

Evaluating mathematical models for N₂O production by ammonia-oxidizing bacteria: towards a unified model

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Abstract

There is increasing evidence showing that ammonia oxidizing bacteria (AOB) are major contributors to N₂O emissions from wastewater treatment plants (WWTPs). However, the mechanisms responsible for N₂O production by AOB are not fully understood. Mathematical modelling provides a means for testing hypotheses related to mechanisms, and can then also become a tool to support the development of mitigation strategies. This study examined the ability of four mathematical model structures to describe two distinct mechanisms of N₂O production by AOB, with the aim to develop a unified model. The production mechanisms evaluated are (1) nitrifier denitrification with NO₂⁻ as terminal electron acceptor and (2) the byproduct of the incomplete oxidation of hydroxylamine (NH₂OH) to NO₂⁻. The four models were compared based on their ability to predict N₂O dynamics observed in three mixed culture studies. Short-term batch experimental data were employed to examine model assumptions related to the (1) production of N₂O due to NH₄⁺ increase, (2) production of N₂O due to dissolved oxygen (DO) variations, (3) N₂O dependency on NO₂⁻ accumulations and (4) production of N₂O with NH₂OH as an externally provided substrate. The modelling results demonstrate that almost all of these models can generally describe the NH₄⁺, NO₂⁻ and NO₃⁻ data. However, none of these models were able to reproduce all measured N₂O data. The results suggest that both the denitrification and NH₂OH pathways may be involved in N₂O production. Therefore, a unified model used to predict N₂O production should provide the ability to capture both mechanisms independently.

Keywords

Ammonia oxidizing bacteria; hydroxylamine; mathematical model; mechanisms; nitrifier denitrification; nitrous oxide

INTRODUCTION

Nitrous oxide (N₂O) is a significant greenhouse gas, with an approximately 300-fold stronger warming effect than carbon dioxide (IPCC, 2001), that can be produced and directly emitted from wastewater treatment systems. Therefore, even low amounts of N₂O emission are unwanted. N₂O is produced during biological nitrogen removal typically attributed to autotrophic ammonia-oxidizing bacteria (AOB) (Tallec *et al.*, 2006; Kampschreur *et al.*, 2009) and heterotrophic denitrifying organisms (Kampschreur *et al.*, 2009; Lu and Chandran, 2010). N₂O emission data show a huge variation in the fraction of nitrogen that is emitted as N₂O (Kampschreur *et al.*, 2009). The large differences in N₂O emissions measured between different wastewater treatment plants and the variations in time strongly contrast with the fixed conversion factors applied to estimate the N₂O

emission in different treatment units (IPCC, 2006). A disadvantage of the use of this type of fixed emission factors is that they do not consider the process itself and the process dynamics and are therefore not realistic for the variety of process conditions encountered in the field. Therefore, mathematical modelling rather than the use of fixed emission factors should be an appropriate method for estimating site-specific emissions of N_2O (CH2MHill, 2008; Ni et al., 2011; Corominas et al., 2012).

N_2O production by heterotrophic denitrification is well understood: N_2O is an intermediate in heterotrophic denitrification. The model describing this process proposed by Hiatt and Grady (2008) has been generally accepted. However, a unified model for N_2O production by AOB is still not available, despite that there are increasing evidences showing that AOB are major contributors to N_2O emission from WWTPs (Kampschreur et al., 2008a; 2008b; Sivret et al., 2008; Yang et al., 2009; Schreiber et al., 2009; Yu et al., 2010; Kim et al., 2010; Ahn et al., 2011). This is due to an incomplete understanding of the mechanisms involved in N_2O production by AOB.

AOB are chemolithotrophs that oxidize NH_4^- to NO_2^- via hydroxylamine (NH_2OH) as their predominant energy-generating metabolism. The first step is catalyzed by ammonia monooxygenase (AMO) where NH_4^- is oxidized to NH_2OH with the reduction of molecular oxygen (O_2). In the second step, NH_2OH is oxidized to NO_2^- by hydroxylamine oxidoreductase (HAO). Although N_2O is not an obligate intermediate in these processes, N_2O can be produced through two proposed pathways. During NH_2OH oxidation, N_2O can be formed through the chemical breakdown of the unstable nitrosyl radical (NOH) (Poughon et al., 2000; Arp and Stein, 2003). AOB are also capable of utilizing nitrite (NO_2^-) and nitric oxide (NO) as an alternative electron acceptor instead of O_2 , producing N_2O as the end product through the nitrifier denitrification pathway (Yu et al., 2010; Kim et al., 2010).

To date, several mechanistic models have been proposed for N_2O production by AOB. The current study attempts to differentiate between these different mechanisms of N_2O production. Four different structures used in the mathematical modelling of N_2O production by AOB are presented in Figure 1. Model I (Ni et al., 2011) and Model II (Mampaey et al., 2011) are based on the nitrifier denitrification pathway. The key difference between the two models is that in Model I, oxygen is assumed to inhibit nitrite and NO reduction by AOB, while in Model II, this inhibition is absent. A further minor difference is that ammonia oxidation is modelled as a two-step (ammonia to hydroxylamine and then to nitrite) process in Model I, but as a one-step process (ammonia to nitrite) in Model II. In contrast, Model III assumes that N_2O production is due to the chemical breakdown of NOH, an intermediate of NH_2OH oxidation (Law et al., 2012), while Model IV assumes reduction of NO, produced from the oxidation of NH_2OH , into N_2O . The key reactions considered by these models are summarised in Figure 1.

