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Evaluating Four Mathematical Models for Nitrous Oxide Production by Autotrophic Ammonia-Oxidizing Bacteria

Bing-Jie Ni,¹ Zhiguo Yuan,¹ Kartik Chandran,² Peter A. Vanrolleghem,³ Sudhir Murthy⁴

¹Advanced Water Management Centre, The University of Queensland, St. Lucia,

Queensland 4072, Australia; telephone: 61-7-3365-4374; fax 61-7-3365-4726;

e-mail: zhiguo@awmc.uq.edu.au

²Department of Earth and Environmental Engineering, Columbia University, New York, New York 10027

³modelEAU, Département de génie civil et de génie des eaux, Université Laval, 1065,

Avenue de la Médecine, Québec, Canada QC G1V 0A6

⁴DC Water and Sewer Authority, 5000 Overlook Ave., SW, Washington,

District of Columbia 20032

ABSTRACT: There is increasing evidence showing that ammonia-oxidizing bacteria (AOB) are major contributors to N2O emissions from wastewater treatment plants (WWTPs). Although the fundamental metabolic pathways for N₂O production by AOB are now coming to light, the mechanisms responsible for N2O production by AOB in WWTP are not fully understood. Mathematical modeling provides a means for testing hypotheses related to mechanisms and triggers for N2O emissions in WWTP, 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. The production mechanisms evaluated are (1) N_2O as the final product of nitrifier denitrification with NO₂⁻ as the terminal electron acceptor and (2) N₂O as a byproduct of 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 effects of (1) NH_{4}^{+} concentration variations, (2) dissolved oxygen (DO) variations, (3) $NO_2^$ accumulations and (4) NH₂OH as an externally provided substrate. The modeling results demonstrate that all these models can generally describe the NH₄⁺, NO₂⁻, and NO₃⁻

Correspondence to: Z. Yuan Contract grant sponsor: University of Queensland Contract grant sponsor: UQ Early Career Researcher Contract grant sponsor: Australia Research Council Contract grant numbers: DP0987204, LP0991765 Contract grant sponsor: National Science Foundation CAREER Contract grant sponsor: Water Environment Research Foundation Received 20 April 2012; Revision received 5 July 2012; Accepted 16 July 2012 Accepted manuscript online 25 July 2012; Article first published online 10 August 2012 in Wiley Online Library (http://onlinelibrary.wiley.com/doi/10.1002/bit.24620/abstract) DOI 10.1002/bit.24620 data. However, none of these models were able to reproduce all measured N_2O data. The results suggest that both the denitrification and NH_2OH pathways may be involved in N_2O production and could be kinetically linked by a competition for intracellular reducing equivalents. A unified model capturing both mechanisms and their potential interactions needs to be developed with consideration of physiological complexity.

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Introduction

Nitrous oxide (N_2O) not only is a greenhouse gas, with an approximately 300-fold stronger warming effect than carbon dioxide (IPCC, 2011), but also reacts with ozone in the stratosphere leading to ozone layer depletion (Portmann et al., 2012). It can be produced and directly emitted from wastewater treatment systems (Ahn et al., 2010a; Foley et al., 2010). N₂O is produced during biological nitrogen removal, typically attributed to autotrophic ammonia-oxidizing bacteria (AOB) (Kampschreur et al., 2009; Tallec et al., 2006) and heterotrophic denitrifying organisms (Kampschreur et al., 2009; Lu and Chandran, 2010). The N₂O emission data collected from wastewater treatment plants (WWTPs) to date show a huge variation in the fraction of influent nitrogen load emitted as N₂O (emission factor) in the range of 0.01–1.8%, and in some

cases even higher than 10% (Ahn et al., 2010a,b; Foley et al., 2010; Kampschreur et al., 2009; Wang et al., 2011). A high degree of temporal variability in N₂O emission has also been observed within the same WWTP (Ahn et al., 2010a). These variabilities in measured emissions strongly contrast with the fixed emission factors currently applied to estimate N2O emissions from wastewater treatment (EPA, 2012; IPCC, 2006). A major problem with the use of fixed emission factors is that the link between emissions and process configurations and operating characteristics is not considered. As such, the estimates do not account for the variety of process conditions encountered in the field. Mathematical modeling should be a more appropriate method for estimating site-specific emissions of N₂O (CH2MHill, 2008; Corominas et al., 2012; Ni et al., 2011).

 N_2O production by heterotrophic denitrification is well understood. N_2O is an intermediate in heterotrophic denitrification. A model describing this process has been presented by Hiatt and Grady (2008), although improvement is still needed (Pan et al., 2012). 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 (Ahn et al., 2011; Foley et al., 2010; Kampschreur et al., 2008a,b; Kim et al., 2010; Schreiber et al., 2009; Yang et al., 2009; Yu et al., 2010). This is mainly due to an incomplete understanding of the mechanisms involved in N_2O production by AOB.

Chemolithoautotrophic AOB oxidize NH₄⁻ to NO₂⁻ via hydroxylamine (NH₂OH) as their predominant energygenerating process (Hooper et al., 1997). The first step is catalyzed by ammonia monooxygenase (AMO) where NH₃ is oxidized to NH₂OH with the reduction of molecular oxygen (O_2) (Arp et al., 2007). In the second step, NH₂OH is oxidized to NO_2^- by hydroxylamine oxidoreductase (HAO) with O₂ as the main terminal electron acceptor (Hooper et al., 1997). It has been proposed that N₂O can be produced during NH₂OH oxidation through two pathways. One is that N₂O is formed as a byproduct of incomplete oxidation of NH₂OH to NO₂⁻ through chemical decomposition of nitroxyl radical (NOH) or biological reduction of NO (Arp and Stein, 2003; Stein, 2011a,b), both of which could form as intermediates during the oxidation of NH₂OH. AOB are also capable of utilizing nitrite (NO_2^-) and subsequently nitric oxide (NO) as alternative electron acceptors in addition to O₂, producing N₂O as the end product through the nitrifier denitrification pathway (Kim et al., 2010; Yu et al., 2010).

