**N₂O and NO emissions during autotrophic nitrogen removal in a granular sludge reactor – a simulation study**

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This contribution deals with NO and N₂O emissions during autotrophic nitrogen removal in a granular sludge reactor. Two possible model scenarios describing this emission by ammonium-oxidizing biomass have been compared in a simulation study of a granular sludge reactor for one-stage partial nitritation–Anammox. No significant difference between these two scenarios was noticed. The influence of the bulk oxygen concentration, granule size, reactor temperature and ammonium load on the NO and N₂O emissions has been assessed. The simulation results indicate that emission maxima of NO and N₂O coincide with the region for optimal Anammox conversion. Also, most of the NO and N₂O are present in the off-gas, owing to the limited solubility of both gases. The size of granules needs to be large enough not to limit optimal Anammox activity, but not too large as this implies an elevated production of N₂O. Temperature has a significant influence on N₂O emission, as a higher temperature results in a better N-removal efficiency and a lowered N₂O production. Statistical analysis of the results showed that there is a strong correlation between nitrite accumulation and N₂O production. Further, three regions of operation can be distinguished: a region with high N₂O, NO and nitrite concentration; a region with high N₂ concentrations and, as such, high removal percentages; and a region with high oxygen and nitrate concentrations. There is some overlap between the first two regions, which is in line with the fact that maximum emission of NO and N₂O coincides with the region for optimal Anammox conversion.

**Keywords:** ammonium oxidizing bacteria; Anammox; nitrogen removal; nitrous oxide; nitric oxide; granular sludge

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**Introduction**

Conventionally, biological nitrogen removal is achieved by nitrification–denitrification over nitrate. However, an alternative and innovative approach is the use of the Anammox (anaerobic ammonium oxidation) process, which requires less energy but is characterized by a relatively low growth rate. The combination of this Anammox process with a partial nitritation process – in for example a granular sludge reactor – has proven its applicability at full scale [1]. This completely autotrophic process has several advantages such as: no need for external carbon addition; less energy and oxygen requirement; and a negligible sludge production [2,3]. Typical wastewaters that can be treated by the process are, among others, reject water and landfill leachate [3–5].

However, as for conventional biological nitrogen removal techniques, there is a growing concern regarding the emission of nitrous oxide (N₂O) during this process. Nitrous oxide is an important greenhouse gas, having a 300-fold stronger effect than CO₂ [6]. Kampuschreuer et al. [7] measured the N₂O emission from a partial nitritation–Anammox and granular sludge reactor to be about 1.2% of the incoming nitrogen load. Okabe et al. [8] determined the N₂O emission from a lab-scale two-reactor partial nitritation–Anammox granular sludge reactor to be respectively 4.0% and 0.1% of the incoming nitrogen load.

According to emission data, fluxes are extremely variable and subject to a large variety of process conditions [9]. However, the IPCC (Intergovernmental Panel on Climate Change) applies the standard emission factor of 0.5% of the nitrogen load to assess N₂O emission from wastewater treatment plants (WWTPs), implicitly assuming no nitrogen is removed during treatment [6]. Modelling N₂O emission, based on the possible underlying mechanisms, offers the potential to obtain a more appropriate estimate. Moreover it could support the development of strategies to reduce N₂O emission in WWTPs.

In this contribution, a simulation study has been carried out with two possible scenarios for N₂O formation. Both scenarios were used, as it is yet unclear which scenario is more likely to occur in practice [10]. The influences of temperature, ammonium load, bulk liquid oxygen level and granule size on the production of NO, a reaction intermediate, and N₂O have been assessed. Further, the difference between the two possible scenarios is shown.
Model description