This paper uses experimental data of three different mixed culture nitrifying reactor studies reported in literature to examine the ability of these different model structures to describe N_2O production in different nitrifying systems. The purpose is to understand how the mathematical structure of the four models presented in Figure 1 influences their ability to reproduce the experimental data.

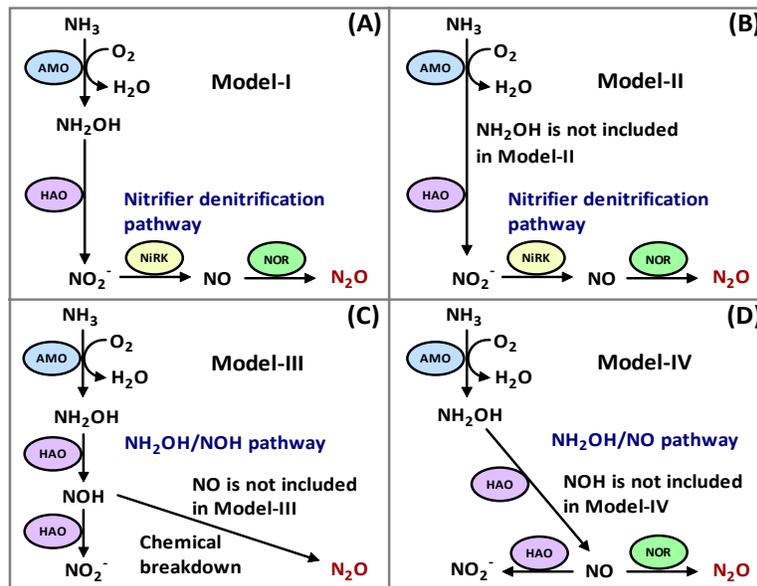


Figure 1. Reaction schemes used in the four N_2O models evaluated in this study: Model I – AOB denitrification pathway with NH_2OH as the electron donor; Model II – AOB denitrification pathway with NH_3 as the electron donor; Model III – the NH_2OH/NOH pathway; and Model IV – the NH_2OH/NO pathway.

MATERIALS AND METHODS

Mechanistic models for N_2O production

The kinetic and stoichiometric matrices for the four mathematical models are provided in Appendix 1. Nomenclature for all state variables is chosen to highlight similarities and differences between modelling approaches and differ slightly from the original publications. All four models employ the following symbols for concentration: ammonium oxidizing organisms (X_{AOB}), nitrite oxidizing organisms (X_{NOB}), particulate undegradable organics (X_U), NH_4^+ (S_{NH_4}), NH_2OH (S_{NH_2OH}), NOH (S_{NOH}), NO_2^- (S_{NO_2}), NO_3^- (S_{NO_3}), NO (S_{NO}), N_2O (S_{N_2O}), N_2 (S_{N_2}), and DO (S_{O_2}). Other biological processes, such as endogenous decay and aerobic NO_2^- oxidation by NOB are included with standard ASM kinetic expressions and parameters values taken from published literature (Koch et al., 2000; Henze et al., 2000) in all four models. Appendix 2 lists the definitions, values, and units of the parameters used in the four models.

In Model I (Appendix 1, Ni et al., 2011), AOB denitrification with NO_2^- as the terminal electron acceptor produces NO and subsequently N_2O by consuming NH_2OH as electron donor. By multiplying the corresponding kinetic rate with a substrate inhibition function for O_2 , Model I describes that NO and N_2O production by nitrifier denitrification increases at low DO concentrations. Similarly, in Model II (Appendix 1, Mampaey et al., 2011), AOB denitrification occurs in parallel with the ammonium oxidation, reducing NO_2^- to NO and then to N_2O with ammonium as electron donor. In addition, Model II employs the same specific ammonium utilization rate of AOB when using O_2 , NO_2^- and NO as electron acceptor. In Model III (Appendix 1, Law et al., 2012), it is hypothesised that N_2O production is due to the chemical breakdown of

NOH, an intermediate produced during NH_2OH oxidation to nitrite. The non-enzymatic decomposition of NOH produces N_2O . It is assumed that the non-enzymatic decomposition of NOH complies with first order kinetics. Model IV (Appendix 1, formulated in this study) is based on the assumption that N_2O is formed through the biological reduction of NO that is formed as an intermediate of NH_2OH oxidation (Stein, 2011), called the $\text{NH}_2\text{OH}/\text{NO}$ pathway here. It is assumed that DO has no inhibitory effect on NO reduction in Model IV, as in Model II.