The current study attempts to differentiate between these different mechanisms of N_2O production with the use of mathematical models. To date, several mechanistic models have been proposed for N_2O production by AOB. Four different structures used in the mathematical modeling 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



Figure 1. Reaction schemes used in the four N₂O models evaluated in this study—(A) Model I: AOB denitrification pathway with NH₂OH as the electron donor; (B) Model II: AOB denitrification pathway with NH₃ as the electron donor; (C) Model III: the NH₂OH/NOH pathway; and (D) Model IV: the NH₂OH/NO pathway. Schematics adapted from Stein (2011a) and Chandran et al. (2011).

difference between these 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 modeled 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 N₂O production is due to the chemical decomposition of the unstable NOH, an intermediate of NH₂OH oxidation (Law et al., 2012), while Model IV assumes reduction of NO, produced from the oxidation of NH₂OH, into N₂O. The key reactions considered by these models are summarized in Figure 1. However, the direct side-by-side comparison of these models with identical data sets has never been done before, although it is highly important to know what these models can and cannot predict, when compared with existing data reported for different systems and under different conditions.

Therefore, the ability of these model structures to describe the experimental N_2O production data reported in literature resulting from batch experiments using three different mixed nitrifying cultures are examined in the article. The purpose is to understand how the mathematical structures of the four models presented in Figure 1 influence their ability to reproduce the experimental data, thus revealing the strengths and limitations of each of these models and the pathways upon which they are built. The findings would guide both future experimental studies aimed to achieve a full understanding of N_2O production by AOB, and modeling efforts aimed to develop practically useful models.

Materials and Methods

Mechanistic Models for N₂O Production

The kinetic and stoichiometric matrices for the four mathematical models are provided in Table I. Nomenclature for all state variables is chosen to highlight similarities and differences between these models, and differs slightly from the original publications. All four models employ the following nomenclature for concentration: ammonium oxidizing organisms (X_{AOB}) , nitrite oxidizing organisms (X_{NOB}) , NH⁺₄ (S_{NH_4}) , NH₂OH $(S_{\text{NH}_2\text{OH}})$, NOH (S_{NOH}) , $NO_{2}^{-}(S_{NO_{2}}), NO_{3}^{-}(S_{NO_{3}}), NO(S_{NO}), N_{2}O(S_{N_{2}O}), N_{2}(S_{N_{2}}),$ and DO (S_{O_2}) . Other biological processes, such as endogenous decay and aerobic NO₂⁻ oxidation by NOB, are included in all four models with standard ASM kinetic expressions and parameters values taken from the published literature (Henze et al., 2000; Koch et al., 2000). Table II lists the definitions, values, and units of the parameters used in the four models.

In Model I (Table I, Ni et al., 2011), AOB denitrification with NO_2^- as the terminal electron acceptor produces NO and subsequently N₂O by consuming NH₂OH as the electron donor. Similarly, in Model II (Table I, Mampaey et al., 2011), AOB denitrification occurs in parallel with ammonium oxidation, reducing NO_2^- to NO and then to N₂O with ammonium as the electron donor. In addition, after personal communication with the authors of Model II, we incorporated an anoxic correction factor into the Monod-expression describing AOB denitrification in Model II to describe a lower specific ammonium utilization rate of AOB when using NO₂⁻ and NO as electron acceptors instead of O₂ (Table I). This anoxic correction factor was not built into the original model reported in Mampaey et al. (2011). In Model III (Table I, Law et al., 2012), it is assumed that the chemical decomposition of NOH complies with first order kinetics. Model IV (Table I, formulated in this study) assumed that DO has no inhibitory effect on NO reduction (Yu et al., 2010), as in Model II.

Testing the Predictive Abilities of the Models

Experimental data from three case studies (Kim et al., 2010; Law et al., 2012; Yang et al., 2009) 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

Table I. Process matrices for the four N₂O models evaluated in this study.

