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Further investigation into roles of dissolved oxygen and nitrite accumulation inside sludge flocs in N₂O production

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Abstract: This work aims to further clarify the impact of dissolved oxygen (DO) on N₂O production by ammonium-oxidizing bacteria (AOB) without significant bulk NO₂⁻ accumulation (<0.2 mg N/L) as well as to address the role of potential nitrite accumulation inside sludge flocs in this regard. To this end, an augmented nitrifying sludge was enriched which was used for systematically designed batch tests. Mathematical modelling was applied to facilitate the interpretation of batch experimental data, thus shedding light on the relationship between N₂O production pathways and key process parameters (i.e., DO and NO₂⁻ accumulation inside sludge flocs). The findings of this work provide further insights and useful information for understanding N₂O production during biological wastewater treatment.

Keywords: Nitrous oxide (N₂O); nitrite accumulation; ammonium-oxidizing bacteria (AOB); modelling

Introduction

Nitrous oxide (N₂O), a significant greenhouse gas as well as a major scavenger of stratospheric ozone leading to ozone layer depletion, can be undesirably produced at wastewater treatment plants. Nitrification, which requires oxygen as the electron acceptor to convert ammonium (NH₄⁺) to nitrite (NO₂⁻) via hydroxylamine (NH₂OH) as an intermediate, is mediated by ammonium-oxidizing bacteria (AOB) and regarded as the major contributor to N₂O production during biological nitrogen removal from wastewater. Although two pathways have been proposed for N₂O production by AOB during nitrification, i.e., the NH₂OH pathway and the AOB denitrification pathway, consensus has not yet been reached in terms of the effects of key process parameters such as dissolved oxygen (DO) and NO₂⁻ on N₂O production by AOB.

Therefore, this work aims to clarify the impact of DO on N₂O production by AOB without significant bulk NO₂⁻ accumulation (<0.2 mg N/L) as well as to address the role of potential nitrite accumulation inside sludge flocs in this regard.

Materials and Methods

An augmented nitrifying sludge comprising mainly nitrite-oxidizing bacteria (NOB) with lesser coexistence of AOB in same flocs was enriched. Using the augmented nitrifying sludge, nine sets of batch tests were carried out, targeting eight DO levels from 0.2 to 3.0 mg O₂/L. During the entire batch test, NH₄⁺ concentration was controlled at 10 mg N/L through periodical dosing of a NH₄⁺ stock solution. In addition to bulk NO₂⁻, NH₄⁺ and NO₃⁻ concentrations, N₂O in both liquid and gas phases were consistently monitored. High-output sonication was applied to release any nitrite potentially accumulated inside the sludge flocs. Indexes including biomass specific ammonium oxidation rate (AOR), biomass specific N₂O production rate (N₂OR) and N₂O emission factor were calculated for each batch test.

The two-pathway N₂O model proposed by Ni et al. (2014), which links the oxidation and reduction processes through a pool of electron carriers, was employed to interpret the batch experimental data. The augmented nitrifying sludge flocs were modelled as a

particle system to facilitate the investigation into the role of potential NO_2^- accumulation inside the sludge flocs in N_2O production by AOB. Following the method of Ni et al. (2009), the number of sludge flocs for each batch test was calculated, assuming a uniform floc diameter of $114 \mu\text{m}$ (i.e., the measured D_{50}). AOB and NOB biomass in the model was set based on the measured volatile suspended solids (VSS) concentration together with the microbial abundance quantified by fluorescence *in-situ* hybridization and 16S rRNA gene pyrosequencing analyses.