Testing the predictive abilities of the models

Experimental data from three case studies (Yang et al., 2009; Kim et al., 2010; Law et al., 2012) concerning N_2O dynamics were used for testing the predictive abilities of the four mathematical models. Yang et al. (2009) demonstrated that nitrifier denitrification was mainly responsible for N_2O production by AOB in an activated sludge treating domestic wastewater (Case 1). Kim et al. (2010) examined N_2O production by AOB in an activated sludge system treating swine wastewater using NH_2OH as a substrate instead of NH_4^+ (Case 2). Law et al. (2012) investigated the correlation between the ammonia oxidation rate and N_2O production rate of an enriched AOB culture treating anaerobic sludge digestion liquor (Case 3). As such, the four models are tested for a wide range of systems with different reactor types (e.g., sequencing batch reactor (SBR) and batch reactor), feed compositions (e.g., NH_4^+ influents and NH_2OH influents), biomass types (e.g., different nitrifying biomass enrichments), and operational conditions (e.g., different DO and NH_4^+ concentrations). The parameters describing N_2O production in each model evaluated here were estimated based on the measured N_2O production and nitrogen conversion during the nitrification processes.

Parameter estimations were performed using AQUASIM (Reichert, 1998). Parameter values estimated in the current study are provided in Appendix 2 together with other parameter values used in the current analysis. Not all parameters were identifiable from the experimental data due to parameter correlation. Hence, our methodology has been to use typical parameter values for nitrification processes and available N_2O production kinetics reported for wastewater applications, whenever possible (see Appendix 2). We only estimate those parameters, which are unique to each model (e.g., $\mu_{\text{AOB,AMO}}$, $\mu_{\text{AOB,HAO}}$, $K_{\text{S1,O2_AOB}}$, $K_{\text{I,O2_AOB}}$ and $\eta_{\mu_{\text{AOB,Ax}}}$ in Model I; μ_{AOB} , $K_{\text{NO,AOB}}$ and $K_{\text{O2,AOB}}$ in Model II; $q_{\text{AOB,2,max}}$, $q_{\text{AOB,3,max}}$ and $q_{\text{AOB,4,max}}$ in Model III; and $\mu_{\text{AOB,AMO}}$, $\mu_{\text{AOB,HAO}}$, $K_{\text{S1,O2_AOB}}$, $K_{\text{S2,O2_AOB}}$ and $\eta_{\mu_{\text{AOB,Ax}}}$ in Model IV), by fitting the presented experimental data in each case study. The appropriate parameters were adjusted to capture the trends in the experimental data such that the models approximately predicted the timing and magnitude of the concentration changes as closely as possible. It should be highlighted that the aim of the modelling work is to verify if various model structures (rather than parameter calibrations) could explain the trend of the experimentally observed N_2O production, because having a solid model structure is a key step in the right direction towards true prediction of N_2O emissions.

RESULTS

Figures 2-4 show the evaluation results of the four mathematical models for N_2O production in three different case studies, respectively. Below, we comment on the model evaluation results for case no. 1 in detail to illustrate the ability of the four mathematical model structures to describe two distinct mechanisms of N_2O production by AOB. The remaining model evaluations (cases no. 2-3) are briefly described, highlighting the differences from case no. 1.

Evaluation of mathematical models: case no. 1

Here, the four mathematical models in Appendix 1 are evaluated based on their ability to capture the observed N₂O production results in the experiments of Yang et al. (2009), which were presented in Figure 2. Data derived from individual nitrification batch experiments (Figure 2) were used to obtain kinetic parameter values related to N₂O production associated with nitrification processes (Appendix 2). The models and calibrated parameter sets (Appendix 2) were then further tested for their ability to predict N dynamics in other sets of experiments reported by Yang et al. (2009): effects of NH₄⁺ and DO concentrations on N₂O production by AOB.

For Model I (Figures 2A-C), agreement between simulated and experimental results was good for all fitted variables in all experiments. N₂O production increased with the decrease of NH₄⁺ concentration and the increase of NO₂⁻ (Figure 2A). Higher NH₄⁺ concentrations led to higher NO₂⁻ accumulation and subsequently to increased N₂O production (Figure 2B). N₂O concentration peaked at DO = 1.5 g O₂ m⁻³ (Figure 2C). N₂O production is also stimulated when low DO concentrations combine with high NO₂⁻ concentration (Figure 2C). This combination occurs at 1.5g O₂ m⁻³. Model I captures all these trends and dynamics, suggesting that it is appropriate to describe the N₂O production in this nitrifying system. The observed N₂O dynamics of decrease in N₂O concentration with the increase of DO were captured in the mathematical model prediction by a low K_{I,O_2_AOB} (DO inhibition for N₂O production) value (0.112 g DO m⁻³) (Figure 2C). This is consistent with the observation that N₂O produced at DO concentrations below 1.5 g DO m⁻³ by Yang et al. (2009), indicating that AOB dinitrification should be the major pathway that caused the N₂O production in this case.

For Model II, the model structure forces an increase in N₂O production when the NH₄⁺, NO and O₂ concentrations increase. The time dependent variation of the N components in the nitrification experiments is shown in Figure 2D together with the corresponding model output for effects of NH₄⁺ (Figure 2E) and DO concentrations (Figure 2F). The predicted profiles shown in Figures 2D and E match the observed experimental trend very well. The kinetic nature of the N₂O production with NH₄⁺ as electron donor within Model II forces the N₂O concentration to follow changes in the NH₄⁺ concentration (Figure 2E). However, the N₂O prediction based on this model structure does not approximate the experimental observations in Figure 2F, although Model II applied the same N₂O production pathway (AOB denitrification) as Model I.