	Model components								
Process	S_{O_2}	$S_{\rm NH_4}$	$S_{\rm NH_2OH}$	S _{NOH}	$S_{\rm NO_2}$	$S_{\rm NO}$	S_{N_2O}	X _{AOB}	Kinetic rate expressions
Model I	—AOB deni	trification path	way (Ni	et al., 2	2011)				
I-1	-1.14	-1	1						$R1 = \mu_{AOPANO} \frac{s_{O_2}}{s_{NH_4}} \frac{s_{NH_4}}{s_{NH_4}} X_{AOP}$
I-2	$-\frac{2.29-Y_{AOB}}{Y_{AOB}}$	i _{N_AOB}	$-\frac{1}{Y_{AOB}}$		$\frac{1}{Y_{AOB}}$			1	$R2 = \mu_{\text{AOB,HAO}} \frac{S_{\text{S}_{1},\text{O}_{2},\text{AOB}+\text{S}_{2}}}{K_{\text{S}_{1},\text{O}_{2},\text{AOB}+\text{S}_{2}}} \frac{S_{\text{NH}_{4},\text{AOB}+\text{S}_{\text{NH}_{4}}}}{S_{\text{NH}_{2},\text{OH}}} X_{\text{AOB}}$
I-3			$^{-1}$		-3	4			$R3 = \mu_{AOB,HAO} \eta_{\mu AOB,AX} \frac{K_{I,O_2-AOB}}{S_{O_2} + K_{I,O_2-AOB}} \frac{S_{NO_2}}{K_{NO_2-AOB} + S_{NO_2}} \frac{S_{NH_2OH}}{K_{NH_2OH} + AOB + S_{NH_2OH}} X_{AOB}$
I-4			-1		1	-4	4		$R4 = \mu_{\text{AOB,HAO}} \eta_{\mu\text{AOB,AX}} \frac{\kappa_{I,02,\text{AOB}}}{\varsigma_{0} + \kappa_{I,02,\text{AOB}}} \frac{s_{N0}}{\kappa_{N0,\text{AOB}} + s_{N0}} \frac{s_{N120\text{H}}}{\kappa_{N14,\text{OH,AOB}} + s_{N14,\text{OH}}} X_{\text{AOB}}$
Model I	AOB den	itrification path	nway (Ma	ampaey	et al., 2	2011)			
II-1	$-\frac{3.43-Y_{AOB}}{Y_{AOB}}$	$-\frac{1}{Y_{AOB}}-i_{N_AOB}$			$\frac{1}{Y_{AOB}}$			1	$R1 = \mu_{AOB} \frac{S_{O_2}}{K_{O_2,AOB} + S_{O_2}} \frac{S_{NH_4}}{K_{NH_4,AOB} + S_{NH_4}} X_{AOB}$
II-2	$-\frac{2.29-Y_{AOB}}{Y_{AOB}}$	$-\frac{1}{Y_{AOB}}-i_{N_AOB}$			$-\frac{1}{Y_{AOB}}$	$\frac{2}{Y_{AOB}}$		1	$R2 = \mu_{AOB} \eta_{\mu AOB,Ax} \frac{S_{O_2}}{K_{O_2,AOB} + S_{O_2}} \frac{S_{NH_4}}{K_{NH_4,AOB} + S_{NH_4}} \frac{S_{NO_2}}{K_{NO_2,AOB} + S_{NO_2}} X_{AOB}$
II-3	$-\frac{2.29-Y_{AOB}}{Y_{AOB}}$	$-\frac{1}{Y_{AOB}}-i_{N_AOB}$			$\frac{1}{Y_{AOB}}$	$-\frac{2}{Y_{AOB}}$	$\frac{2}{Y_{AOB}}$	1	$R3 = \mu_{AOB}\eta_{\mu AOB,AX} \frac{S_{O_2}}{K_{O_2}} \frac{S_{NH_4}}{K_{O_1}} \frac{S_{NH_4}}{K_{N_1}} \frac{S_{NO}}{K_{NO}} X_{AOB}$
Model II	I—NH ₂ OH	/NOH pathway	(Law et	al., 20	12)				
III-1	-1^{-1}	-1	1						$R1 = q_{AOB,1,max} \frac{S_{O_2}}{K_{S_1,O_2-AOB}+S_{O_2}} \frac{S_{NH_4}}{K_{NH_4,AOB}+S_{NH_4}} X_{AOB}$ (when
									$R1 \le R2 + R3$; $R1 = R2 + R3$ (when $R1 > R2 + R3$)
III-2			$^{-1}$	1					$R2 = q_{\text{AOB},2,\text{max}} \frac{S_{\text{O}_2}}{K_{\text{S}_2,\text{O}_2} - \text{AOB} + S_{\text{O}_2}} \frac{S_{\text{NH}_2\text{OH}}}{K_{\text{NH}_2\text{OH},\text{AOB}} + S_{\text{NH}_2\text{OH}}} X_{\text{AOB}}$
III-3				-1	1				$R3 = q_{\text{AOB},3,\text{max}} \frac{S_{\text{O}_2}}{K_{\text{S}_3,\text{O}_2-\text{AOB}} + S_{\text{O}_2}} \frac{S_{\text{NOH}}}{K_{\text{NOH},\text{AOB}} + S_{\text{NOH}}} X_{\text{AOB}}$
III-4				$^{-1}$			$\frac{1}{2}$		$R4 = q_{AOB,4,max}S_{NOH}$
III-5	-1						_		$R5 = \frac{1}{2}(R2 + R3 - R1)$
Model I	V—NH ₂ OH	/NO pathway (†	this stud	y)					2
IV-1	-1.14	-1	1						$R1 = \mu_{\text{AOB,AMO}} \frac{S_{\text{O2}}}{K_{\text{S}_{1},\text{O2}},\text{AOB}+S_{\text{O2}}} \frac{S_{\text{NH}_{4}}}{K_{\text{NH}_{4},\text{AOB}}+S_{\text{NH}_{4}}} X_{\text{AOB}}$
IV-2	$-\frac{1.71-Y_{AOB}}{Y_{AOB}}$	$i_{\rm N_AOB}$	$-\frac{1}{Y_{AOB}}$			$\frac{1}{Y_{AOB}}$		1	$R2 = \mu_{AOB,HAO} \frac{S_{O_2}}{K_{S_2,O_2-AOB} + S_{O_1}} \frac{S_{NH_2OH}}{K_{NH_3OH,AOB} + S_{NH_3OH}} X_{AOB}$
IV-3	$-\frac{0.57-Y_{AOB}}{Y_{AOB}}$	$i_{ m N_AOB}$			$\frac{1}{Y_{AOB}}$	$-\frac{1}{Y_{AOB}}$		1	$R3 = \mu_{AOB,HAO} \frac{S_{O_2}}{K_{S_1,O_2}} \frac{S_{NO}}{AOB + S_{O_2}} \frac{S_{NO}}{K_{NO} AOB + S_{NO}} X_{AOB}$
IV-4			-1		1	-4	4		$R4 = \mu_{\text{AOB,HAO}} \eta_{\mu\text{AOB,AX}} \frac{S_{\text{NO}}}{K_{\text{NO,AOB}} + S_{\text{NO}}} \frac{S_{\text{NH}_2\text{OH}}}{K_{\text{NH}_2\text{OH,AOB}} + S_{\text{NH}_2\text{OH}}} X_{\text{AOB}}$

For explanation of processes see Figure 1.

