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Chapter 7

Modelling N₂O Production and Emissions

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Summary

Mathematical modelling of N₂O emissions is of great importance for the understanding and reduction of the environmental impact of wastewater treatment systems. This chapter reviews the current status of the modelling of N₂O emissions from wastewater treatment. The existing mathematical models describing all known microbial pathways for N₂O production are reviewed and discussed. These include N₂O production and consumption by heterotrophic denitrifiers, N₂O production by ammonia-oxidizing bacteria (AOB) through the hydroxylamine oxidation pathway and the AOB denitrification pathway and the integration of these pathways in single-pathway N₂O models. The two-pathway models are compared to single-pathway models. The calibration and validation of these models using lab-scale and full-scale experimental data is also reviewed. The mathematical modelling of N₂O production, while still being enhanced by new knowledge development, has reached a maturity that facilitates the estimation of site-specific N₂O emissions and the development of mitigation strategies for wastewater treatment plants taking into account the specific design and operational conditions of the plant.

Keywords – AOB pathways, calibration, heterotrophic denitrification, modelling, N₂O

Terminology

Term	Definition
Mathematical model	A system of mathematical equations that describes physical and biological processes. It is a simplified representation of the real process.
Model parameters	Model parameters are model constituents (stoichiometric and kinetic) determined according to model applications. The value of a parameter for the given application is ideally constant.
State variables	State variables represent time-varying concentrations or other properties to be determined by a solver based on their derivatives.
Model calibration	The estimation and adjustment of model parameters to enhance the agreement between model output and experimental data.
Model validation	The comparison of model simulated output with real observations using data not used in model development. The model is validated if the simulation during the validation period lies within acceptable limits around the observations.
Kinetics	Kinetics describe the rate of chemical or biological reactions, by considering factors that influence the rate of reactions. Kinetics are associated with the fundamental mechanisms of the reaction.
Stoichiometric relationship	The quantitative relationship among the amounts of substances consumed or produced in a chemical or biological reaction.
Metabolic pathway	A series of biochemical reactions occurring within microorganisms. The reactants, products, and intermediates of an enzymatic reaction which are known as metabolites, are linked by the metabolic pathway.
Emission factor (N ₂ O)	The N ₂ O emission factor is defined as the ratio between N ₂ O nitrogen emitted and the ammonium nitrogen converted.

7.1 INTRODUCTION

Mathematical models have been widely applied to the prediction of nitrogen removal in wastewater treatment, and are gaining increasing attention for the prediction of N₂O accumulation and emission during nitrification and denitrification processes (CH2MHill, 2008; Hiatt and Grady, 2008; Ni *et al.*, 2011; Corominas *et al.*, 2012; Pocquet *et al.*, 2013; Guo and Vanrolleghem, 2014; Harper *et al.*, 2015; Mannina *et al.*, 2016). The ability to predict N₂O production by modelling provides an opportunity to include N₂O production as an important consideration in the design, operation and optimization of biological nitrogen removal processes (Ni *et al.*, 2011, 2013a). Furthermore, mathematical modelling should be a more appropriate method for estimating site-specific emissions of N₂O than oversimplified models with fixed N₂O emission factors (Ni *et al.*, 2011, 2013a; Corominas *et al.*, 2012; Mampaey *et al.*, 2013; Pocquet *et al.*, 2013; Guo and Vanrolleghem, 2014). In addition, mathematical modelling provides a method for verifying hypotheses related to the mechanisms for N₂O production, and thus serves as a tool to support the development of mitigation strategies (Ni *et al.*, 2013b; Zaborowska *et al.*, 2019; Duan *et al.*, 2020; Vasilaki *et al.*, 2020).

N₂O modelling has evolved rapidly in the past few years, with models based on various production pathways proposed. These models have been calibrated with data obtained from laboratory reactors and full-scale wastewater treatment plants operated under various conditions. Each of these models has its underlying assumptions and has been calibrated/validated to various degrees based on the understanding of the processes of the distinct model creators. These models displayed various predictive abilities (usually good fit with own data but failure with foreign data). Despite the obvious importance of N₂O modelling, and the increasing number of publications, model comparisons and comprehensive reviews are rare (Mannina *et al.*, 2016; Sperandio *et al.*, 2016). This chapter aims to compare these models and provide guidance for their use. The existing mathematical models describing all known microbial and chemical pathways for N₂O production and consumption, as well as their underlying assumptions, are reviewed, discussed and compared.

This work includes the single-pathway and two-pathway models of ammonia-oxidizing bacteria (AOB), the N₂O models of heterotrophic denitrifiers, and the integrated N₂O models including both AOB and heterotrophic denitrifier activities. An overview of the model evaluations using lab-scale and full-scale experimental data is also presented to provide insights into the applicability of these N₂O models under various conditions.

7.2 N₂O KINETIC MODEL STRUCTURES

N₂O is produced during biological nitrogen removal in wastewater treatment, typically attributed to autotrophic AOB (Tallec *et al.*, 2006; Kampschreur *et al.*, 2009; Chandran *et al.*, 2011) and heterotrophic denitrifiers (Kampschreur *et al.*, 2009; Lu and Chandran, 2010; Pan *et al.*, 2012). There are three main microbial pathways involved in N₂O formation, namely the NH₂OH oxidation, nitrifier (AOB) denitrification, and heterotrophic denitrification pathways (Wunderlin *et al.*, 2012, 2013). The latter pathway is the only known microbial pathway that allows N₂O consumption. Table S1 in the supplementary information (SI) lists the definitions of the all the state variables used in the models described in this chapter.