The increase in DO and therefore the decrease in N₂O can be translated to a substrate inhibition function for O₂ in Model I (Appendix 1). Predicting the observed experimental N₂O results using Model I (Appendix 1) could then be achieved by the low O₂ substrate inhibition parameter as shown in Appendix 2. The Model II structure, however, does not contain a term describing O₂ substrate inhibition for N₂O and, therefore, cannot describe these experimental data (Figure 2F). The inability to predict the decrease of N₂O production under high DO concentration in AOB denitrification is a shortcoming of Model II in real applications because low DO concentration has been recognized to be a key operational parameter leading to N₂O emission in many nitrifying systems (Kampschreur et al., 2009; Yang et al., 2009).

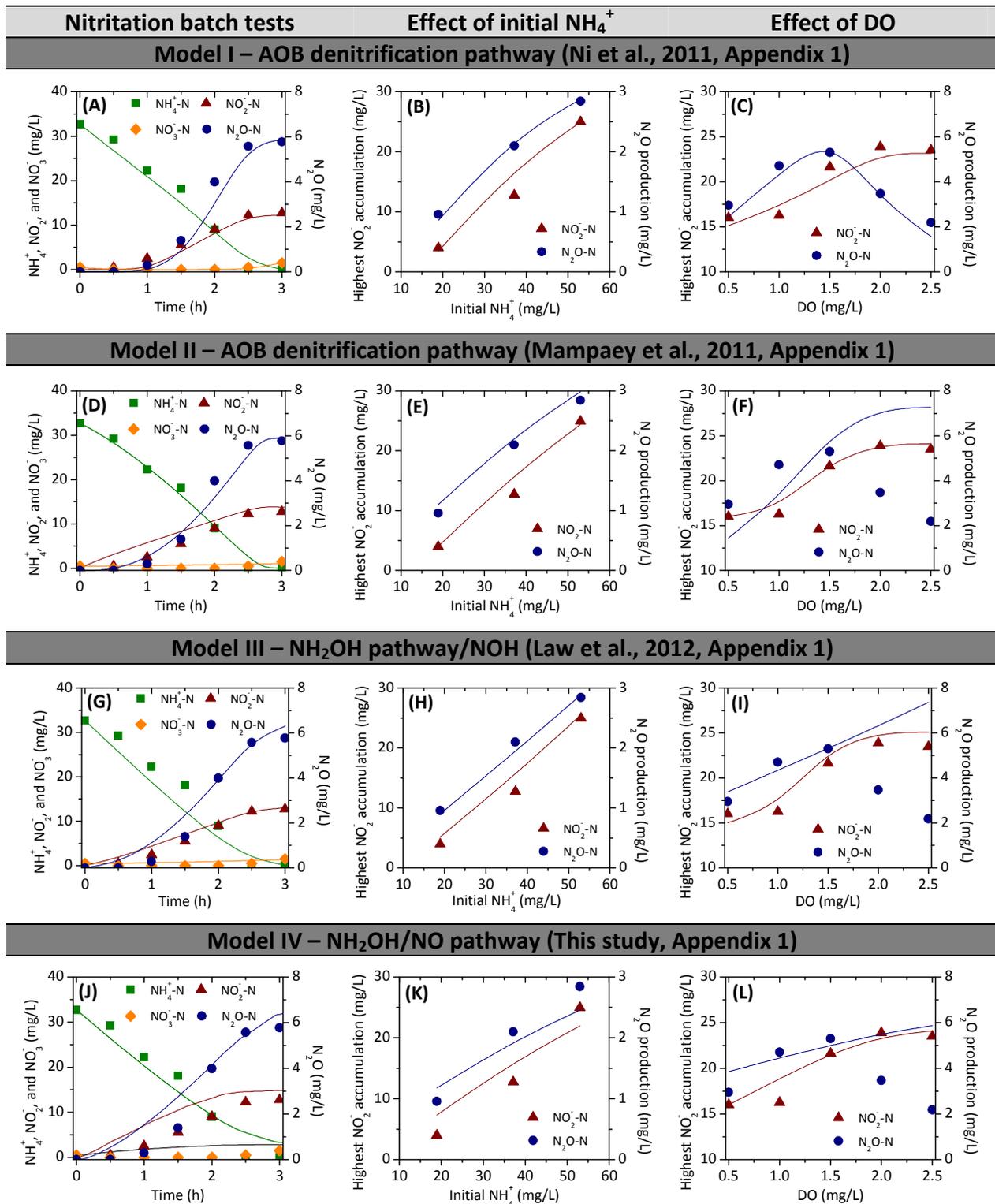


Figure 2. Experimental results (symbols) and model predictions (lines) for N_2O productions in nitritation batch tests, under different initial NH_4^+ and DO concentrations in case study 1: (A-C) Model-I evaluation; (D-F) Model-II evaluation; (G-I) Model-III evaluation; and (J-L) Model-IV evaluation.