Table II. B	Best-fit parameters	of the four models	(I-IV)) describing	N_2O	production i	in three	different	nitrification	case studies
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Parameter	Definition	Case 1	Case 2	Case 3	Source		
Model I—AOB d	enitrification pathway						
Y_{AOB}	Yield coefficient for AOB $g \text{COD} g^{-1} N$	0.150	0.150	0.150	Ni et al. (2011)		
i _{N AOB}	Nitrogen content of biomass $(gNg^{-1}COD)$	0.07	0.07	0.07	Ni et al. (2011)		
$\mu_{AOB,AMO}$	Maximum AMO-mediated reaction rate (h ⁻¹)	0.122	0.074	0.115	Estimated		
$\mu_{AOB,HAO}$	Maximum HAO-mediated reaction rate (h^{-1})	0.092	0.183	0.134	Estimated		
K_{S_1,O_2} _AOB	S_{O_2} affinity constant for S_{NH_4} oxidation (g DO m ⁻³)	0.043	0.031	0.051	Estimated		
K_{S_2,O_2} _AOB	S_{O_2} affinity constant for S_{NH_2OH} oxidation (g DO m ⁻³)	0.6	0.6	0.6	Ni et al. (2011)		
K _{I.O₂_AOB}	S_{O_2} substrate inhibition parameter (g DO m ⁻³)	0.112	0.088	0.107	Estimated		
$\eta_{\mu AOBAX}$	Anoxic reduction factor	0.074	0.092	0.071	Estimated		
K _{NH4.AOB}	$S_{\rm NH_4}$ affinity constant for AOB, g N m ⁻³	2.4	2.4	2.4	Ni et al. (2011)		
K _{NH2OH.AOB}	$S_{\rm NH_2OH}$ affinity constant for AOB (g N m ⁻³)	2.4	2.4	2.4	Ni et al. (2011)		
K _{NO2} AOB	S_{NO_2} affinity constant for AOB (g N m ⁻³)	0.14	0.14	0.14	Ni et al. (2011)		
K _{NO AOB}	$S_{\rm NO}$ affinity constant for AOB (g N m ⁻³)	0.0084	0.0084	0.0084	Ni et al. (2011)		
Model II—AOB o	denitrification pathway						
Y _{AOB}	Yield coefficient for AOB $(g \text{COD} g^{-1} \text{N})$	0.150	0.150	0.150	Mampaey et al. (2011)		
i _{N AOB}	Nitrogen content of biomass $(gNg^{-1}COD)$	0.07	0.07	0.07	Mampaey et al. (2011)		
μ_{AOB}	Maximum AOB growth rate (h^{-1})	0.076	0.029	0.0431	Estimated		
$\eta_{\mu m AOB,Ax}$	Anoxic reduction factor	0.72	0.083	0.006	Estimated		
$K_{\rm NH_4,AOB}$	$S_{\rm NH_4}$ affinity constant for AOB (g N m ⁻³)	1.0	1.0	1.0	Mampaey et al. (2011)		
K _{NO,AOB}	$S_{\rm NO}$ affinity constant for AOB (g DO m ⁻³)	3.91	0.18	2.35	Estimated		
$K_{\rm NO_2,AOB}$	$S_{\rm NO_2}$ affinity constant for AOB (g N m ⁻³)	8.0	8.0	8.0	Mampaey et al. (2011)		
$K_{O_2,AOB}$	S_{O_2} affinity constant for AOB (g DO m ⁻³)	0.13	1.05	2.13	Estimated		
Model III—NH ₂ O	DH/NOH pathway						
qAOB,1,max	Specific maximum rate of $R1 \pmod{g^{-1} VSS h^{-1}}$	14.75	14.75	14.75	Law et al. (2012)		
qAOB,2,max	Specific maximum rate of R2 (mmol g^{-1} VSS h^{-1})	14.98	7.75	22.86	Estimated		
qAOB,3,max	Specific maximum rate of R3 $(\text{mmol g}^{-1}\text{VSS h}^{-1})$	4.42	5.52	13.42	Estimated		
qAOB,4,max	Maximum reaction rate of R4 (mmol mmol ^{-1} h ^{-1})	0.31	0.26	0.79	Estimated		
$K_{\rm NH_4,AOB}$	$S_{\rm NH_4}$ affinity constant for AOB (mmol L ⁻¹)	0.17	0.17	0.17	Law et al. (2012)		
K _{NH2OH,AOB}	$S_{\rm NH_2OH}$ affinity constant for AOB (mmol L ⁻¹)	0.05	0.05	0.05	Law et al. (2012)		
K _{NOH.AOB}	S_{NOH} affinity constant for AOB (mmol L ⁻¹)	0.05	0.05	0.05	Law et al. (2012)		
K_{S_1,O_2} _AOB	S_{O_2} affinity constant for R1 (mmol L ⁻¹)	0.019	0.019	0.019	Law et al. (2012)		
K_{S_2,O_2} _AOB	S_{O_2} affinity constant for R2 (mmol L ⁻¹)	0.0019	0.0019	0.0019	Law et al. (2012)		
K_{S_3,O_2} _AOB	S_{O_2} affinity constant for R3 (mmol L ⁻¹)	0.0019	0.0019	0.0019	Law et al. (2012)		
Model IV-NH ₂ O	DH/NO pathway						
Y_{AOB}	Yield coefficient for AOB $(g \text{COD} g^{-1} \text{N})$	0.150	0.150	0.150	Wiesmann (1994)		
i _{N AOB}	Nitrogen content of biomass $(gNg^{-1}COD)$	0.07	0.07	0.07	Henze et al. (2000)		
$\mu_{AOB,AMO}$	Maximum AMO-mediated reaction rate (h ⁻¹)	0.145	0.09	0.205	Estimated		
$\mu_{AOB,HAO}$	Maximum HAO-mediated reaction rate (h^{-1})	0.075	0.24	0.065	Estimated		
K_{S_1,O_2} _AOB	S_{O_2} affinity constant for S_{NH_4} oxidation (g DO m ⁻³)	0.043	0.021	0.4	Estimated		
K_{S_2,O_2} _AOB	S_{O_2} affinity constant for S_{NH_2OH} oxidation (g DO m ⁻³)	0.6	0.43	0.056	Estimated		
$\eta_{\mu AOBAX}$	Anoxic reduction factor	0.45	0.38	0.23	Estimated		
$K_{\rm NH_4,AOB}$	$S_{\rm NH_4}$ affinity constant for AOB (g N m ⁻³)	2.4	2.4	2.4	Wiesmann (1994)		
K _{NH2OH.AOB}	$S_{\rm NH_2OH}$ affinity constant for AOB (g N m ⁻³)	2.4	2.4	2.4	Ni et al. (2011)		
K _{NO,AOB}	$S_{\rm NO}$ affinity constant for AOB (g N m ⁻³)	0.0084	0.0084	0.0084	Schreiber et al. (2009)		