In addition N₂O might be potentially produced through chemical pathways (Schreiber *et al.*, 2009; Harper *et al.*, 2015). Such processes involve hydroxylamine with different oxidants (HNO₂, Fe³⁺, O₂) or hydroxylamine disproportionation, or HNO₂ reduction by Fe²⁺. The kinetic model structure for such a chemical pathway is relatively simple, based on the first order regarding the reactants for instance, as proposed for the reaction between hydroxylamine and free nitrous acid (Harper *et al.*, 2015). Moreover the recent work of Su *et al.* (2019) demonstrated that these chemical reactions are strongly influenced by pH and become important only at acidic pH (≤ 5). Consequently abiotic N₂O production contributes little (< 3% of total N₂O production) to total N₂O emissions in typical nitrification reactor systems between pH 6.5 and 8. Hence, in this chapter the description will be focused on biological models.

7.2.1 MODELLING OF N₂O PRODUCTION AND CONSUMPTION BY HETEROTROPHIC DENITRIFIERS

7.2.1.1 Introduction

N₂O is a known intermediate in heterotrophic denitrification (von Schulthess and Gujer, 1996; Pan *et al.*, 2012; 2013a). Heterotrophic denitrification converts the nitrate and/or nitrite generated from autotrophic nitrification to nitrogen gas (N₂) and thus removes nitrogen from wastewater. It consists of four consecutive steps, which produce three obligatory intermediates, namely NO₂⁻, NO and N₂O. These steps are individually catalysed by four different denitrification reductases, i.e. nitrate reductase (Nar), nitrite reductase (Nir), NO reductase (NOR) and N₂O reductase (N₂OR). N₂O is produced by the sequential action of the NO₃⁻, NO₂⁻ and NO reductases.

Many factors could affect the denitrification process and thus impact N₂O emission, such as chemical oxygen demand (COD) to N ratios, the substrate and biomass types, pH levels and temperature, among others (Lu and Chandran, 2010; Pan *et al.*, 2012, 2013a). On the other hand, the four parallel denitrification steps could also exert influence on each other through electron competition, which could result in accumulation of various intermediates including N₂O. The four denitrification steps all require electrons from carbon oxidation, and they could face competition for electrons when the electron supply rate from carbon oxidation does not meet the demand for electrons by the four steps of denitrification combined (Pan *et al.*, 2013a).

To predict denitrification intermediates accumulation, denitrification needs to be modelled as a multiple-step process (von Schulthess and Gujer, 1996). Figure 7.1 gives an overview of the major models. Four-step denitrification models have been proposed and widely applied to predict the accumulation of all denitrification intermediates including N₂O (Kampschreur *et al.*, 2007; Hiatt and Grady, 2008; Ni *et al.*, 2011; Pan *et al.*, 2013b). To date, two distinct concepts have been proposed (Table 7.1), which are represented by the activated sludge model for nitrogen (ASMN) (Hiatt and Grady, 2008) and the activated sludge model with indirect coupling of electrons (ASM-ICE) (Pan *et al.*, 2013b), respectively. Table S2 in the SI lists the kinetic and stoichiometric matrices for the two models, which are fundamentally different in describing the electron allocation among different steps of heterotrophic denitrification (Table 7.1).

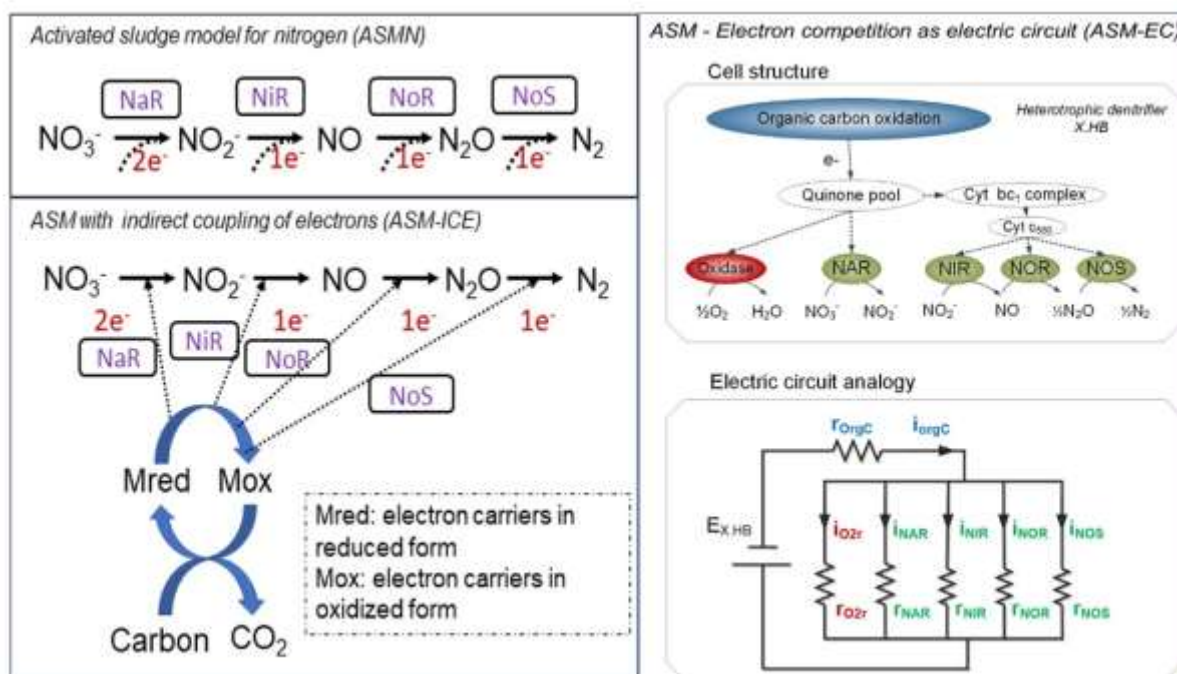


Figure 7.1 Schematic illustration of denitrification models. ASMN: Hiatt and Grady (2008); ASM-ICE: Pan *et al.* (2013b); ASM-EC electric circuit analogy: Domingo-Félez and Smets (2020).

