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Dynamics of N₂O production pathways in a full-scale activated sludge system analysed by ¹⁵N/¹⁸O dual isotope labelling

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Introduction

Nitrous oxide emissions from biological nitrogen removal can contribute substantially to the CO₂-equivalent footprint of wastewater treatment, but the pathways and regulation of N₂O production are poorly understood. Measurements of N₂O in full-scale activated sludge plant showed large variation in N₂O concentration during different phases of operation (Ekström et al. 2016), which indicated the involvement of different N₂O production pathways in N₂O emission. A partitioning of these pathways and analysis of their dynamics is therefore important for establishment of strategies to mitigate N₂O release..

Nitrifier nitrification (NN), nitrifier denitrification (ND) and heterotrophic denitrification (HD) are recognised as the three main pathways of N₂O production. We developed a ¹⁵N/¹⁸O dual isotope labelling technique to distinguish and quantify these pathways in wastewater treatment systems. The use of ¹⁸O-labeled O₂ specifically permits the partitioning of NN and ND, which cannot be achieved with certainty with ¹⁵N labelling alone. In this study we applied the dual isotope labelling technique in an analysis of N₂O 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 N₂O 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/O₂ atmosphere with the addition of ¹⁵N-labeled ammonium, nitrite, or nitrate and/or ¹⁸O-labeled O₂. 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 ¹⁸O-labeled O₂. Incubations were sampled for the measurement of bulk and ¹⁵N-labeled NH₄⁺, NO₂⁻ and NO₃⁻, of ¹⁵N-labeled N₂, and of ¹⁵N and ¹⁸O-labeled N₂O. Isotope exchange of ¹⁸O between NO_x⁻ and H₂O was further quantified in order to obtain NN rates from ¹⁸O incubations. Isotope analysis was performed by continuous flow GC-IRMS with conversion of DIN species to N₂.

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 $1.87 \pm 0.06 \mu\text{mol gVSS}^{-1} \text{min}^{-1}$ in the batch incubations at 1 and 3 mg L⁻¹ DO respectively compared to $1.65 \pm 0.07 \mu\text{mol gVSS}^{-1} \text{min}^{-1}$ in the plant. Similarly, the NO₃⁻ reduction rate during the anoxic phase was $1.59 \pm 0.01 \mu\text{mol gVSS}^{-1} \text{min}^{-1}$ compared to $1.26 \pm 0.13 \mu\text{mol gVSS}^{-1} \text{min}^{-1}$ 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 $0.61 \text{ nmol gVSS}^{-1} \text{min}^{-1}$, 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

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

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

Ekström, S., Jensen, M.M., Lemaire, R., Plósz, B.G., Thornberg D., Smets, B.F. (2016) . Quantification of N₂O emissions and their effect on the CO₂ footprint of a full-scale phased isolation nutrient removal system. MEWE2016 conference abstract.