Biofilm model

Emissions of NO and N₂O during a single-stage partial nitritation–Anammox process were modelled based on existing models for autotrophic nitrogen removal in a granular sludge reactor [11] and a mechanistic model for NO and N₂O production by autotrophic ammonia oxidizing bacteria (AOB) [10]. The resulting one-dimensional biofilm model was implemented in the Aquasim software [12]. The total reactor volume was 500 m³ (cross-section area $A = 36.6 \text{m}^2$). Spherical biomass granules were grown from an initial radius of 0.01 mm to a steady-state value, and the number of granules was calculated such that the reactor contained 100 m³ of particulate material, comprising active biomass and inert matter originating from endogenous respiration. The biofilm porosity was assumed to be constant ($\varepsilon_w = 0.8$), determined by the initial fractions of particulate components ($\varepsilon_{X,\text{AOB}} = 0.1$; $\varepsilon_{X,\text{NOB}} = \varepsilon_{X,\text{AN}} = 0.05$; $\varepsilon_{X,\text{I}} = 0$). The biomass concentration in the granules was set to 70,000 g VSS m⁻³, corresponding to 93,333 g COD m⁻³ (for a typical conversion factor of 0.75 g VSS g⁻¹ COD, see Henze et al. [13]).

The completely mixed bulk liquid made up 300 m³ (excluding granules), the remaining 100 m³ was assigned to a gas phase. This gas phase was added to the model in order to consider interphase transport between the gas phase and bulk liquid for O₂, NO, N₂O and N₂, based on Henry’s law:

$$TR_i = K_{La O_2} \left( \frac{S_i^G}{H_i} - S_i \right)$$

in which $TR_i$ represents the gas transfer rate of O₂, N₂O, NO or N₂; $K_{La}$, $S_i^G$, $H_i$ and $S_i$ represent the corresponding interphase transport coefficient, the gas phase concentration, the Henry coefficient and the liquid phase bulk concentration, respectively.

The interphase transport coefficient, $K_{L,a O_2}$, is related linearly to superficial gas velocity, $v_G$, which is directly proportional to the air flow rate, Q [14]:

$$K_{L,a O_2} = 0.6v_G = 0.6 \frac{Q}{A}$$

The transport coefficients of N₂O, NO and N₂ were calculated based on the respective diffusion coefficients, as follows:

$$K_{L,a i} = K_{L,a O_2} \sqrt{ \frac{D_{i}}{D_{O_2}} }$$

The influent, fed at a flow rate of 2500 m³ d⁻¹, contained mainly ammonia. A negligible amount of nitrite and nitrate (0.01 g N·m⁻³) was added in order to avoid numerical errors. Initial concentrations of solubles in the bulk liquid were assumed to be equal to influent concentrations. A constant reactor pH of 7 was assumed; temperature was also considered constant but was varied in the range 25–40°C between different simulation runs.

A reference simulation was defined as corresponding to a reactor temperature of 30°C, a granule size of 0.75 mm and an ammonium concentration of 300 g N·m⁻³. In further simulations, the granule size was altered between three different sizes (0.5, 0.75, 1 mm); the ammonium loads were varied from 100 to 1000 g N·m⁻³. In total 130 different simulations were performed. Further, these simulations were performed sufficiently long enough to ensure steady state.

Biochemical conversion model

This model describes growth and endogenous respiration of AOB, nitrite oxidizing bacteria (NOB) and Anammox bacteria. Heterotrophic growth was not considered, as the influent used for simulation did not contain organic carbon.

A distinction was made between two hypothetical scenarios for NO and N₂O production by autotrophic AOB [10]. These scenarios differ in the origin of the reducing equivalents for AOB denitrification of nitrite to NO and subsequently to N₂O, which can be either ammonia (scenario A) or AOB biomass (scenario B). The reduction rates are proportional to the AOB concentration and contain substrate limitation terms. In scenario A, NO formation is limited by O₂, ammonia and nitrite, while N₂O formation is limited by O₂, ammonia and NO. In scenario B, NO formation is limited by nitrite, and N₂O formation is limited by NO [10]. In contrast to the model used by Mampaey et al. [10], the same maximum growth rate ($\mu_{\text{AOB}}$) is used for all AOB processes (ammonium oxidation, NO formation and N₂O formation). The Gujer matrix, incorporating reaction stoichiometry and kinetics for both scenarios, is presented in Appendix 1 and is according to Mampaey et al. [10].