7.2.1.2 Activated sludge model for nitrogen (ASMN)

The “*direct coupling approach*”, represented by ASMN (Model OHO-A in Table 7.1, Hiatt and Grady, 2008), directly couples the carbon oxidation and nitrogen reduction processes in the model. This model describes each of the four steps as a separate and independent oxidation-reduction reaction (Table S2 in SI), with the kinetics of each step modelled according to the nitrogen reduction reaction kinetics and using a stoichiometric relationship obtained through an electron balance. Model OHO-A ignores the fact that the nitrogen oxides reduction and carbon oxidation are carried out by different enzymes with their specific kinetics, and consequently either of the two processes could limit the rate of denitrification. In addition, this coupling approach describes each denitrification step independently with its rate not being affected by other denitrification steps that draw electrons from the same electron supply. Essentially, the carbon oxidation rate is modelled as the sum of the carbon requirements by all denitrification steps, with the underlying assumption that electron supply will always be able to meet the predicted total electron demand.

This model’s structure is close to what wastewater modellers are used to, i.e. kinetics depending on soluble and particulate components, and less on detailed metabolic pathway information. The importance of N₂O accumulation and emission logically depend on respective consumption and production rates. For instance it could be mentioned that this model predicts more N₂O production in the case of organic matter limitation by using a higher K_s value for organic matter in the last reaction (original set of parameter values by Hiatt and Grady, 2008). This is an important point in terms of acceptability and usability in the profession.

7.2.1.3 Activated sludge model with indirect coupling of electrons (ASM-ICE)

The “*indirect coupling approach*”, proposed by Pan *et al.* (2013b) and named ASM-ICE, decouples the carbon oxidation and nitrogen reduction processes. Electron carriers are introduced as a new component in this model to link carbon oxidation to nitrogen oxides reduction, in which carbon oxidation reduces carriers and nitrogen oxides reduction oxidizes carriers (Model OHO-B in Table 7.1, Pan *et al.*, 2013b). In this way, each step of heterotrophic denitrification can be regulated by both the nitrogen reduction and the carbon oxidation processes. The possibility

of either the carbon oxidation or electron transfer being a limiting step in denitrification is thus considered in the model. In heterotrophic denitrifiers, competition for electrons may occur between the four reduction steps when the electron supply rate from the oxidation process cannot meet the demand for electrons from the four reduction steps (Pan *et al.*, 2013b), which plays an important role in the accumulation and emission of N₂O (Pan *et al.*, 2013a). The electron competition between the four denitrifying steps can be modelled by assigning different values to the affinity constants responsible for Processes 2, 3, 4 and 5 with respect to *Mred*, which are provided by Process 1. Model OHO-B can be used as a practical tool for predicting N₂O accumulation during denitrification, with the complex biochemical reactions and electron transfer processes involved in biological denitrification by different microbial species being lumped into one oxidation and four reduction reactions that are linked through electron carriers.

7.2.1.4 Activated sludge model – electron competition (ASM-EC)

Almeida *et al.* (1997) proposed that the electron flow through the respiratory chain can be modelled similarly to electron flow across resistors in an electric circuit. A model structure describing four-step denitrification, aerobic respiration, and organic carbon oxidation was proposed using the analogy between electron competition during respiration and electron distribution in a multi-resistor electric circuit (Domingo-Félez and Smets, 2020). A potential is created by the presence of heterotrophic denitrifying bacteria that mediate electron transfer between an electron donor and an electron acceptor pair. The competition for electrons between multiple denitrifying enzymes is analogous to the electron flow through parallel resistors. Reaction rates are analogous to the current intensity through a resistor (Ohm's law). Individual resistances vary with the substrate concentrations, and the electron current released through electron donor (i.e. organic substrates) oxidation will be distributed between reduction rates. Following conservation of potential and conservation of charge, the current through any resistor can be calculated. The ASM-EC model can substitute the process rates describing denitrification in ASM-type models and includes fewer parameters than ASMN or ASM-ICE.

7.2.2 MODELLING N₂O PRODUCTION BY AOB

7.2.2.1 Introduction

AOB are chemolithotrophs that oxidize ammonia (NH₃) to nitrite (NO₂⁻) via hydroxylamine (NH₂OH) as their predominant energy-generating metabolism (Arp and Stein, 2003; Arp *et al.*, 2007) (Figure 7.2A). The first step is catalysed by ammonia monooxygenase (AMO) where NH₃ is oxidized to NH₂OH with the reduction of molecular oxygen (O₂). In the second step, NH₂OH is oxidized to NO₂⁻ by hydroxylamine oxidoreductase (HAO), with O₂ as the primary electron acceptor. However, AOB contain a periplasmic copper-containing nitrite reductase (NirK) and a nitric oxide reductase (Nor) (Hooper *et al.*, 1997; Chandran *et al.*, 2011) (as shown in Figure 7.2A). NirK could speed up NH₂OH oxidation by channelling electrons from the cytochrome pool to NO₂⁻ (to form NO) and thus play a facilitative role in NH₃ oxidation itself (Hooper *et al.*, 1997; Chandran *et al.*, 2011). AOB also possess the inventory to alternatively convert NO into N₂O, using a haem-copper nitric oxide reductase, sNOR (Chandran *et al.*, 2011).