wastewater (Case 1). Five nitritation batch experiments were carried out at different controlled DO levels, varying from 0.5 to 2.5 g O_2 m⁻³. The initial NH₄⁺ concentrations for all the five tests were around 35 mg N L⁻¹. Kim et al. (2010) examined N₂O production by AOB in an activated sludge system treating swine wastewater (Case 2). Two nitrification batch experiments were performed with the enriched nitrifying sludge by using NH₄⁺ (5 mM) and NH₂OH (5 mM) as the externally provided substrate, respectively. Law et al. (2012) investigated the correlation between the ammonia oxidation rate and N₂O production rate of an enriched AOB culture treating anaerobic sludge digestion liquor (Case 3). Experiments were carried out both directly in the parent sequencing batch reactor (SBR) and in a batch reactor, during which the ammonia oxidation rate was varied by adjusting the DO level and the initial $\rm NH_4^+$ concentration. The total $\rm NO_2^-$ concentration in all tests was 500 \pm 50 mg N/L.

Given the very different culturing and experimental conditions used in these three studies, the four models are tested for a wide range of systems with different reactor types (SBR vs. batch reactor), feed compositions (NH_4^+ vs. NH_2OH as the electron donor), biomass types (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 rates during the nitrification processes.

Parameter estimations were performed using AQUASIM (Reichert, 1998). Parameter values estimated in the current study are provided in Table II, together with other parameter values used in the model simulations. Not all parameters were identifiable from the experimental data due to parameter correlation. Hence, our methodology has been to use typical parameter values reported in literature for nitrification processes including N2O production kinetics, whenever possible (see Table II). We only estimated those parameters, which are unique to each model ($\mu_{AOB,AMO}$, $\mu_{AOB,HAO}$, K_{S_1,O_2} _AOB, K_{I,O_2} _AOB, and $\eta_{\mu AOB,Ax}$ in Model I; μ_{AOB} , $K_{NO,AOB}$, $K_{O_2,AOB}$, and $\eta_{\mu AOB,Ax}$ in Model II; qAOB,2,max, qAOB,3,max, and qAOB,4,max in Model III; and $\mu_{AOB,AMO}$, $\mu_{AOB,HAO}$, K_{S_1,O_2_AOB} , K_{S_2,O_2_AOB} , and $\eta_{\mu AOB,Ax}$ in Model IV), by fitting model predictions with the experimental data presented in the case studies. It should be highlighted that the aim of the modeling work is to verify if various model structures (rather than parameter calibrations) could explain the trend of the experimentally observed N₂O production, because having a solid model structure is a key step towards reliable prediction of N₂O emissions.

Results

Figures 2–5 show the evaluation results of the four mathematical models against the experimental data in the three case studies, respectively. Below, we comment on the model evaluation results for Case 1 in detail to illustrate the ability of the four mathematical model structures to describe the Case 1 data. The remaining model evaluations (Cases 2–3) are briefly described, highlighting the differences from Case 1.

Evaluation of Mathematical Models: Case 1

Here, the four mathematical models in Figure 1 are evaluated based on their ability to capture the observed N₂O production results in the experiments of Yang et al. (2009), which are presented in Figure 2. An individual nitritation batch experiment at $DO = 1.5 \text{ g} O_2 \text{ m}^{-3}$ (left column of Fig. 2) was used to obtain kinetic parameter values associated with nitrification including N₂O production (Table II). The models and the calibrated parameter sets (Table II) were then used to predict the relationship between DO concentrations and the total amount of N₂O production in each of the five batch tests reported by Yang et al. (2009) (right column of Fig. 2).