Numerical values for rate constants at 30°C were taken mainly from Volcke et al. [11] and Mampaey et al. [10]. A maximum specific growth rate of 1.36 d⁻¹, 0.79 d⁻¹ and 0.052 d⁻¹ were used for AOB, NOB and Anammox respectively, while the decay coefficients were assumed to be 5% of these specific growth rates. The affinity constants for ammonium and nitrite of Anammox were assumed to be 0.03 g NH₄⁻N·m⁻³ and 0.005 g NO₂⁻N·m⁻³, respectively. The affinity constants for nitrite and oxygen of NOB were assumed to be 2.71 g NO₂⁻N·m⁻³ and 1.1 g O₂·m⁻³, respectively. Other parameter values are indicated in Table 1.

Temperature dependency

To investigate the reactor performance at different temperatures, a temperature dependency was considered for the maximum microbial growth rates and decay rates, the affinity constants for total ammonium and total nitrite, the interphase transport rates, the Henry coefficients and the diffusion coefficients.

The temperature dependency of the maximum specific growth rates, $\mu$, and the decay coefficients, $b$, was
Table 1. Model parameter values at 30 °C and pH 7.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>YAOB</td>
<td>0.2</td>
<td>g COD·g⁻¹ N</td>
<td>[11,15]</td>
</tr>
<tr>
<td>iNMB</td>
<td>0.07</td>
<td>g N·g⁻¹ COD</td>
<td>[13]</td>
</tr>
<tr>
<td>μmax,AOB</td>
<td>1.36</td>
<td>d⁻¹</td>
<td>[16]</td>
</tr>
<tr>
<td>KNH,AOB</td>
<td>1.31</td>
<td>g NH₄·N·m⁻³</td>
<td>[17]</td>
</tr>
<tr>
<td>KO₂,AOB</td>
<td>0.30</td>
<td>g O₂·m⁻³</td>
<td>[11]</td>
</tr>
<tr>
<td>KNO₂,AOB</td>
<td>7.25</td>
<td>g NO₂·N·m⁻³</td>
<td>[17]</td>
</tr>
<tr>
<td>KNO₃,AOB</td>
<td>1.00</td>
<td>g N·m⁻³</td>
<td>[10]</td>
</tr>
</tbody>
</table>

incorporated according to an Arrhenius type equation [13]:

\[
k(T) = k(T_r)e^{\theta(T-T_r)}
\]

where \( k(T) \) is the kinetic parameter (μ or b) at the actual temperature \( T \), \( T_r \) is the reference temperature (30 °C) and \( \theta \) is the Arrhenius constant, which is calculated based on the activation energy (\( E_{\text{act}} \)) of the corresponding biomass [13]:

\[
\theta = \frac{E_{\text{act}}}{R \cdot T \cdot T_r}
\]

These activation energies were taken as 68 kJ mol⁻¹ for AOB [18], 44 kJ mol⁻¹ for NOB [18] and 70 kJ mol⁻¹ for Anammox bacteria [19]. R denotes the universal gas constant (8.31 J·mol⁻¹·K⁻¹).

Equation (4) does not take into account the decrease of activity at higher temperatures (above 40 °C). However, all simulation studies were performed at temperatures between 25 and 40 °C, a range where the Arrhenius type equation appears valid [20].

It is generally known that the uncharged components, ammonia (NH₃) and nitrous acid (HNO₂), are the true substrates for ammonium and nitrite oxidation, respectively [21]. Although the affinity constants for these uncharged components are independent of temperature (and pH), the affinity constants expressed in terms of total ammonium (NH₃ + NH₄⁺) and total nitrite (HNO₂ + NO₂⁻), respectively, depend on temperature (and pH) (see, for example, Van Hulle et al. [20]), according to the following relationships which are based on the chemical equilibrium equations:

\[
K_{\text{NH}_3,i}(T) = K_{\text{NH}_3,i}(T_r) \frac{10^{-pH} + K_{\text{NH}_3}(T)}{10^{-pH} + K_{\text{NH}_3}(T_r)}
\]

\[
K_{\text{NO}_2,i}(T) = K_{\text{NO}_2,i}(T_r) \frac{10^{-pH} + K_{\text{NO}_2}(T)}{10^{-pH} + K_{\text{NO}_2}(T_r)}
\]

with \( i \) referring to a certain type of biomass (AOB, NOB or Anammox); \( K_{\text{NH}_3} \) and \( K_{\text{NO}_2} \) denote the acid/base equilibrium constants, the temperature dependency of which has been modelled according to Anthonisen et al. [21].