Although N₂O is not an obligate intermediate in NH₃ oxidation, N₂O can be produced by AOB through two major pathways according to the current understanding (Figure 7.2A): i) N₂O as a by-product of incomplete oxidation of NH₂OH to NO₂⁻, typically referred to as the NH₂OH oxidation pathway (Poughon *et al.*, 2000; Chandran *et al.*, 2011; Stein, 2011a; Law *et al.*, 2012), and ii) N₂O as the final product of AOB denitrification with NO₂⁻ as the terminal electron acceptor and NO as an intermediate, the so-called nitrifier or AOB denitrification pathway (Chandran *et al.*, 2011; Stein, 2011b; Ni *et al.*, 2013b).

It is generally accepted that NO₂⁻ and NO reduction for N₂O production is carried out by AOB under oxygen limiting or completely anoxic conditions (Kampschreur *et al.*, 2009; Law *et al.*, 2013). Increased N₂O production under high NO₂⁻ concentrations has been suggested to be due to AOB denitrification (Yang *et al.*, 2009; Yu *et al.*, 2010). On the other hand, there is also evidence supporting N₂O production from NH₂OH oxidation by AOB. The higher NH₃ oxidation rate could result in the accumulation of NH₂OH and other reaction intermediates such as NO or NOH (Law *et al.*, 2012), which in turn result in N₂O formation with detailed reactions yet to be fully elucidated (Chandran *et al.*, 2011; Stein, 2011a).

As the fundamental metabolic pathways for N₂O production by AOB are now coming to light (Kampschreur *et al.*, 2007; Schreiber *et al.*, 2009; Yu *et al.*, 2010; Okabe *et al.*, 2011; Stein, 2011a; Perez-Garcia *et al.*, 2014; Castro-Barros *et al.*, 2015; Harris *et al.*, 2015), several mechanistic models have been proposed for N₂O production by AOB in mixed culture based on one or two of the known N₂O production pathways of AOB, i.e. AOB denitrification and NH₂OH oxidation pathways. To date, two categories of N₂O production models by AOB in mixed culture have been proposed, which are represented by single-pathway models and two-pathway models.

7.2.2.2 Single-pathway models

Six different single-pathway model structures available in literature are presented in Table S3 in the SI, detailed with their kinetic and stoichiometric matrices. Table 7.1 presents the key differences among the model structures of these single-pathway models by AOB.

Model A (Ni *et al.*, 2011) and Model B (Mampaey *et al.*, 2013) are based on the AOB denitrification pathway. In Model A (Table 7.1, Ni *et al.*, 2011), AOB denitrification with NO₂⁻ as the terminal electron acceptor produces NO

and subsequently N₂O by consuming NH₂OH as the electron donor. Similarly, in Model B (Table 7.1, Mampaey *et al.*, 2013), AOB denitrification occurs in parallel with ammonium oxidation, reducing NO₂⁻ to NO and then to N₂O with ammonium as the electron donor. The key difference between these two models is that in Model A, dissolved oxygen (DO) is assumed to inhibit nitrite and NO reduction by AOB, while in Model B, 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 A, but as a one-step process (ammonia to nitrite) in Model B.

Model A1 (Pocquet *et al.*, 2013) and Model B1 (Guo and Vanrolleghem, 2014) are also based on the AOB denitrification pathway, and are the two modified versions of Models A and B which describe N₂O production in several studies (Pocquet *et al.*, 2013; Guo and Vanrolleghem, 2014). In Model A1 (Table 7.1, Pocquet *et al.*, 2013), the oxygen inhibition of the AOB denitrification pathway was removed. In addition, free ammonia (FA) and free nitrous acid (FNA) were considered as the substrate for the AOB reactions, in order to explicitly consider the effect of pH variation. In Model B1 (Table 7.1, Guo and Vanrolleghem, 2014), oxygen limitation and inhibition was added through a Haldane function in the kinetics of both nitrite reduction and NO reduction processes (Guo and Vanrolleghem, 2014). Inhibition by FA was also considered in Model A1 and inhibition by both FA and FNA were included in Model B1.

Model C (Law *et al.*, 2012) and Model D (Ni *et al.*, 2013b) are based on the NH₂OH oxidation pathway. Model C assumes that N₂O production is due to the chemical decomposition of the unstable NOH, an intermediate of NH₂OH oxidation (Law *et al.*, 2012). In contrast, Model D assumes that the reduction of NO, produced from the oxidation of NH₂OH, resulted in N₂O production by consuming NH₂OH as the electron donor. Model D (Table 7.1, Ni *et al.*, 2013b) assumes that DO has no inhibitory effect on NO reduction (Yu *et al.*, 2010), as in Model B.

7.2.2.3 Two-pathway models

The two N₂O production pathways of AOB (NN: Hydroxylamine pathway, ND: Nitrifiers denitrification) have been integrated into different two-pathway models. Table 7.1 and Figure 7.2 compare the key differences between these four models (E–H). Two of them (E–F) are based on the decoupling approach with electron carriers (Ni *et al.*, 2014; Peng *et al.*, 2015a) whereas the two others (G–H) are based on the coupling approach (Pocquet *et al.*, 2016; Domingo-Félez and Smets, 2016).

