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## N2O and NO dynamics in AOB-enriched and mixed-culture biomass: Experimental Observations and Model Calibration

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# Abstract

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Nitrous oxide (N<sub>2</sub>O) is emitted during biological nitrogen removal (BNR) in wastewater treatment operations. The main organisms responsible for N<sub>2</sub>O production are ammonia-oxidizing bacteria (AOB) and heterotrophic denitrifying bacteria (HB). AOB produce N<sub>2</sub>O (1) during incomplete ammonium (NH<sub>4</sub><sup>+</sup>) oxidation to nitrite (NO<sub>2</sub><sup>-</sup>) (nitrifier nitrification, NN) and (2) under low dissolved oxygen (DO) conditions using NO<sub>2</sub><sup>-</sup> as the terminal electron acceptor (nitrifier denitrification, ND). In heterotrophic denitrification N<sub>2</sub>O is an obligate intermediate of respiration that can be released under low carbon-to-nitrogen ratios or in the presence of DO (HD).

Mechanistic models can be useful to synthesize the complex interrelationships within BNR to ultimately develop mitigation strategies for N<sub>2</sub>O emissions. In this study a combination of experimental and modelling tools were used to study and describe N<sub>2</sub>O dynamics from N-removing processes.

N<sub>2</sub>O production dynamics were investigated using targeted batch respirometric assays with two biomass types: an AOB-enriched biomass (type A) and a mixed liquor with a lower AOB abundance from a full-scale BNR plant (type B). Nitrogenous substrates (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>2</sub>OH) were added at varying oxygen concentrations while responses in N<sub>2</sub>O, nitric oxide (NO), dissolved oxygen and pH were monitored.

Under aerobic conditions net N<sub>2</sub>O production with biomass type A and B was higher during NH<sub>2</sub>OH oxidation compared to NH<sub>4</sub><sup>+</sup>; no N<sub>2</sub>O was produced in the sole presence of NO<sub>2</sub><sup>-</sup>. At the onset of anoxia the N<sub>2</sub>O production significantly increased, accumulating in the bulk. Biomass type B showed a much higher N<sub>2</sub>O consumption rate compared to type A, explained by a larger fraction of N<sub>2</sub>O reducers. NO production in both systems was triggered by NH<sub>2</sub>OH pulses under any DO level and NO<sub>2</sub><sup>-</sup> pulses at low DO concentrations.

A newly developed pseudo-mechanistic model distinguishing N<sub>2</sub>O production pathways from autotrophic (NN, ND) and heterotrophic bacteria (HD) successfully described the experimental data.

The model considers NH<sub>3</sub> as substrate of AOB and could describe NH<sub>4</sub><sup>+</sup> oxidation at varying NH<sub>4</sub><sup>+</sup> and pH. NH<sub>2</sub>OH and NO - intermediates of AOB and HB metabolism - were the key precursors of N<sub>2</sub>O production. The model captured a pH dependence of the N<sub>2</sub>O consumption rate of biomass type B (pH optimum = 8).

Parameter sets were estimated for each biomass type (maximum rates, substrate affinities) and highlighted differences in microbial community composition. For example, the estimated NH<sub>3</sub> affinity differed, probably due to the different NH<sub>4</sub><sup>+</sup> and pH levels at which the biomasses operated (NH<sub>4</sub><sup>+</sup>\_type\_A > NH<sub>4</sub><sup>+</sup>\_type\_B → K<sub>NH<sub>3</sub></sub>\_type\_A > K<sub>NH<sub>3</sub></sub>\_type\_B). The fractions of NH<sub>4</sub><sup>+</sup> oxidized and NO<sub>2</sub><sup>-</sup> reduced to N<sub>2</sub>O by AOB (NN and ND pathways) also varied between systems. The model could also describe the sequential accumulation of heterotrophic denitrification intermediates in biomass type B from NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>O via NO<sub>2</sub><sup>-</sup> and NO. Overall, while biomass type B showed a larger N<sub>2</sub>O production rate, the net emission remained lower as heterotrophic denitrifiers acted as an N<sub>2</sub>O sink.