Results and Discussion

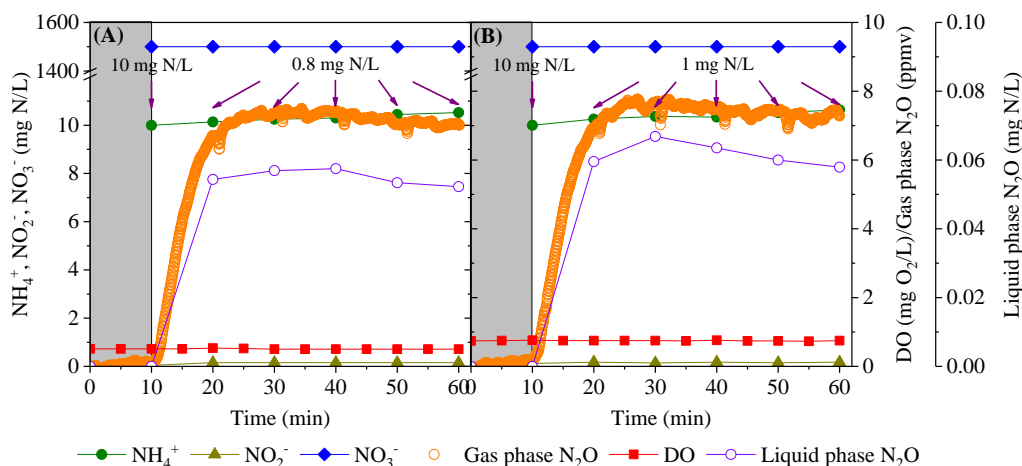


Figure 1. Batch test results at DO level of (A) 0.5 mg O_2 /L and (B) 0.75 mg O_2 /L.

The potential N_2O consumption by heterotrophic denitrifiers was ruled out by N_2O consumption control tests (data not shown). Experimental data for all batch tests studied exhibited similar trends including NO_2^- , NH_4^+ and N_2O , and **Figure 1** shows examples at DO levels of 0.5 and 0.75 mg O_2 /L. No N_2O was detected during the first 10 min control phase, indicating the negligible N_2O production contribution from heterotrophic denitrification. Applicable to both gas phase and liquid phase data, N_2O concentration reached steady state in about 10 min after dosage of 10 mg N/L of NH_4^+ . NO_2^- concentration in the bulk liquid during the entire batch test was below 0.2 mg N/L.

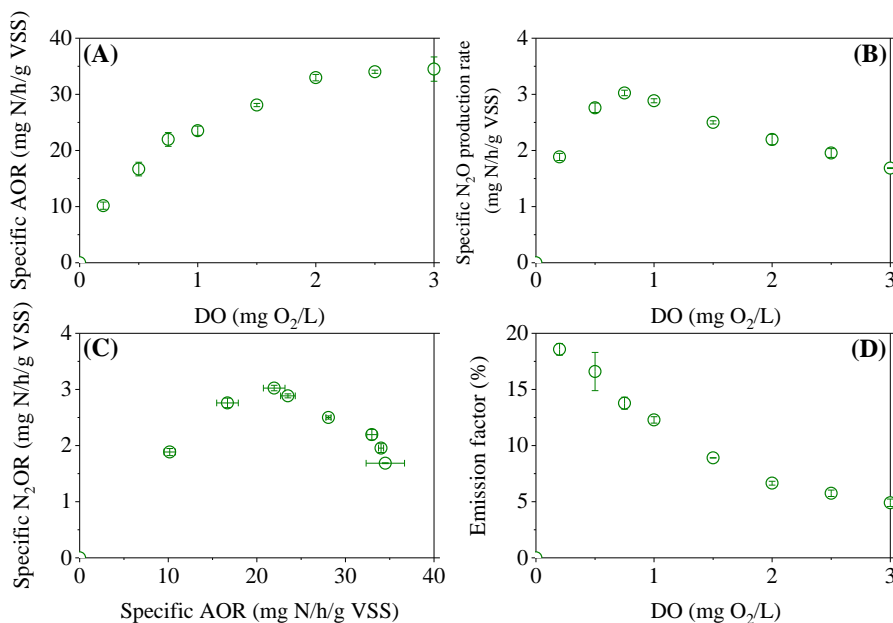


Figure 2. Relationship between (A) AOR and DO, (B) N_2OR and DO, (C) N_2OR and AOR and (D) N_2O emission factor and DO detected in batch tests (error bars stand for standard deviation, $n=3$).