In Model E (Table 7.1, Ni *et al.*, 2014), the complex biochemical reactions and electron transfer processes involved in AOB metabolism are lumped into three oxidation and three reduction reactions (Figure 7.2). Electron carriers are introduced as a new component in the model to link the electron transfer from oxidation to reduction. By decoupling the oxidation (E-1 to E-3 in Figure 7.2) and reduction (E-4 to E-6 in Figure 7.2) reactions through the use of electron carriers, the electron distribution between O₂, NO₂⁻ and NO as electron sinks is modelled by assigning different kinetic values to Processes E-4, E-5 and E-6 with respect to electron carriers. These electron carriers are regenerated by Processes E-2 and E-3. In this way, the model can predict the relative contribution of the two pathways to total N₂O production by AOB, as well as the shifts in the dominating pathway at various DO and nitrite level conditions.

Model F (Peng *et al.*, 2015a) is based on the decoupling approach with both electron and energy (adenosine triphosphate) balances, which are proposed by the extension of Model E to describe the dependency of N₂O production by AOB on the inorganic carbon (IC) concentration (Peng *et al.*, 2015a). In addition to the electron carriers that link electron transfer from oxidation to reduction, adenosine triphosphate (ATP)/adenosine diphosphate (ADP) are also introduced as components in the model (Table 7.1) to link energy generation to IC fixation for biomass growth (Figure 7.2). The energy distribution between ammonia oxidation, NO₂⁻ reduction and oxygen reduction as energy source (ATP) is modelled through assigning different kinetic values to Processes F-1, F-5 and F-6 with respect to ADP, which is consumed by Process F-7 with IC as substrate for AOB growth. In this way, the possible effect of IC on AOB growth, and subsequently the N₂O production from different pathways by AOB, can be explicitly described when the IC concentration in the bioreactor varies temporally or spatially, with N₂O production increasing with an increase in IC concentrations.

In Model G (Table 7.1, Pocquet *et al.*, 2016) the two pathways are combined based on a direct coupling approach. The model includes five enzymatic reactions (Figure 7.2). As in models E and F, NO is considered as an intermediary compound during oxidation of hydroxylamine into nitrite and N₂O is supposed to be produced by both the reduction of NO (hydroxylamine pathway) and the reduction of nitrite (AOB denitrification). Free ammonia and free nitrous acid are considered as substrate for nitrification and denitrification respectively. As in model E and F, the NO intermediary is not considered in the denitrification pathway which avoids the NO loop (i.e. production via nitrite reduction and re-oxidation into nitrite). The inhibition of AOB denitrification by oxygen is considered by a modified Haldane equation as in Guo and Vanrolleghem (2014).

In Model H (Table 7.1, Domingo-Félez and Smets, 2016) the two pathways are also combined based on a direct coupling approach and it includes five enzymatic reactions (Figure 7.2). Free ammonia and free nitrous acid are considered the substrates for nitrification and denitrification, respectively. Hydroxylamine is oxidized aerobically producing nitrite and is independent of oxygen presence producing NO (hydroxylamine pathway). The inhibition of AOB denitrification by oxygen is considered by an inverse Michaelis-Menten-like equation and produces NO (AOB denitrification). Hence, NO is an intermediate of the two pathways with different dependencies on oxygen and free nitrous acid concentrations. A single autotrophic N₂O-producing process accounts for the combined NO reduction.