Models III and IV adopted the other N₂O production pathway (incomplete oxidation of NH₂OH), which was different with that of the Models I and II (Appendix 1). The nitrification experiments and effects of NH₄⁺ and DO concentrations predicted by Models III and IV are provided in Figures 2G-L. Although the N concentration profiles in nitrification experiments (Figures 2G and J) are predicted well due to the AOB kinetics nature of the NH₄⁺ consumption and NO₂⁻ formation in Models III and D, this model is not able to capture the observed experimental trends for decrease of N₂O production under high DO concentration (Figures 2I and L) due to AOB denitrification, which was not included as N₂O production pathway in Models III and IV. Contrasting to the experimental data, the N₂O production and ammonium oxidation activities both increased with increasing DO concentration (Figures 2I and L).

NH₄⁺ versus NH₂OH as substrate: case no. 2

In the second case study, N₂O production by a nitrifying enrichment was monitored in batch reactors using NH₄⁺ and NH₂OH as the externally supplied substrate, respectively (Kim *et al.*, 2010). The four mathematical models were evaluated to test their ability to describe N₂O dynamics in these experiments. Figure 3A show that the measured NH₄⁺, N₂O, NO₂⁻ and NO₃⁻ data are very well described by Model A, in the experiment with NH₄⁺ as substrate. The N₂O production when using NH₂OH as the substrate can also be described relatively well (Figure 3B). The N₂O production rate slightly increased with NO₂⁻ accumulation, but rapidly ceased as NH₄⁺ was consumed (Figure 3A). By contrast, with NH₂OH as the substrate (Figure 3B), the N₂O production rates were two orders of magnitude higher (Figure 3B).

In the experiment with NH₄⁺ as substrate, the N₂O peak observed at the highest NO₂⁻ accumulation, caused by a dynamic response upon NH₄⁺ oxidation to NH₂OH and nitrite availability, cannot be explained by Model II. The kinetic nature of Model II forces that the N₂O production rate is dependent on oxygen, ammonium, and nitrite availability. The AOB denitrification occurs in parallel with the standard nitrification with ammonium as electron donor, instead of NH₂OH. Thus, Model II predicts that N₂O should peak at high availability of both NH₄⁺ and NO₂⁻, and therefore, cannot describe the experimental data (Figure 3C). In addition, Model II employed the same specific substrate removal rate of AOB when using O₂, NO₂⁻ and NO as electron acceptor, resulting in a much higher N₂O production rate in its prediction (Figure 3C). The higher predictions for N₂O production also lead to lower NO₂⁻ and NO₃⁻ concentration profiles (Figure 3C). Furthermore, there is no direct NH₂OH production and consumption term in Model II (Appendix 1), thus, Model II is not able to describe the N₂O dynamics by using NH₂OH as externally provided substrate.

In Models III and IV, the NO₂⁻ and NO₃⁻ concentrations increase with ammonium oxidation, giving a good fit with these experimental profiles. However, the description of the N₂O production is not accurate. In the predictions of Models III and IV (Figures 3E-H), the production of N₂O during NH₄⁺ oxidation, quickly leads to a linear increase of the N₂O production rate, which is only related to the increasing ammonium oxidation rate. The N₂O production decreases when ammonium is depleted even though nitrite is high, because the process is linked to incomplete NH₂OH oxidation and not dependent on nitrite availability.