Figure 2 shows the indexes including biomass specific AOR and N₂OR as well as N₂O emission factor calculated based on the batch experimental results obtained. AOR increased with DO but reached a steady level when DO exceeded 2.5 mg O₂/L (**Figure 2A**), resulting from the limited conversion capacity of AOB. N₂OR first increased with DO, peaked at DO of 0.75 mg O₂/L, and thereafter kept decreasing until DO reached the maximum studied level of 3.0 mg O₂/L (**Figure 2B**). The correlation between N₂OR and AOR was positive when the AOR was below 22.0 ± 1.2 mg N/h/g VSS, above which the N₂OR was negatively correlated to AOR (**Figure 2C**). The trends in **Figures 2B** and **2C** revealed that a DO level higher than 0.75 mg O₂/L would exhibit an inhibitive effect on the specific N₂O production of AOB, irrespective of the higher AOR induced, which was analogous to the reported inhibitive impact of DO on the AOB denitrification pathway for N₂O production. Nevertheless, the negative dependency of the overall N₂O emission factor on DO was still commensurate with literature reported finding (**Figure 2D**).

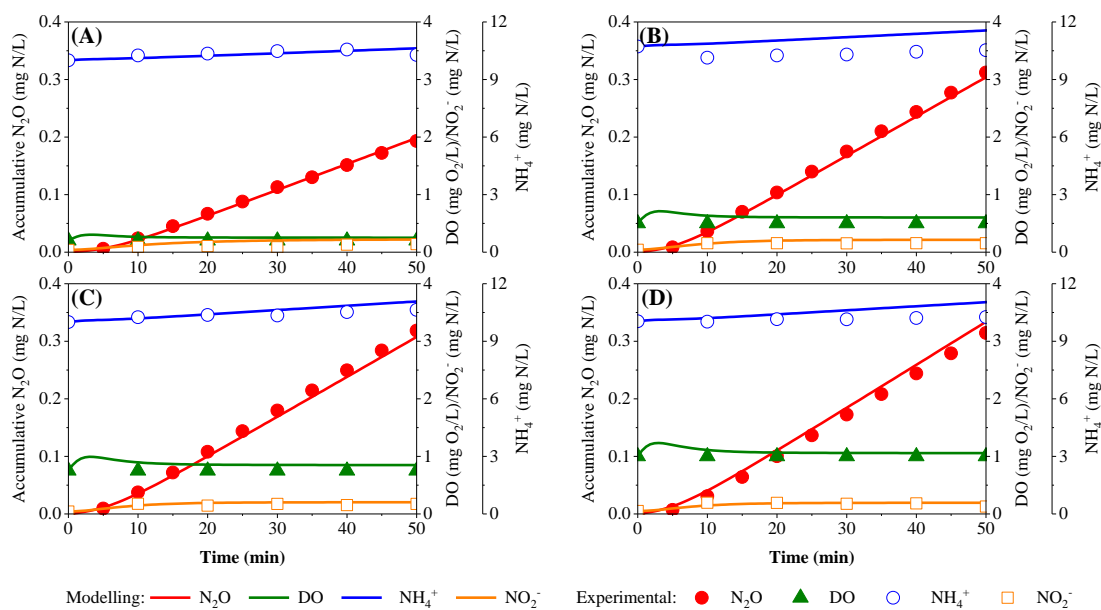


Figure 3. Model calibration and validation results (note that only four DO levels are presented).

The two most sensitive parameters identified by the sensitivity analysis were estimated by fitting all the batch experimental data obtained at five different DO levels. The batch experimental data at DO levels remaining were used for model calibration. As the shown in **Figure 3**, the model calibration/validation process was successfully carried out with a good match between modelling and experimental results proving the validity of the calibrated model. The contributions of the two pathways to the N₂O production under different DO concentration conditions were

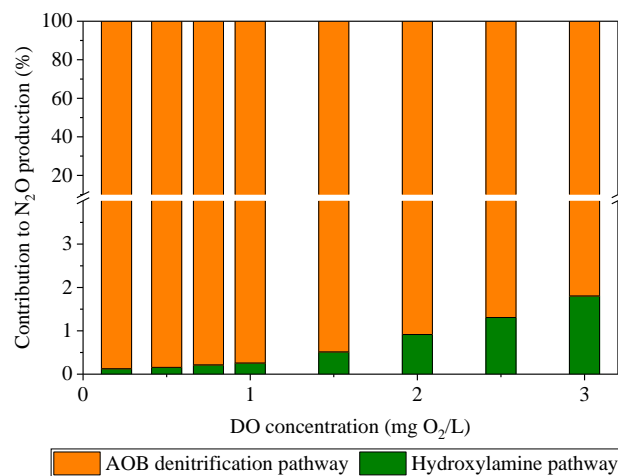


Figure 4. Contribution of the two pathways to the N₂O production at different DO concentrations.