For Model I (Fig. 2A–C), agreement between simulated and experimental results was good ($R^2 = 0.9247$) for all fitted variables in all experiments. The N₂O emission rate increased with the decrease of NH₄⁺ concentration and the



increase of NO₂⁻, and then decreased after NH₄⁺ was consumed to become lower than 5 mgN L⁻¹ (Fig. 2A). The total cumulative N₂O production was highly dependent on the DO concentration. As shown in Figure 2B, the cumulative N₂O production initially increased and then decreased with the increase of DO, peaking at DO = $1.5 \text{ g O}_2 \text{ m}^{-3}$. 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 production with the increase of DO were captured in the mathematical model by the oxygen inhibition term on nitrite reduction with a low K_{I,O_2-AOB} value of 0.112 g DO m⁻³ (Fig. 2B).

For Model II, the model-predicted variation of the N components in the nitritation experiments at DO = 1.5 g O_2 m⁻³ is compared to the experimental data in Figure 2C, while the model output for the effect of DO concentration on N₂O production is compared to the experimental data in Figure 2D. The predicted profiles shown in Figure 2C match

the observed experimental trends very well ($R^2 = 0.9065$). The kinetic structure within Model II that the N₂O production is dependent on the NH₄⁺ concentration forces the N₂O concentration to follow changes in the NH₄⁺ concentration (Fig. 2C). However, the N₂O prediction predicted by this model structure does not approximate the experimental observations in Figure 2D, although Model II assumed the same N₂O production pathway (AOB denitrification) as used by Model I. This was caused by the absence of oxygen inhibition on AOB denitrification in Model II.

Models III and IV adopted the other N₂O production pathway (incomplete oxidation of NH₂OH), which is different from that of Models I and II (Table I). The nitritation experiments and the effect of DO concentration predicted by Models III and IV are presented in Figure 2E– H. Although the measured N concentration profiles in the nitritation batch test at DO = $1.5 \text{ g O}_2 \text{ m}^{-3}$ are predicted reasonably well ($R^2 = 0.8509$, Fig. 2E and $R^2 = 0.8295$, Fig. 2G), these two models are not able to capture the observed dependency of N₂O production on DO concentration (Fig. 2F and H). Contrasting to the experimental data, the predicted N₂O production increased with increasing DO in the entire DO range studied (Fig. 2F and H).

In summary, Model I produced the best description of the experimental data of this case study, especially the observed trend of decrease in N_2O production at high DO concentrations.

NH₄⁺ Versus NH₂OH as Substrate: Case 2

In the second case study, N_2O production by a nitrifying enrichment was monitored in batch reactors using NH_4^+ and NH_2OH as the externally supplied substrate, respectively (Kim et al., 2010). The four mathematical models were evaluated to test their ability to describe the N_2O dynamics observed in these experiments.

Figure 3A shows that the measured NH_4^+ , N_2O , NO_2^- , and NO_3^- data in the experiment with NH_4^+ as substrate is very well described by Model I ($R^2 = 0.9117$). The N_2O production rate increased with NO_2^- accumulation, but rapidly ceased when NH_4^+ was depleted (Fig. 3A). This correlation is well captured by Model I. With NH_2OH as the substrate (Fig. 3B), the N_2O production rates were significantly higher in comparison to the case with NH_4^+ as the substrate (Fig. 3B). When NH_2OH is employed instead of NH_4^+ , the absence of the AMO activity (oxidation of NH_3 to NH_2OH oxidation) makes more electrons gained from NH_2OH oxidation available for respiration (Chandran and Smets, 2008), leading to increased N_2O production. Model I captured this feature very well.

In comparison, Model II could not describe well the N_2O peak observed at the highest NO_2^- accumulation. According to Model II, NH_3 rather than NH_2OH is the direct electron donor and nitrite is the terminal electron acceptor for AOB denitrification. The dependency of



Figure 3. Experimental results (symbols) and model predictions (lines) for N₂O productions in nitritation batch test with NH_4^+ (left) or NH_2OH (right) as substrate in Case 2 (Kim et al., 2010): (A) and (B) Model-I evaluations; (C) and (D) Model-II evaluations; (E) and (F) Model-III evaluations; and (G) and (H) Model-IV evaluations.

the rate of AOB denitrification on the NH_4^+ and $NO_2^$ concentrations is modeled using Monod kinetics. Therefore, the AOB denitrification rate and hence the N₂O production rate peaked when both NH₄⁺ and NO₂⁻ concentrations were relatively high. The fact that the Model II-predicted peak appeared ahead of the experimentally observed peak (Fig. 3C), while Model I was able to correctly predict this peak, suggests that the N2O peak is likely related to the dynamics of NH₂OH. According to the Model I prediction, NH₂OH, the true electron donor for AOB denitrification reached a constant level soon after the commencement of NH_{4}^{+} oxidation, and remained at a relatively constant level until the depletion of NH₄⁺. This has led to the cooccurrence of the NO_2^- and N_2O peaks. The above comparison further suggests that it is important to consider NH₂OH as an intermediate of NH₄⁺ oxidation in N₂O modeling. Furthermore, Model II is not able to describe the N₂O dynamics by using NH₂OH as the externally provided substrate, as the production and consumption of NH_2OH is not considered in Model II (Table I).

In Models III and IV, the NO₂⁻ and NO₃⁻ concentrations increased with ammonium oxidation, giving a good fit $(R^2 = 0.8991)$ with these experimental profiles. However, the description of the N₂O production is not accurate. In the predictions of Models III and IV (Fig. 3E-H), the production of N₂O during NH_4^+ oxidation quickly leads to a linear increase of the N2O concentration, which is related to the constant 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. With NH₂OH as the substrate (Fig. 3F and H), the predictions of Models III and IV for NO₃⁻ were higher than experimental observations, although Models III and IV could roughly describe the N₂O production data. This is due to the fact that nitrite would be oxidized to nitrate other than reduced to N₂O in Models III and IV because N₂O production is only related to incomplete NH₂OH oxidation, not AOB denitrification.