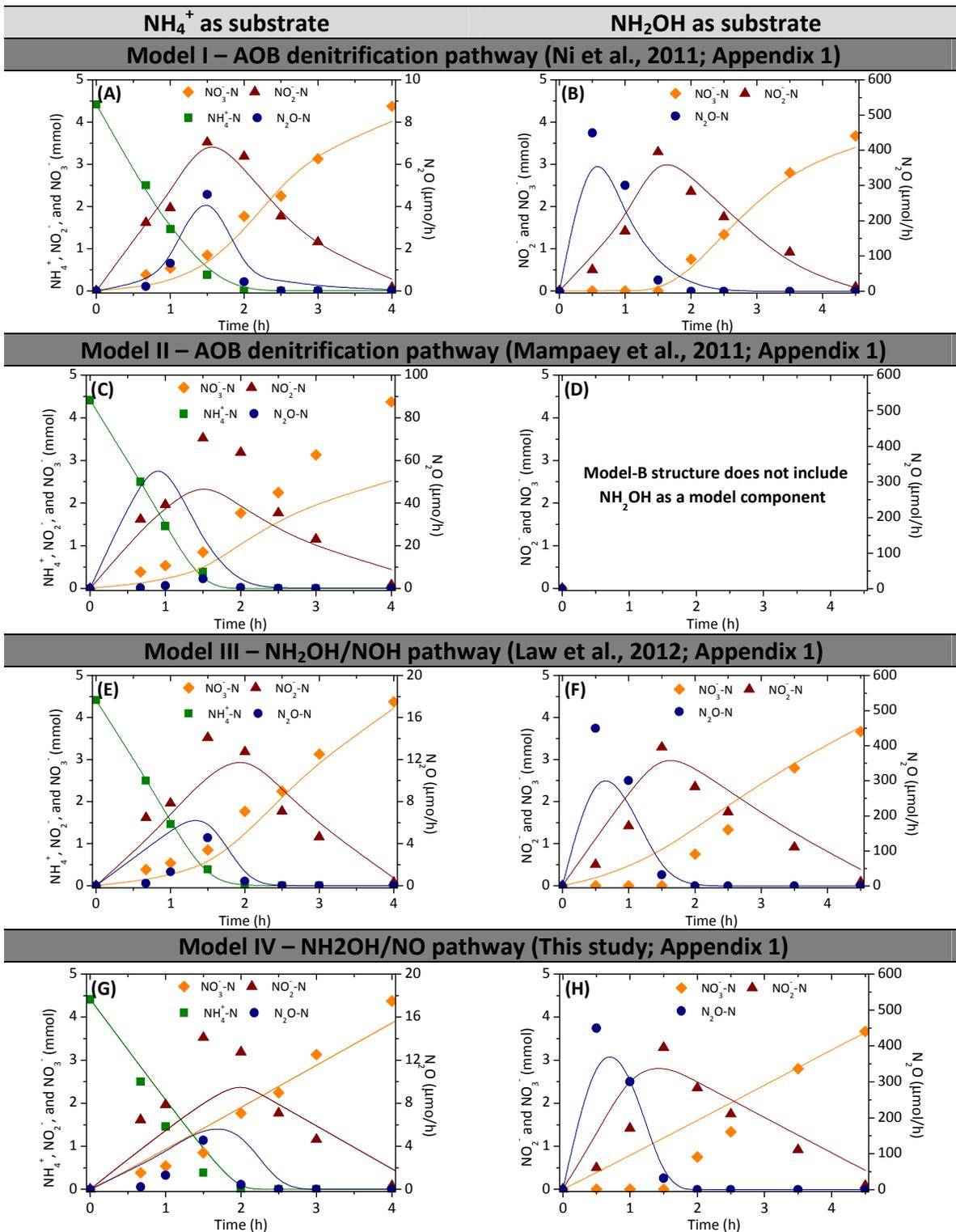


Figure 3. Experimental results (symbols) and model predictions (lines) for N₂O productions in nitrification batch test with NH₄⁺ (left) or NH₂OH (right) as substrate in case study 2 (Kim et al., 2010): (A-B) Model-I evaluations; (C-D) Model-II evaluations; (E-F) Model-III evaluations; and (G-H) Model-IV evaluations.

In our opinion, Model I, with the AOB denitrification pathway, enables the best description of the experimental data in this case study. Because the N_2O production is linked to availability of NH_2OH (the electron donor for the denitrification), the higher N_2O production rate during NH_2OH oxidation is well described by Model I: when NH_2OH is employed instead of NH_4^+ , omission of the AMO (oxidation of NH_4^+ to NH_2OH) reaction makes all four electrons per mole of N available for respiration. Because NO_2^- is the substrate for the AOB denitrification, the N_2O production increases with nitrite availability.

In sum, Model I enables the best description of the experimental data, especially the observed trend of decrease in N_2O concentration with the increase of DO in this case study. Because a substrate inhibition function for O_2 is employed in Model I, this N_2O dynamics is well described by Model I, oppositely to predictions of Models II, III and IV.

Step-wise variation of DO concentrations: case no. 3

In the last case study, N_2O production of an enriched AOB culture was investigated in a batch reactor with a step-wise decrease in DO concentrations (Law *et al.*, 2012). Figure 4C shows that the measured NH_4^+ , NO_2^- and NO_3^- data are very well described by Model I. However, the model output for N_2O production of Model I predicts a step-wise increase in N_2O production rate with a step-wise decrease in DO concentrations (Figure 4B), which is opposite to the experimental observations.

Although the kinetic nature of Model II ensures that the N_2O production rate is dependent on oxygen availability, resulting in a similar N_2O dynamic trend with experiments (Figure 4E), the predictions of Model II for N_2O production rate are substantially higher than the experimental data. For this model, the maximum ammonium oxidation rates coupled to oxygen and nitrite reduction were assumed to be identical. This leads to an extremely high N_2O production rate as both the NH_4^+ and NO_2^- concentrations are very high in the system.

On the contrary, Models III and IV were both able to reproduce all the experimental data in this case study (Figures 4G-L), which could not be reproduced by Models I and II based on the nitrifier denitrification pathway. The N_2O production increased/decreased with increasing/decreasing DO concentration indicating that incomplete NH_2OH oxidation was the major pathway that caused the N_2O production in this nitrifying system. This was captured well by Models III and IV.

In summary, Model III and IV, with the incomplete NH_2OH oxidation pathway, enables the best description of the experimental data in this case study. Because the N_2O production and ammonia oxidation activities both increased/decreased with increasing/decreasing DO concentration.

