrations higher than 50 mg NO2-N L−1 (Figs. 3C and 4), it is not possible to clearly discern if the actual inhibitor is the nitrite or the produced NO. However, since NO accumulation increases with increasing nitrite concentration (Fig. 4), NO is the consequence of increasing nitrite and thus, nitrite could be indirectly linked to the nitrifying inhibition in the hypothetical case that the real inhibitor was the NO.

3.6. Main mechanism producing N2O and NO

It is clear that AOB are the organisms producing N2O and NO from biological processes in the present study. Regarding the main pathway, aerobic conditions promote the hydroxylamine route [14], while nitrifier denitrification is stimulated improved at high nitrite concentrations and although it is enhanced under limited oxygen concentrations [13] it also occurs under aerobic conditions (up to 3.5 mg O2 L−1) [47]. Our findings suggest that during nitrite accumulation, nitrifier denitrification is the main pathway for the N2O and NO production. All tests assessed with nitrite (pulse addition and step-wisely increasing nitrite concentration) showed an improved production of NO, suggesting that the nitrite added is the precursor for the NO and N2O production, and not hydroxylamine. NO clearly cannot be neglected as intermediate during nitrifier denitrification, in contrast to other studies [48], when studying the N2O and NO emissions during nitrification of low strength ammonium wastewater. In accordance to our results, Daelman et al. [24], in a N2O monitoring campaign from a full-scale municipal WWTP, found nitrifier denitrification the dominant pathway for N2O emission when nitrite accumulates in the system.

During normal reactor operation (no accumulation of nitrite), the hydroxylamine oxidation pathway could have played an important role in N2O formation. Peng et al. [47] showed in a nitrifying reactor treating high strength ammonium wastewater that the contribution of the hydroxylamine oxidation pathway to the N2O production was only important at very high oxygen concentrations (3.5 mg O2 L−1) and very low levels of nitrite (<10 mg NO2-N L−1). In the present study, nitrite did not accumulate during normal reactor operation and the oxygen concentration was not limited (2.0–2.5 mg O2 L−1), which suggests that the role of hydroxylamine oxidation pathway may have been important during normal reactor operation; note however that N2O emissions were very small in this case (0.16 ± 0.11% of the ammonium converted being emitted as N2O-N) and no emission of NO was detected. Furthermore, the likely enzymatic inhibition caused by accumulation of nitrite during the step-wisely increasing nitrite concentration experiments strengthens the hypothesis of nitrifier denitrification as main mechanism during accumulation of nitrite. The increase of N2O production is stopped at 100 mg NO2-N L−1 and then even reduced at 150 mg NO2-N L−1 (Fig. 3C). At these nitrite concentrations it was discussed that the levels of enzymes involved in the hydroxylamine pathway (amoA) are inhibited (mentioned in Section 3.5), reducing their activity. Thus, the production of N2O at these high nitrite concentration could be linked to nitrifier denitrification.

4. Conclusions

- Nitrite pulses in a nitrification reactor resulted in an increase in N2O and NO emissions. These emissions decreased to original levels when nitrite was completely oxidised to nitrate.
- Step-wise addition of nitrite (reactor concentrations from 0 to 150 mg NO2-N L−1) during ammonium oxidation caused an increase in the N2O (from 0.16% to 1.5%) and NO (from negligible values to 3.8%) emission factors (N2O or NO emitted per NH4-N converted).
- NO emissions were higher than those of N2O when biomass was exposed to high nitrite concentrations (>50 mg NO2-N L−1).
- The release of the intermediate NO in the nitrifier denitrification N2O production pathway cannot be neglected.
- High nitrite concentrations showed an inhibitory effect on the nitrifying activity of non-adapted bacterial groups to nitrite, likely due to NO accumulation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2015.10.121.
References