**(Statistical analysis)**

For statistical analysis of the simulation output (130 simulations in total, as described above), a principal component analysis (PCA) was run with the software program SPSS (version 20, http://www.spss.com). Principal component analysis is a pattern recognition method and aims to reduce a large number of variables to a smaller number of representative variables (principal components or PCs [29]). Based
on the Scree plot, two PCs were extracted to explain the underlying data structure. These two PCs can explain 63% of the total variance.

In total, 13 factors were used: the concentration of N$_2$, N$_2$O and NO in the gas phase, the concentration of N$_2$, N$_2$O, NO, dissolved oxygen (DO), nitrite, nitrate and ammonium in the liquid phase, and the applied temperature, influent load and particle size. Given 130 cases and 13 factors, the criterion that the ratio ‘Cases to Factors’ should be at least 5/1 is met. The Kaiser–Meyer–Olkim criterion for sampling adequacy (KMO) was used to verify that correlations between items were sufficiently large for PCA. This KMO value should be above 0.5 [30].

### Results and discussion

#### Reference case

Figure 2 displays a typical steady-state biomass distribution profile at standard conditions, similar to that obtained by Volcke et al. [11], although in that contribution no N$_2$O and NO production was considered. AOB are located at the outside of the granule, in the presence of oxygen and ammonia. Nitrite-oxidizing bacteria (NOB) are located (if present) at the inside of the AOB outer layer. In this layer some oxygen is still present along with nitrite generated by AOB. Anammox organisms are situated in the inner anoxic part, where nitrite and ammonia are present as a result of diffusion. The symbol X$_I$ stands for inert particulate matter and is present at the centre of the granules.

In Figure 3a, it can be seen that, at high DO concentrations (>2 g O$_2$·m$^{-3}$), nitrite is oxidized to nitrate by NOB rather than being converted into N$_2$ by Anammox. This is due to the fact that, for high bulk oxygen concentrations, the oxygen flux through the granules is high, allowing NOB ingrowth and causing Anammox inhibition.

Figure 3b summarizes the steady-state simulation results concerning the influence of bulk oxygen concentration on total flux of N$_2$O, NO and N$_2$ in the gas phase and liquid effluent stream. At low DO (0.3 g O$_2$·m$^{-3}$), all three curves attain a maximum. At DO between 0 and 0.3 g O$_2$·m$^{-3}$, there is nitrite accumulation caused by the fact that AOB accumulate in the reactor. In this operating zone, NOB are outcompeted because of their low oxygen affinity and because of the competition with Anammox bacteria. Anammox organisms require nitrite and ammonia, coming from the outer layer of the granule. As DO increases, more nitrite will be formed and will diffuse towards the Anammox organisms. Optimal Anammox activity is achieved at DO = 0.3 g O$_2$·m$^{-3}$, as reflected by a peak in the N$_2$ production. The maximum NO and N$_2$O production is caused by a maximum concentration of nitrite near the surface of the granules, because the kinetic rate expression describing NO production by AOB is related to the nitrite concentration by a Monod relation. This means that approximately optimal autotrophic N-removal process conditions also result in a maximum NO production, which subsequently results in a maximum N$_2$O production.

Figure 4 illustrates that more than 92% of the produced N$_2$O and over 97% of the produced NO are present in the off-gas, owing to the limited solubility of both gases. This is in accordance with the observations of Desloover et al. [31] and implies that these gases are released into the atmosphere and N$_2$O can act as a greenhouse gas. As a result, it is important to include gas–liquid interphase transport when modelling NO and N$_2$O formation. Without interphase transport, NO would accumulate in the bulk liquid during simulation, causing an overestimated N$_2$O production by the current model. In these simulations the NO concentration in the bulk liquid amounts to 0.23 g NO·N·m$^{-3}$, which is the limiting factor for N$_2$O production.