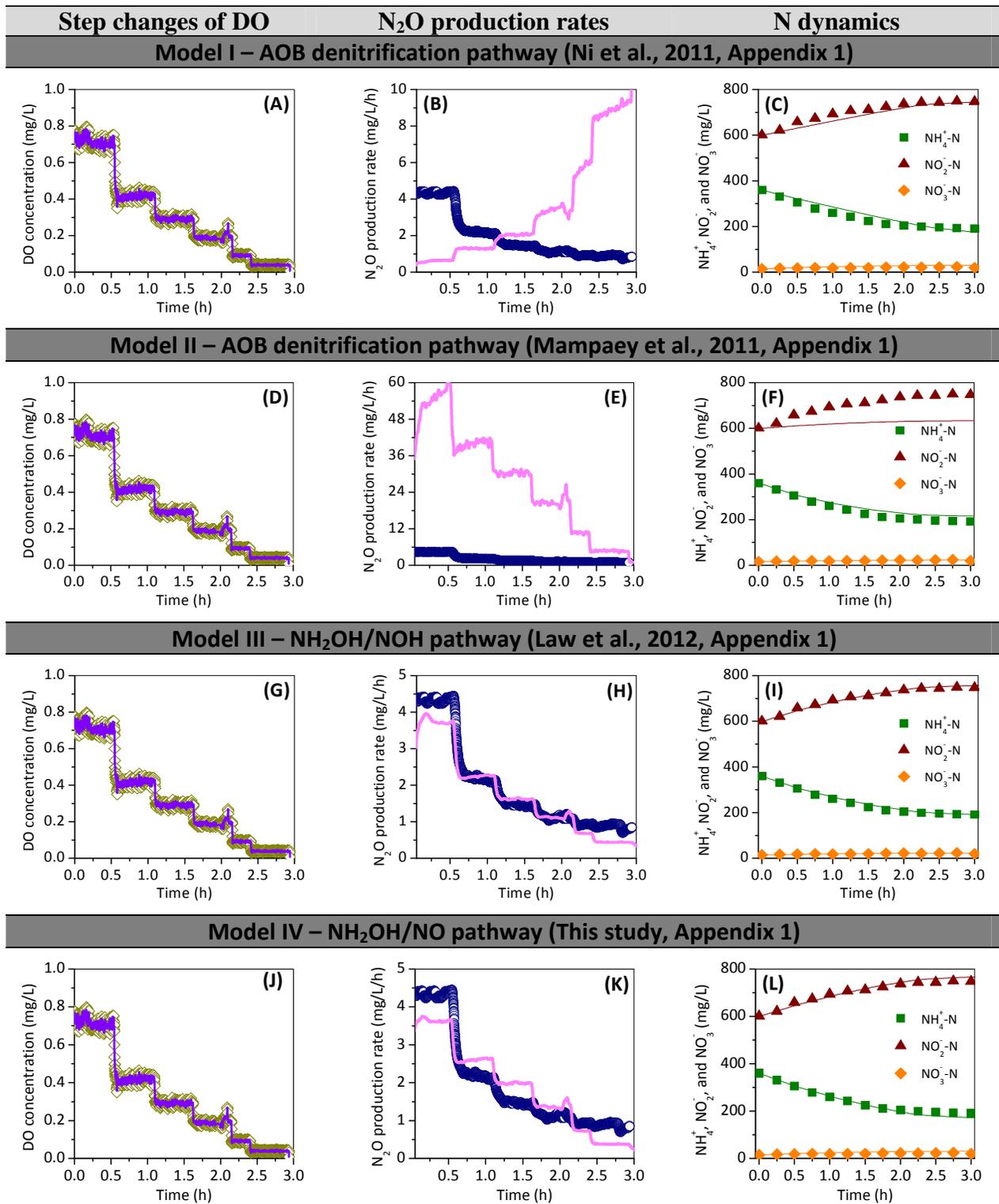


Figure 4. Experimental results (symbols) and model predictions (lines) for N₂O productions in nitrification batch tests with step changes of DO in Case 3: (A-C) Model-I evaluation; (D-F) Model-II evaluation; (G-I) Model-III evaluation; and (J-L) Model-IV evaluation.

DISCUSSION

It is well known that AOB can contribute to the formation of N_2O , an important greenhouse gas, in wastewater treatment (Kampschreur *et al.*, 2009; Foley *et al.*, 2010). Mathematical modelling of N_2O production is an important step towards understanding the full environmental impact of wastewater treatment. The ability to predict N_2O production provides an opportunity to use N_2O production as a biological nitrogen removal process design and optimization variable. However, the N_2O production mechanisms by AOB are very complex and involved with distinct production pathways (Poughon *et al.*, 2000; Arp and Stein, 2003; Ni *et al.*, 2011). Hence, there exists a trade-off between model complexity and parameter identifiability. Often, more complex mathematical models more closely represent the processes the model is meant to capture but if the parameters in the model are not identifiable, the complex model loses its practical usefulness to predict accurately across a wide range of process conditions. On the other hand, over-simplification can also lead to the poor prediction of relevant parameters.