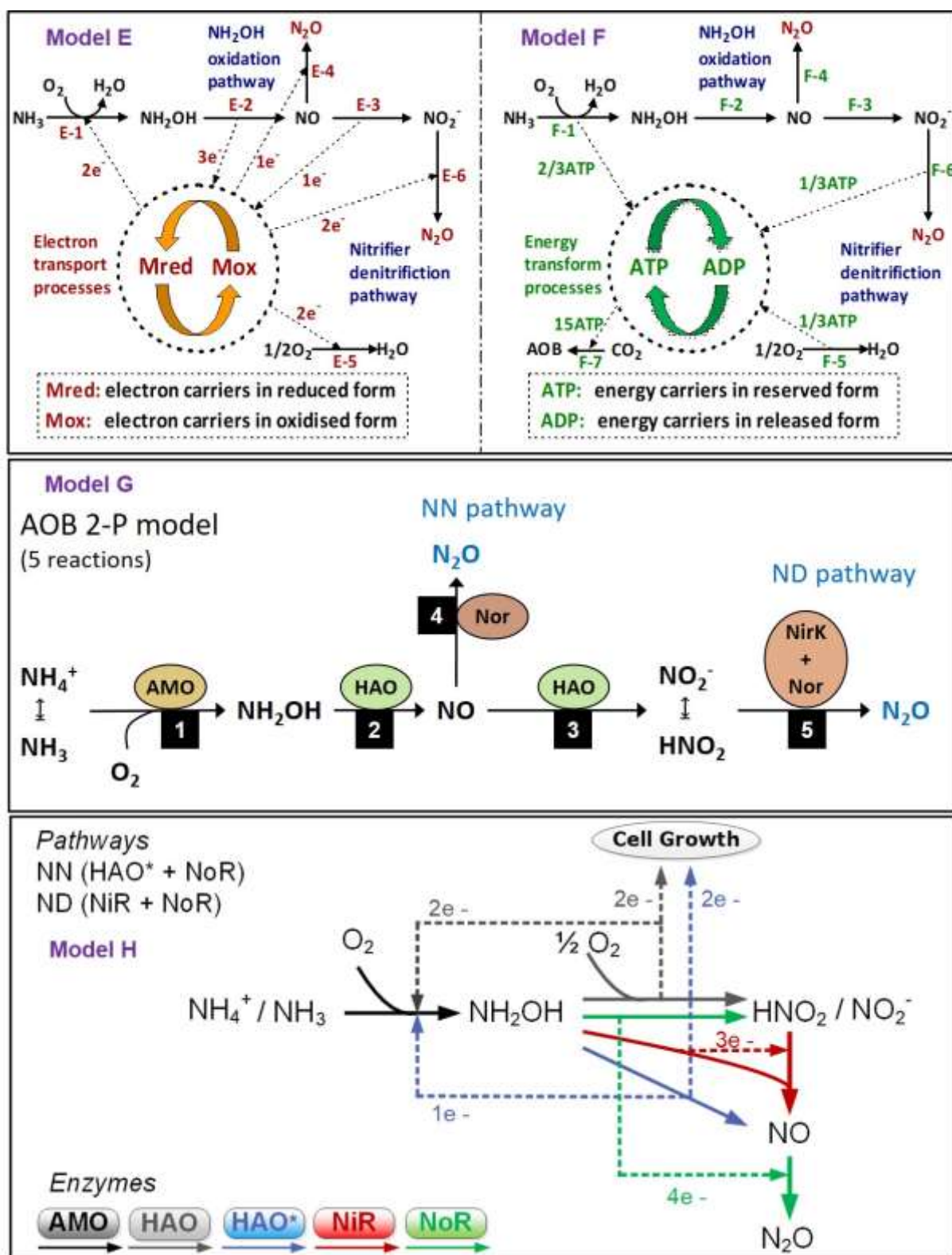


Figure 7.2 General description of the two-pathway AOB models (E: Ni *et al.*, 2014, F: Peng *et al.*, 2015a, G: Pocquet *et al.*, 2016; H: Domingo-Félez and Smets, 2016). NN: hydroxylamine pathway, ND: nitrifier denitrification pathway

Table 7.1 Key differences among the N₂O models by heterotrophs, single-pathway models by AOB and two-pathway models by AOB

N ₂ O models		Model components	Stoichiometric	Kinetic	Reference
N ₂ O models by heterotrophs	Model OHO-A	Without electron carriers.	Coupling carbon oxidation and nitrogen reduction (4 processes).	Without electron competition concept.	Hiatt and Grady, 2008
	Model OHO-B	With electron carriers.	Decoupling carbon oxidation and nitrogen reduction (5 processes).	With electron competition concept.	Pan <i>et al.</i> , 2013b
Single-pathway models by AOB	Model A – AOB denitrification	Using S _{NH4} and S _{NO2} ; With S _{NH2OH} .	Two-step NH ₄ ⁺ oxidation; Two-step NO ₂ ⁻ reduction; Cell growth during NH ₂ OH oxidation.	Two different oxygen affinity constants; Oxygen inhibition on NO ₂ ⁻ and NO reductions; Anoxic reduction factor.	Ni <i>et al.</i> , 2011
	Model A1 – AOB denitrification	Using S _{NH3} and S _{HNO2} ; With S _{NH2OH} .	Same as Model A.	Two different oxygen affinity constants; Without oxygen inhibition; NH ₃ inhibition on NH ₃ oxidation; Anoxic reduction factor.	Pocquet <i>et al.</i> , 2013
	Model B – AOB denitrification	Using S _{NH3} and S _{HNO2} ; Without S _{NH2OH} .	One-step NH ₄ ⁺ oxidation; Two-step NO ₂ ⁻ reduction; Cell growth during all 3 processes.	Only one oxygen affinity constant; Without oxygen inhibition; Anoxic reduction factor.	Mampaey <i>et al.</i> , 2013
	Model B1 – AOB denitrification	Same as Model B.	Same as Model B.	Only one oxygen affinity constant; NH ₃ and HNO ₂ inhibitions on NH ₃ oxidation; Haldane function for oxygen limitation/inhibition; Anoxic reduction factor.	Guo and Vanrolleghem, 2014
	Model C – NH ₂ OH pathway (via NOH)	Using S _{NH4} and S _{NO2} ; With S _{NOH} .	Three-step NH ₄ ⁺ oxidation via NOH; Cell growth during NH ₂ OH oxidation.	Two different oxygen affinity constants; NOH breakdown to produce N ₂ O.	Law <i>et al.</i> , 2012
	Model D – NH ₂ OH pathway (via NO)	Using S _{NH4} and S _{NO2} ; With S _{NO} .	Three-step NH ₄ ⁺ oxidation via NO; Cell growth during NH ₂ OH oxidation.	Two different oxygen affinity constants; NO reduction to produce N ₂ O; Without oxygen inhibition.	Ni <i>et al.</i> , 2013b
Two-pathway models by AOB	Model E	Using S _{NH4} and S _{NO2} ; With electron carriers.	Three-step NH ₄ ⁺ oxidation; One-step NO ₂ ⁻ reduction; Without cell growth.	Applying electron competition concept; Without oxygen inhibition; Without anoxic reduction factor.	Ni <i>et al.</i> , 2014
	Model F	Mostly same as Model E; With S _{CO2} ; With energy carriers.	Mostly same as Model E; With energy carriers involved; With cell growth considered.	Mostly same as Model E; With energy carriers involved; With effect of inorganic carbon considered.	Peng <i>et al.</i> , 2015a