Similar results for scenario A are observed. This implies an optimal experimental design to allow model selection cannot be set up based on the given simulation results.
Further, the $\text{N}_2\text{O}$ production amounts to 25% of the incoming nitrogen load. Full-scale data reported in the literature indicated that generally $\text{N}_2\text{O}$ emission from WWTPs was between 1% and 15% of the influent total nitrogen load, although in most cases it was below 3% [9,31,32]. As such, this is much higher than what was recently observed experimentally. Therefore the model needs to be calibrated with experimental data, although the qualitative conclusions drawn in this study still hold. Probably some model modifications, such as a reduction of the nitrogen oxide formation rate, will have to be performed [10].

**Influence of ammonium load**

Figure 5 shows that the maximum nitrogen gas production decreases with an increasing ammonium load. Further, a broader DO range for optimal $\text{N}_2$ production is associated with a higher ammonium load, as was also observed by Volcke et al. [11]. A better N-removal efficiency is made possible by a lower conversion to $\text{N}_2\text{O}$ at low DO ($<0.5 \text{ g O}_2\cdot\text{m}^{-3}$) or $\text{NO}_3^-$ at higher DO ($>0.5 \text{ g O}_2\cdot\text{m}^{-3}$). At higher nitrogen loads, a higher conversion to $\text{N}_2\text{O}$ is also observed.

When a higher ammonium load is applied, it is necessary to increase the aeration flow rate to maintain the optimal production of $\text{N}_2$ at a certain DO set-point, which can be understood by the fact that more ammonium needs to be oxidized to nitrite in order to be converted into $\text{N}_2$ by Anammox. This higher aeration flow rate results in higher $\text{N}_2\text{O}$ emissions. Also, an increased nitrite accumulation occurs at the DO concentration at which maximum $\text{N}_2\text{O}$ emissions occur.

According to Veys et al. [33], an increased ammonium load could lead to a decrease in reactor performance, because the aeration flow rate cannot be increased further since this could lead to an irreversible loss of Anammox activity as a breakthrough of nitrite and/or oxygen occurs.

Both scenarios yielded similar results, although at increased influent concentrations (especially 1000 g $\text{NH}_4^-\cdot\text{m}^{-3}$) the $\text{N}_2\text{O}$ peak seems to be situated at higher DO concentrations.

**Influence of granule size**

The maximal production level of $\text{N}_2$ does not depend on the size of the granules, in case the granules are large enough to allow optimal Anammox activity and enough nitrite is available. This can be seen clearly in Figure 6.
certain threshold thickness, a thicker biofilm does not imply improved N removal, but an elevated DO is required in order to obtain optimal N removal. Increasing the biofilm thickness, an increased DO gradient is necessary to diffuse deep enough into the biofilm to reach the entire AOB layer, so that sufficient nitrite is produced to achieve good ammonium removal.

With increasing granule size, the maximal N₂O production increases and occurs at higher DO concentrations, which is shown in Figure 6. The increasing size of the AOB layer in the granule, possibly causing elevated production of nitrite could explain the significant increase of produced N₂O. Overall, it can be concluded that the granule size needs to be large enough not to limit optimal Anammox activity at a fixed DO level. Larger particles imply an elevated N₂O production and an increased DO to obtain maximal N removal.

**Influence of temperature**

The influence of temperature on the production of N₂ and N₂O is illustrated in Figure 7. As can be seen, increasing the temperature from 25 to 40 °C implies an increase in the maximal production of N₂, which is reached at lower DO concentrations. An increasing temperature also results in a decline in the maximal production of N₂O as well as a lower DO concentration at which this maximal N₂O production occurs. A high temperature thus increases the autotrophic N-removal efficiency and reduces N₂O emission. This indicates that particular attention will need to be paid to N₂O emissions during autotrophic nitrogen removal at low wastewater temperatures, as envisaged in future energy-positive WWTPs [34]. However, expected lower influent concentrations in these systems will also result in a lower N₂O production (see Figure 5).
The reduced N\textsubscript{2}O production at increased temperatures can be explained by the fact that Anammox bacteria have an optimal growing temperature of about 35 °C. A decreasing temperature increases the oxygen penetration depth, limiting the anoxic zone of the granule, necessary to shelter Anammox bacteria. As such, the necessary DO for optimal N\textsubscript{2} removal increases.

