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Dynamics of N$_2$O production pathways in a full-scale activated sludge system analysed by $^{15}$N/$^{18}$O dual isotope labelling

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Keywords: Ammonium oxidation; denitrification; activated sludge; DO effects

Introduction
Nitrous oxide emissions from biological nitrogen removal can contribute substantially to the CO$_2$-equivalent footprint of wastewater treatment, but the pathways and regulation of N$_2$O production are poorly understood. Measurements of N$_2$O in full-scale activated sludge plant showed large variation in N$_2$O concentration during different phases of operation (Ekström et al. 2016), which indicated the involvement of different N$_2$O production pathways in N$_2$O emission. A partitioning of these pathways and analysis of their dynamics is therefore important for establishment of strategies to mitigate N$_2$O release.

Nitrifier nitrification (NN), nitrifier denitrification (ND) and heterotrophic denitrification (HD) are recognised as the three main pathways of N$_2$O production. We developed a $^{15}$N/$^{18}$O dual isotope labelling technique to distinguish and quantify these pathways in wastewater treatment systems. The use of $^{18}$O-labeled O$_2$ specifically permits the partitioning of NN and ND, which cannot be achieved with certainty with $^{15}$N labelling alone. In this study we applied the dual isotope labelling technique in an analysis of N$_2$O production pathways during different operational phases of a full-scale activated sludge plant. Short-term incubations were performed on site under simulated operational conditions, along with real-time measurement of N$_2$O in the reactor.

Material and Methods
The study was performed at the largest Danish wastewater treatment plant Lynetten, with sludge sampled from an activated sludge plant with phased isolation nutrient removal reactors. Nitrification and denitrification take places in the continuous flow system by alternating process conditions as well as influent and effluent flows in surface aerated reactors. Sludge was sampled during different operational phases and incubated immediately in several parallel closed batches under a He/O$_2$ atmosphere with the addition of $^{15}$N-labeled ammonium, nitrite, or nitrate and/or $^{18}$O-labeled O$_2$. The operational conditions were simulated and incubation times were similar to the aeration phases of the reactor. DO was monitored by a non-invasive optical oxygen sensor and adjusted by addition of unlabelled or $^{18}$O-labeled O$_2$. Incubations were sampled for the measurement of bulk and $^{15}$N-labeled NH$_4^+$, NO$_2^-$ and NO$_3^-$, of $^{15}$N-labeled N$_2$, and of $^{15}$N and $^{18}$O-labeled N$_2$O. Isotope exchange of $^{18}$O between NO$_3^-$ and H$_2$O was further quantified in order to obtain NN rates from $^{18}$O incubations. Isotope analysis was performed by continuous flow GC-IRMS with conversion of DIN species to N$_2$. 
Results and Conclusions

Substrate consumption rates in the batch incubations were similar to those in the plant during the phases tested. Ammonium oxidation rates during the oxic phase were 1.58±0.04 and 1.87±0.06 µmol gVSS⁻¹ min⁻¹ in the batch incubations at 1 and 3 mg L⁻¹, DO respectively compared to 1.65±0.07 µmol gVSS⁻¹ min⁻¹ in the plant. Similarly, the NO₃⁻ reduction rate during the anoxic phase was 1.59±0.01 µmol gVSS⁻¹ min⁻¹ compared to 1.26±0.13 µmol gVSS⁻¹ min⁻¹ in the plant. Denitrification was strongly suppressed by oxygen but remained detectable at both 1 and 3 mg L⁻¹ DO with rates corresponding to 4.3 and 2.9% of the anoxic rate, respectively.

The net production of N₂O in the anoxic incubations was 0.61 nmol gVSS⁻¹ min⁻¹, resulting from HD, but production was approx. 3 and 1.7 times higher at 1 and 3 mg L⁻¹ DO, respectively (Table 1). The large production at 1 mg L⁻¹ DO was mainly attributed to ND, which peaked at 73% of total N₂O production and still dominated as N₂O source at 3 mg L⁻¹ DO. The remaining N₂O production was mainly due to NN. Although a slight gross production of N₂O from HD was detected in the oxic incubations, denitrification was a small net sink for N₂O, i.e. N₂O reduction exceeded the production from HD even at 3 mg L⁻¹ DO.

Table 1  N₂O production rates (nmol gVSS⁻¹ min⁻¹) and relative contributions of the three main N₂O production pathways derived from short-term batch incubations

<table>
<thead>
<tr>
<th>Simulated phase</th>
<th>NN rate</th>
<th>NN%</th>
<th>ND rate</th>
<th>ND%</th>
<th>HD rate</th>
<th>HD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anoxic</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.61±0.083</td>
<td>100</td>
</tr>
<tr>
<td>Oxic, DO = 1 mg L⁻¹</td>
<td>0.64±0.15</td>
<td>24.4</td>
<td>1.92±0.71</td>
<td>73.3</td>
<td>0.057±0.001</td>
<td>2.2</td>
</tr>
<tr>
<td>Oxic, DO = 3 mg L⁻¹</td>
<td>0.43±0.04</td>
<td>40.0</td>
<td>0.65±0.11</td>
<td>59.1</td>
<td>0.017±0.021</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Our results demonstrate that all three pathways were operative in the Lynetten activated sludge plant, each responding differently to the changes that occur during the different phases of operation. Dual ¹⁵N/¹⁸O isotope labelling is a robust approach to distinguish different N₂O production pathways in biological nitrogen removal plants, and it can contribute to the development of operational strategies to minimise N₂O emissions.

References