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	Model G	Using S_{NH_3} and S_{HNO_2} With $S_{\text{NH}_2\text{OH}}$.	Two-step NH_3 oxidation; One-step HNO_2 reduction; Cell growth during NH_2OH oxidation.	With oxygen inhibition of denitrification; With reduction factor; Three different oxygen affinity constants.	Pocquet <i>et al.</i> , 2016
	Model H	Using S_{NH_3} and S_{HNO_2} With $S_{\text{NH}_2\text{OH}}$.	Two-step NH_3 oxidation; Two-step HNO_2 reduction; Cell growth during NH_2OH oxidation; O_2 -independent NH_2OH oxidation.	With oxygen inhibition of denitrification; With reduction factor; Two different oxygen affinity constants; Two different NH_2OH affinity constants.	Domingo-Félez and Smets, 2016

7.3 MODEL INTEGRATION, USE AND CALIBRATION

7.3.1 INTEGRATED N₂O MODELS

N₂O can be generally produced by both AOB and heterotrophic denitrifiers in WWTPs and consumed by heterotrophic denitrifiers (Kampschreur *et al.*, 2009; Law *et al.*, 2012; Guo and Vanrolleghem, 2014). Therefore, integrated N₂O models incorporating N₂O production/consumption by both AOB and heterotrophic denitrifiers would contribute to more powerful models that predict the N₂O dynamics more accurately in WWTPs, which could also be a useful tool for the development of N₂O mitigation strategies.

Two approaches have been reported to integrate the N₂O production/consumption by both AOB and heterotrophic denitrifiers into a comprehensive N₂O model: i) ASM-type models that combine one of the single-pathway models of AOB (e.g. Models A–D, Table S3) with ASMN of heterotrophic denitrifiers (Model OHO-A, Table S2) (Ni *et al.*, 2011; Pocquet *et al.*, 2013; Guo and Vanrolleghem, 2014; Spérandio *et al.*, 2016), and ii) electron balance-based models that integrate the electron carrier-based two-pathway model of AOB (Model E, Table S4) and ASMN (Model OHO-A, Table S2) (Ni *et al.*, 2015). Both modelling approaches have been successfully applied to describe N₂O emissions from mixed culture nitrification-denitrification systems and to identify the relative contributions of AOB and heterotrophic denitrifiers to total N₂O production (Ni *et al.*, 2011, 2013b, 2015; Spérandio *et al.*, 2016). A third potential approach to integrate the N₂O production/consumption by both AOB and heterotrophic denitrifiers could be a full electron balance-based model integrating the electron carrier-based two-pathway model of AOB (Model E, Table S4) and the electron carrier-based model of heterotrophs (Model OHO-B, Table S2), this requires future testing though. Model H integrates a two-pathway AOB model, ASMN of heterotrophic denitrifiers and abiotic reactions considering free nitrous acid and hydroxylamine.

It should be noted that the possible consumption of N₂O by heterotrophic denitrification as an N₂O sink may occur and reduce overall N₂O production in an integrated model under the conditions of high COD to N ratio and/or low DO level.

7.3.2 MODEL EVALUATION AGAINST EXPERIMENTAL DATA

The N₂O models have to be tested to predict N₂O emission data from experiments in order for the models to become useful tools in practical applications. During recent years, many measurement campaigns have been performed. All available N₂O models have been evaluated with experimental data collected from different systems to reveal their performance under various process conditions and shed light on the conditions under which each of the models would be suitable for application.

7.3.2.1 Heterotrophic denitrification

For denitrifying N₂O models, Model OHO-A was found generally able to reproduce the nitrate, nitrite and N₂O profiles when only one nitrogen oxide species was added (Ni *et al.*, 2011; Pan *et al.*, 2015), but Model OHO-A failed to reproduce the results when two or more nitrogen oxide species were added together. In contrast, Model OHO-B was shown to be able to describe general COD consumption, nitrate reduction and nitrite accumulation by an enriched denitrifying culture (Pan *et al.*, 2015), and the influence of nitrite and N₂O addition on nitrate reduction, as well as the experimental results when one or more nitrogen oxide species were added (Pan *et al.*, 2015). Therefore the decoupling approach of Model OHO-B might be essential to describe complex conditions with addition of multiple nitrogen oxide species, but in the many situations for which only nitrate and nitrite are provided Model OHO-A is still applicable. For both models it can be noted that an independent calibration of each of the successive steps of denitrification has rarely been possible. As heterotrophic denitrification is an important N₂O mechanism to be considered for future mitigation strategies, more measurements of intermediates is recommended in future studies to improve the robustness of the models calibration.

7.3.2.2 Single-pathway AOB models

The six single-pathway AOB models (Models A–D, Table 7.1) were evaluated and compared (Ni *et al.*, 2011; Ni *et al.*, 2013a; Spérandio *et al.*, 2016) based on their ability to capture the observed N₂O production results from