In our opinion, Model I, based on the AOB denitrification pathway and with NH₂OH considered in the model structure, enables the best description of the experimental data in this case study.

N_2O Production Rate Versus Ammonium Oxidation Rate: Case 3

In this case study, N₂O production by an enriched AOB culture was investigated in both SBR operation and extensive batch experiments with a step-wise change in DO concentration (Law et al., 2012). Figure 4C shows that the measured NH₄⁺, NO₂⁻, and NO₃⁻ data are very well described by Model I ($R^2 = 0.9744$). However, Model I predicted a stepwise increase in the N₂O production rate with a stepwise decrease in DO concentration (Fig. 4B), which is opposite to the experimental observation.

The kinetic structure of Model II ensures that the N₂O production rate is dependent on oxygen availability, resulting in a N₂O dynamic trend similar to that shown by the experimental data (Fig. 4E). In order to reasonably predict the N₂O production rate when both the NH⁺₄ and NO⁻₂ concentrations are very high in this case study (400 and 600 mg N L⁻¹, respectively), an anoxic reduction factor ($\eta_{\mu AOB,Ax} = 0.006$) that is substantially lower than that used in the Case 1 and Case 2 studies (0.72 and 0.083, respectively) had to be employed due to the fact that the N₂O production rate is dependent on ammonium and nitrite concentrations in Model II. The use of the anoxic factors found for Cases 1 and 2 would lead to extremely high N₂O production rates in Case 3, as both the NH⁺₄ and NO⁻₂ concentrations are very high in the system.

Models III and IV were both able to reproduce all the experimental data in Figure 4G–L of this case study, which could not be reproduced by Model I based on the nitrifier denitrification pathway. The N_2O production increased/

decreased with increasing/decreasing DO concentration indicating that incomplete NH₂OH oxidation was likely the major pathway that caused the N₂O production in this nitrifying system.

In addition, the experimental data of this case study indicated a clear exponential relationship between the biomass specific N₂O production rate (N₂OR) and ammonia oxidation rate (AOR; Fig. 5). In the experimental studies, AOR and hence N₂OR were altered by independently changing the ammonium and DO concentrations in batch tests. Models I, II, and IV could not describe the experimental data (Fig. 5A, B, and D). When DO was varied to alter AOR, the relationship between N₂OR and AOR predicted by Model I exhibited a trend that is opposite to that displayed by the experimental data (Fig. 5A). In all other cases, Models I, II, and IV predicted a linear rather than an exponential relationship between N₂OR and AOR (Fig. 5A, B, and D). In contrast, Model III could predict the N₂OR and AOR relationship well (Fig. 5C).

In summary, Model III, based on the incomplete NH_2OH oxidation pathway with the assumption of N_2O being produced from the chemical decomposition of NOH, produces the best description of all experimental data in this case study.

Discussion

Validity of the Four Mathematical Models

It is well known that AOB can contribute to the formation of N_2O , an important greenhouse gas, in wastewater treatment (Foley et al., 2010; Kampschreur et al., 2009). Mathematical modeling of N_2O production is an important step towards understanding the full environmental impact of wastewater treatment. The ability and validity of models to predict N_2O production provides an opportunity to include N_2O production as an important consideration in the design and operation of biological nitrogen removal processes.

The modeling results of this work demonstrate that all four models can generally describe the NH_4^+ , NO_2^- , and NO_3^- data (Figs. 2–4).

The N₂O production observed in Cases 1 and 2 could be described well by the AOB denitrification pathway, especially by Model I. The Model II structure does not contain a term describing DO inhibition on AOB denitrification, therefore, cannot describe the experimentally observed relationship between N₂O production and DO concentration observed by Yang et al. (2009) (Fig. 2D). Similarly to Model II, Model III and Model IV could not adequately describe the Case 1 and Case 2 N₂O data.

However, Model I, based on the AOB denitrification pathway, could not predict the N_2O data in Case 3 (Figs. 4 and 5). Indeed, Model I predicted the opposite trend of the N_2O production dependency on DO experimentally observed in this case (Fig. 4B). While Models II, III, and IV



Figure 4. Experimental results (symbols) and model predictions (lines) for N_2O productions in nitritation batch tests with step changes of DO in Case 3: (A)–(C) Model-I evaluations; (D)–(F) Model-II evaluations; (G)–(I) Model-III evaluations; and (J)–(L) Model-IV evaluations.

could all reasonably describe the N_2O data in Figure 4, Models II and IV failed to predict the exponential relationship between the biomass specific AOR and N_2OR (Fig. 5). In addition, Models II, III, and IV all failed to predict the dependency of N_2O production on DO observed in Case 1 (Fig. 2).

In summary, none of the four models evaluated can solely describe all experimental data in the three case studies.

Mechanisms Responsible for N₂O Production by AOB

The inability of any of the models assessed to fully describe all N_2O data used in this study could be related to the fact

that they each have been proposed based on a single N_2O production pathway, while the N_2O production mechanisms by AOB are complex and likely involve multiple production pathways operational under different conditions (Arp and Stein, 2003; Chandran et al., 2011; Stein, 2011a).

The model evaluation results in this work suggest that both the AOB denitrification and NH_2OH pathways could be involved in N_2O production, as summarized in Chandran et al. (2011). The two alternate pathways are likely differently affected by DO concentrations.