Unfortunately, reactor temperatures of about 20 °C can incite a significant amount of N\textsubscript{2}O emission unless Anammox is sufficiently shielded from oxygen. Therefore, especially in winter time, single-stage partial nitritation–Anammox granular sludge reactors are expected to emit large amounts of N\textsubscript{2}O, which is analogous to conventional biological nitrogen removal by a combination of nitrification and denitrification [35].

From Figures 5, 6 and 7, the maximum N\textsubscript{2} production and corresponding N\textsubscript{2}O production were summarized and are shown in Figure 8 for scenario A. A comparison was made between the amount of produced N\textsubscript{2} and the amount of produced N\textsubscript{2}O at optimal nitrogen removing conditions for scenario A. It can be seen that increasing the influent load, decreasing the temperature and increasing the particle size have a negative effect on the nitrogen gas production.

### Principal component analysis

The KMO value for the sampling adequacy of the PCA analysis was equal to 0.526. This indicates that correlations between items were sufficiently large to apply PCA [30]. In Table 2 the resulting component matrix is given. This component matrix quantifies the relationship between the original variables and the principal components. In total three different groups can be distinguished. A first group is related to positive values of PC1 and PC2 and contains points that are high in N\textsubscript{2}O, NO and nitrite concentration. This is in accordance with Kampschreur et al. [9] and suggests that an increased nitrite concentration is associated with N\textsubscript{2}O and NO production. A second group is related to positive values of PC1 and negative values of PC2 and indicates high N\textsubscript{2} concentrations and, as such, high removal percentages. A third group is related to a negative PC1 and indicates high oxygen and nitrate concentrations. Indeed, if a high DO concentration exists in the liquid, the NOB can survive in the biofilm, which causes an increased nitrate production. In Figure 9 the 130 data points used for the PCA analysis are plotted against PC1 and PC2. As discussed above, three different regions exist: one with high nitrogen removal, one with high N\textsubscript{2}O production and one with complete nitritation to nitrate. There is some overlap between the two first regions, which is in line with the fact that maximum emissions of NO and N\textsubscript{2}O coincide with the region for optimal Anammox conversion.

#### Table 2. The component matrix for the PCA analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>-0.252</td>
<td>-0.015</td>
</tr>
<tr>
<td>Particle size</td>
<td>0.071</td>
<td>0.141</td>
</tr>
<tr>
<td>Influent load</td>
<td>0.444</td>
<td>-0.342</td>
</tr>
<tr>
<td>S\textsubscript{N\textsubscript{2}}</td>
<td>0.644</td>
<td>-0.676</td>
</tr>
<tr>
<td>S\textsubscript{N\textsubscript{2}O}</td>
<td>0.932</td>
<td>0.256</td>
</tr>
<tr>
<td>S\textsubscript{NO}</td>
<td>0.861</td>
<td>0.487</td>
</tr>
<tr>
<td>S\textsubscript{NO\textsubscript{2}}</td>
<td>-0.693</td>
<td>0.234</td>
</tr>
<tr>
<td>S\textsubscript{NO\textsubscript{3}}</td>
<td>0.696</td>
<td>-0.620</td>
</tr>
<tr>
<td>S\textsubscript{NH}</td>
<td>0.929</td>
<td>0.263</td>
</tr>
<tr>
<td>S\textsubscript{NH\textsubscript{4}}</td>
<td>0.352</td>
<td>-0.726</td>
</tr>
<tr>
<td>S\textsubscript{NO}</td>
<td>0.859</td>
<td>0.487</td>
</tr>
<tr>
<td>S\textsubscript{NO\textsubscript{2}}</td>
<td>0.539</td>
<td>0.504</td>
</tr>
<tr>
<td>S\textsubscript{NO\textsubscript{3}}</td>
<td>-0.650</td>
<td>0.261</td>
</tr>
<tr>
<td>Eigenvalue</td>
<td>5.69</td>
<td>2.49</td>
</tr>
<tr>
<td>% of explained variance</td>
<td>43.8</td>
<td>19.1</td>
</tr>
</tbody>
</table>
Conclusions and perspectives

In this contribution, the effect of the bulk oxygen concentration, ammonium load, granule size and temperature on NO and N\textsubscript{2}O emissions during autotrophic nitrogen removal in a granular sludge reactor has been assessed for two possible scenarios for N\textsubscript{2}O formation.