The modelling results of this work demonstrate that almost all of the four models can generally describe the NH_4^+ , NO_2^- (except Model II) and NO_3^- data (Figures 2-4). However, none of these models were able to reproduce all sets of measured N_2O data. Dynamic N_2O data coupled with an understanding of the triggers causing the changes provide opportunities to evaluate the relevance of mathematical models to successfully predict these dynamic changes. The structures of the Models I and II were based on the AOB denitrification pathway. On contrast, Models III and IV employed the NH_2OH pathway in its structure. While these four mathematical models do not specifically exclude N_2O production mechanisms, their model structures inherently emphasize one mechanism over another.

As shown in Figures 2 and 3, the N_2O production observed in case studies no. 1 and 2 could be described well by the AOB denitrification pathway, especially by Model I. Model II was not flexible enough in kinetic structure to capture some of the data (Figures 2F and 3C) analysed in case studies no. 1 and 2 due to its over-simplification. Thus, by comparing modeling results of Models I and II, some key kinetic feature should be included when modeling the AOB denitrification pathway for N_2O production:

- NH_2OH , instead of NH_4^+ , should be the true electron donor substrate for AOB denitrification.
- A lower specific substrate utilization rate of AOB should be applied when using NO_2^- and NO as electron acceptor instead of O_2 .
- A substrate inhibition function for O_2 should be included to describe N_2O production by AOB denitrification at low DO concentrations.

However, neither Model I nor Model II, both based on the AOB denitrification pathway, could predict the N_2O data in Case 3 (Figure 4). Indeed, Model I based on the AOB denitrification pathway predicted the opposite trend (Figure 4B). Instead of an increase in N_2O production under decreased DO conditions, the batch tests showed increased N_2O production by the AOB mainly under increased DO conditions. In fact, the N_2O production rate was lowest under low DO supply (Figure 4H). The analogous response of the N_2O production rate and DO concentrations coupled with the Models III and IV data interpretation strongly suggests that the enriched AOB culture used in case study no. 3 likely produced N_2O through the NH_2OH oxidation pathway. While Models III and IV could describe the N_2O data in Case 3 (Figure 4), they failed to predict the dependency of N_2O production on DO observed in Case 1 (Figure 2).

The results suggest that both the denitrification and NH_2OH pathways may be involved in N_2O production. Thus, both nitrifier denitrification and NH_2OH pathways should be the contributors to N_2O generation by AOB. The two alternate pathways are likely differently affected by dissolved oxygen concentrations. In the case of the current paper, none of the four models can solely describe all the experimental data in the three case studies, as they have been proposed based on different N_2O production pathways. Combining a nitrifier denitrification production term with a term to predict N_2O production by NH_2OH oxidation is necessary to explain the experimental results extensively. Hence, the combined version of these models based on the two different N_2O production pathways is required to predict the dynamic changes in N_2O production accurately. This new model structure is the most complex but this was required to accurately capture N_2O concentrations resulting from different production mechanisms.

While complex, this new model may also be necessary to capture the relevant N_2O production mechanisms across a wide range of wastewater treatment process conditions. For instance, the NH_4^+ concentration, a key parameter in all nitrifying systems, is known to influence the concentration and dynamics of N_2O (Yang et al., 2009; Butler et al., 2009; Law et al., 2012). The total concentration of N_2O tends to increase with increasing DO at extremely high NH_4^+ concentration (e.g., 400 mg N L^{-1} in case 3) (Law et al., 2012) and decrease with increasing DO at low NH_4^+ concentration (e.g., 40-60 mg N L^{-1} in cases 1 and 2) (Yang et al., 2009; Ni et al., 2011). These differences suggest that production of N_2O by the AOB denitrification may be more important at lower NH_4^+ concentration while production by the NH_2OH oxidation pathway could dominate at much higher NH_4^+ concentration. Further research on the relative importance of the mechanisms of N_2O production under various process conditions could provide a more detailed overview of the physiology of N_2O production by AOB, and provide insight into how to appropriately capture the production of AOB with a more comprehensive mathematical model. One possibility/hypothesis is that the intracellular concentration of reducing power or electrons in the system may lead to a kinetic competition between the two pathways.

Furthermore, while the dynamic reactor data and batch experiments presented here provide strong evidence of the importance of the N_2O production mechanisms evaluated, additional research to determine model parameters describing the production N_2O is required as model parameter values (Appendix 2) vary across the examined systems, although they are, consistently, within a decal range.

CONCLUSION

In this work, four mathematical model structures to describe two distinct mechanisms of N_2O production by AOB were compared based on their ability to predict N_2O dynamics observed in three mixed culture studies. The modelling results demonstrate that almost all of these models can generally describe the NH_4^+ , NO_2^- and NO_3^- data. However, none of these models were able to reproduce all measured N_2O data. The N_2O production observed in Cases 1 and 2 could be described well by the AOB denitrification pathway, especially by Model I. However, neither Model I nor Model II, both based on the AOB denitrification pathway, could predict the N_2O data in Case 3. While Models III and IV could describe the N_2O data in Case 3, they failed to predict the dependency of N_2O production on DO observed in Case 1.

The results suggest that both the denitrification and NH_2OH pathways may be involved in N_2O production. Therefore, a unified model used to predict N_2O production should provide the ability to capture both mechanisms independently. The intracellular concentration of reducing power or electrons in the system may possibly lead to a kinetic competition between the two pathways.

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