AOB denitrification has been identified as the predominant N_2O producing pathway in many mixed



Figure 5. The experimentally observed (symbols) and model-predicted (lines) relation between the specific N_2O production rate and the specific ammonia oxidation rate in Case 3: (A) Model-I evaluations; (B) Model-II evaluations; (C) Model-III evaluations; and (D) Model-IV evaluations. The change of specific ammonia oxidation rate was simulated by varying DO (thin line) and ammonium concentrations (thick line).

and enriched nitrifying cultures, as demonstrated in Cases 1 and 2 (Kim et al., 2010; Yang et al., 2009), which were both described well by the Model I based on AOB denitrification pathway. In Case 3, 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 the lowest DO supply (Fig. 4H). This response of the N₂O production rate to DO concentration coupled with the Models III and IV predictions strongly suggest that the enriched AOB culture used in Case 3 likely produced N₂O through the NH₂OH oxidation pathway. Case 3 was an exception to Cases 1 and 2, as it represented high ammonium and nitrite conditions that may have provided conditions of abiotic reactions. It may be possible that the conditions of high levels (approximately 500 mg N L^{-1}) of NH₄⁺ and NO₂⁻ in Case 3 inhibited N₂O production by the AOB denitrification pathway.

Unified Model for N₂O Production by AOB

By comparing modeling results of Models I–IV, combining a nitrifier denitrification production term with a term to predict N₂O production by incomplete NH₂OH oxidation is likely necessary in a unified model to explain all the experimental results resulting from different culturing and operational conditions.

When modeling the AOB denitrification pathway for N₂O production, NH₂OH, instead of NH₄⁺, should be the electron donor substrate for AOB denitrification. A substrate inhibition function for O_2 in NO_2^- reduction should be included to describe N₂O production by AOB denitrification at low DO concentrations. It should be noted that Model I assumed that NO reduction in AOB denitrification was also inhibited by O2. However, it cannot be conclusively said that NO reduction is indeed inhibited by O₂. We performed additional simulation studies using Model I by removing the DO inhibitory term in NO reduction, and obtained the same fit between modelpredicted and experimentally observed N2O data. This is because the rate-limiting step for N₂O production by AOB denitrification is NO₂⁻ reduction. Fundamentally, Yu et al. (2010) demonstrated that the expression of the NO reductase cluster (norB) for NO reduction by AOB recovered upon moving from anoxic to aerobic conditions, suggesting norB to be up-regulated by O2. The mutually distinct transcription and expression of NO₂⁻ reductase (*nirK*) and *norB* in AOB indicated that NO_2^- and NO reduction in AOB may have a different response to O₂ presence. In addition, this is also in contrast to the parallel expression of these two genes in chemoorganoheterotrophic denitrification (Yu et al., 2010). Thus, NO reduction in AOB denitrification is not similar to heterotrophic denitrification, in which O_2 and NO compete for energy synthesis.

For modeling the NH_2OH oxidation pathway, it is yet to be fully clarified if NO, NOH or indeed NH_2OH is the direct source of N_2O . Indeed, it cannot be excluded that N_2O is formed from all these compounds. The exponential relationship between N_2OR and AOR reported in Law et al. (2012) suggest the likely involvement of chemical decomposition (Law et al., 2012).

It is however unclear under what conditions each of the pathways will become the dominating pathway. A simple combination of the models based on the two N_2O production pathways would not adequately describe the shift of the dominating pathway under different conditions. A key difference between the studies reported in Yang et al. (2009) and Law et al. (2012) is the very different NH_4^+ and NO_2^- concentrations. These two parameters could have influenced the dominating pathways for N_2O production. It is possible that the high NH_4^+ and NO_2^- concentrations (i.e., approximately 500 mg N L⁻¹) (Law et al., 2012) could have stimulated the NH_2OH pathway while low NH_4^+ and NO_2^- concentration (i.e., 10–40 mg N L⁻¹ in Case 1) (Yang et al., 2009) led to the domination of the AOB denitrification pathway. Such triggers need to be understood and considered in the unified model for reliable N_2O production.

One possibility to consider such triggers is to consider the intracellular concentration of electron carriers to model the kinetic competition between the two pathways. For example, the imposition of excessive ammonia would trigger a higher ammonia oxidation rate and potentially also a higher AMO gene expression, which could in turn result in NH₂OH accumulation (Chandran et al., 2011). In response, incomplete NH₂OH oxidation to produce N₂O is likely to occur (NH₂OH oxidation pathway). During O₂-suficient conditions, it is unlikely that AOB denitrification is also induced. Upon the conversion of the accumulated NH₂OH or upon achieving a higher rate of NH₂OH conversion into NO_2^- (more electrons generation), the higher $NO_2^$ concentrations could drive AOB denitrification to produce N₂O (AOB denitrification pathway). More studies are required to further clarify on this aspect and provide more insight into how to appropriately capture the kinetic competition of different N2O production pathways by AOB within a unified model.

These proposed modeling approaches only represent our current knowledge on the N_2O production pathways by AOB. It is possible that other pathways, currently unrevealed, could also explain the data reported.

Conclusions

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 modeling results demonstrate that all 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, as they have been proposed based on different N_2O production pathways. The results suggest that both the AOB denitrification and NH_2OH pathways could be involved in N_2O production. The two alternate pathways are likely differently affected by DO concentrations. Thus, mathematical modeling of N_2O production by AOB will benefit from including both nitrifier denitrification and incomplete NH_2OH oxidation pathways. When modeling the AOB denitrification pathway for N_2O production, NH_2OH , instead of NH_4^+ , should be the electron donor substrate. A substrate inhibition function for O_2 in NO_2^- reduction should be included to describe N_2O production by AOB denitrification at low DO concentrations. The intracellular concentration of reducing equivalents in the system may possibly be employed to model the kinetic competition between the two pathways, leading to a truly unified model used to predict N_2O production under varying operational conditions.

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