No significant difference between scenarios A and B is noticed in the steady-state behaviour of a granular sludge reactor for autotrophic nitrogen removal. As a result, this behaviour cannot be used to set up an optimal experimental design to reveal the true N\textsubscript{2}O formation scenario. On the other hand, it also means that the actual formation mechanism will not affect the steady-state reactor behaviour.

Maximum NO and N\textsubscript{2}O emission, caused by denitrifying AOB, coincides with optimum autotrophic ammonium removal (maximal N\textsubscript{2} production). Also, most of the NO and N\textsubscript{2}O are present in the off-gas, owing to limited solubility of both gases. The size of granules needs to be large enough not to limit optimal Anammox activity, but not too large as this implies an elevated production of N\textsubscript{2}O. Also, temperature has a significant influence on N\textsubscript{2}O emission. A higher temperature means a better N-removal efficiency and a lowered N\textsubscript{2}O production.

PCA analysis of the results showed that there is a strong correlation between nitrite and N\textsubscript{2}O production. Further, three regions of operation can be distinguished: a region with high N\textsubscript{2}O, NO and nitrite concentrations; a region with high N\textsubscript{2} concentration and, as such, high removal percentages; and a region with high oxygen and nitrate concentrations.

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References

### Table 1. Gujer matrix representing NO and N$_2$O formation by AOB under both scenarios [10].

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Ammonium $S_{NH}$ [g NH$_4$-N m$^{-3}$]</th>
<th>Nitrite $S_{NO}$ [g NO$_2$-N m$^{-3}$]</th>
<th>Oxygen $S_{O2}$ [g O$_2$ m$^{-3}$]</th>
<th>Nitric oxide $S_{NO}$ [g NO-N m$^{-3}$]</th>
<th>Nitrous oxide $S_{N2O}$ [g N$_2$O-N m$^{-3}$]</th>
<th>AOB $X_{AOB}$ [g COD m$^{-3}$]</th>
<th>Process rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A NO to NO</td>
<td>$-1/Y_{AOB}$ + $i_{NXB}$</td>
<td>$-1/Y_{AOB}$</td>
<td>$1 - 2.29/Y_{AOB}$</td>
<td>$2/Y_{AOB}$</td>
<td>$1$</td>
<td>$\mu_{AOB} \cdot \frac{S_{NO}}{K_{NO2} + S_{NO}} \cdot \frac{S_{NH}}{K_{NH} + S_{NH}} \cdot \frac{S_{N2O}}{K_{N2O2,AOB} + S_{N2O}} \cdot X_{AOB}$</td>
<td></td>
</tr>
<tr>
<td>A NO to N$_2$O</td>
<td>$-1/Y_{AOB}$ + $i_{NXB}$</td>
<td>$1/Y_{AOB}$</td>
<td>$1 - 2.29/Y_{AOB}$</td>
<td>$-2/Y_{AOB}$</td>
<td>$2/Y_{AOB}$</td>
<td>$1$</td>
<td>$\mu_{AOB} \cdot \frac{S_{NO}}{K_{NO2} + S_{NO}} \cdot \frac{S_{NH}}{K_{NH} + S_{NH}} \cdot \frac{S_{N2O}}{K_{N2O2,AOB} + S_{N2O}} \cdot X_{AOB}$</td>
</tr>
<tr>
<td>B NO to NO</td>
<td>$i_{NXB}$</td>
<td>$-1.75$</td>
<td>$1.75$</td>
<td>$-1$</td>
<td></td>
<td>$1$</td>
<td>$\mu_{AOB} \cdot \frac{S_{NO}}{K_{NO22,AOB} + S_{NO}} \cdot X_{AOB}$</td>
</tr>
<tr>
<td>B NO to N$_2$O</td>
<td>$i_{NXB}$</td>
<td>$-1.75$</td>
<td>$1.75$</td>
<td>$-1$</td>
<td></td>
<td>$1$</td>
<td>$\mu_{AOB} \cdot \frac{S_{NO}}{K_{NO22,AOB} + S_{NO}} \cdot X_{AOB}$</td>
</tr>
</tbody>
</table>