then calculated using the developed model and presented in **Figure 4**. Albeit exhibiting a decreasing trend, the AOB denitrification pathway was dominating under all the DO concentration conditions studied in this work. In contrast, the contribution to the N_2O production from the hydroxylamine pathway increased slightly but consistently from 0.1% at DO of 0.2 mg O_2/L to 1.8% at DO of 3.0 mg O_2/L . Therefore, AOB denitrification pathway was dominant with the secondary contribution of hydroxylamine pathway which increased with DO.

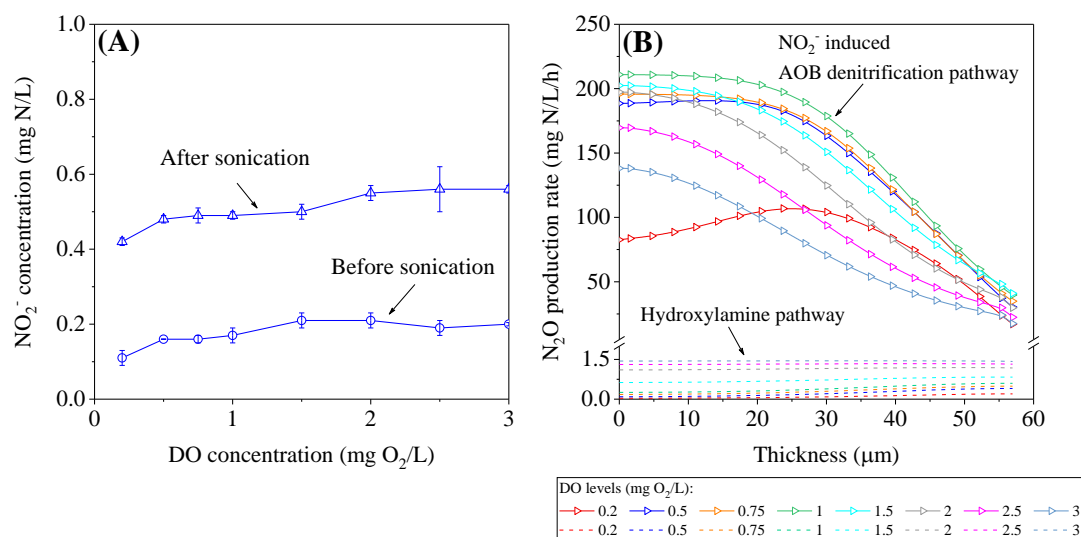


Figure 5. (A) Bulk NO_2^- concentration before and after sonication at different DO levels and (B) model-generated distribution of N_2O production rates in the representative floc at different DO levels.

Despite the low bulk NO_2^- concentration (<0.2 mg N/L) observed under all DO conditions, the mixed liquid underwent an evident increase in NO_2^- concentration to around 0.5 mg N/L after sonication treatment, as shown in **Figure 5A**. This increase suggested a significant NO_2^- accumulation in the flocs despite the trace NO_2^- detected in the bulk liquid outside the flocs and proved the necessity of modelling the enriched sludge flocs as a particle system. As NO_2^- is a key factor/precursor regulating the N_2O production from the AOB denitrification pathway, nitrite accumulation inside the sludge flocs might favour the AOB denitrification pathway which would lead to the trends obtained in this work. **Figure 5B** shows the uneven distribution of N_2O production pathways in the representative floc, which was the result of the concentration gradients of provided/produced substrates. NO_2^- induced AOB denitrification pathway was at rates much higher than hydroxylamine pathway at all DO levels. The rate of AOB denitrification pathway first increased and then decreased with DO, consistent with **Figure 2B**. In contrast, the rate of hydroxylamine pathway kept increasing with DO level.

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