different experiments (Yang *et al.*, 2009; Kim *et al.*, 2010; Law *et al.*, 2012; Spérandio *et al.*, 2016). Model A could predict well the observed trend of a decrease in N₂O production at high DO concentrations (Yang *et al.*, 2009), whereas Model B was not able to predict such a trend due to the absence of oxygen inhibition on AOB denitrification in Model B (Ni *et al.*, 2013a). Model B could not describe well the N₂O peak that is likely related to the dynamics of NH₂OH (Ni *et al.*, 2013a), which was not included in Models B and B1. Models A, A1, B and B1 have been tested and found to reasonably describe N₂O production data with high nitrite accumulation (Figure 7.3) (Pocquet *et al.*, 2013; Spérandio *et al.*, 2016). In contrast, both Models C and D were not able to capture the observed dependency of N₂O production on nitrite availability (Yang *et al.*, 2009; Kim *et al.*, 2010; Spérandio *et al.*, 2016) due to the fact that the two models are linked to incomplete NH₂OH oxidation. However, Models C and D were able to reproduce the experimental observations that the N₂O production increased/decreased with increasing/decreasing DO concentration (Law *et al.*, 2012). The kinetic structure of Model B also ensured that the N₂O production rate was dependent on oxygen availability, resulting in a similar N₂O dynamic trend (increase in the N₂O production rate with an increase in DO concentration). On the contrary, Model A predicted the opposite to such an observation (Law *et al.*, 2012). These results suggest that DO inhibition might be required to describe AOB denitrification and NH₂OH needs to be included as a necessary intermediate. The use of FA and FNA in model structures would be recommended for a better description of the pH effect and possible FNA inhibition. NOH would be preferably used as an N₂O precursor for describing the NH₂OH pathway under extremely high nitrite accumulation conditions, whereas NO could be generally applied as an intermediate for N₂O production from NH₂OH oxidation under common wastewater conditions.

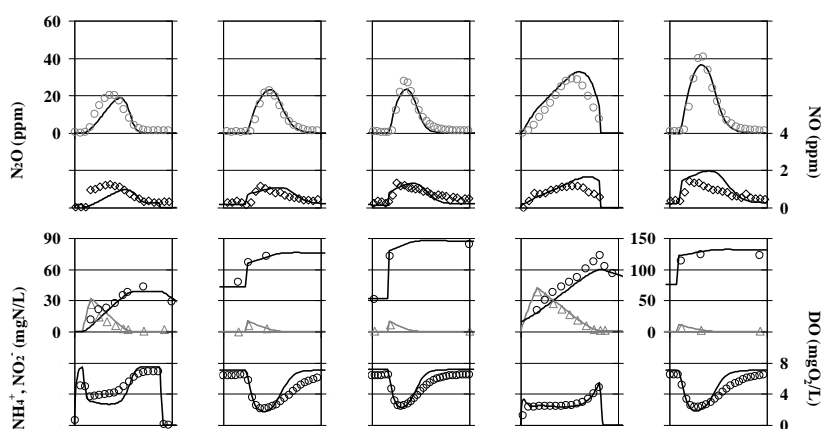


Figure 7.3 Comparison of simulation (A1, Pocquet *et al.*, 2013) and measured data for five batch experiments at different nitrite and ammonium levels. NO in gas phase (\diamond), N₂O in gas phase (\circ), ammonium (Δ), nitrite (\square) and dissolved oxygen (\circ). Duration of experiments: 1 h. N₂O emission factors: 1.39%, 2.58%, 3.86%, 1.83%, 4.52% (gN-N₂O/gN-NH₄⁺), respectively (Spérandio *et al.*, 2016).

7.3.2.3 Two-pathway AOB models

With respect to the two-pathway models of AOB, Model E has satisfactorily described the N₂O data from several different nitrifying cultures (partial nitrification culture or/and full nitrification culture) and under various DO and NO₂⁻ concentration conditions (Figure 7.4) (Ni *et al.*, 2014; Peng *et al.*, 2014; Sabba *et al.*, 2015). Model F has also predicted well these different nitrifying cultures (partial nitrification and full nitrification culture) but also under various IC conditions (Peng *et al.*, 2015a). Although the electron-based two-pathway models (Models E and F) have been demonstrated to be effective, electron carriers may not necessarily be the only approach to the integration of the two pathways into one model. Model G is based on the *coupling approach* without considering electron carriers. It was calibrated with batch experiments and validated with long-term data collected in a sequencing batch reactor performing nitrification and denitrification (Pocquet *et al.*, 2016). A good prediction of the N₂O emissions for varying nitrite concentrations was obtained. Model G is also capable of describing the trends observed for the NO emissions and the variation of the NO/N₂O ratio depending on the pathways'

contribution. The combined effect of nitrite (via free nitrous acid) and dissolved oxygen (DO) is also correctly predicted by Model G (Figure 7.4) (Lang *et al.*, 2017).

Two-pathway models E and G successfully predicted shifts of the dominating pathway at various DO, nitrite and/or IC levels (see Figure 7.5, Lang *et al.*, 2017), consistent with experimental observations that N₂O was produced from both nitrifier denitrification and NH₂OH oxidation pathways by AOB (Ni *et al.*, 2014; Peng *et al.*, 2014; Wunderlin *et al.*, 2013). The model results suggested that the contribution of AOB denitrification decreased as DO increased, accompanied by a corresponding increase in the contribution by the NH₂OH oxidation pathway. This was verified by site preference (SP) isotopic measurements (Peng *et al.*, 2014). The two-pathway models also successfully predicted the increase of the AOB denitrification pathway with nitrite (at low nitrite concentrations) and the inhibition of AOB denitrification at high nitrite concentrations (see Figure 7.5) (Ni *et al.*, 2014; Pocquet *et al.*, 2016).

Model H was calibrated with AOB-enriched biomass and activated sludge mixed liquor biomass (Domingo-Félez *et al.*, 2017; Su *et al.*, 2019; Domingo-Félez and Smets, 2020b). Optimal extant respirometry and anaerobic batch experiments that target endogenous and exogenous processes (of both autotrophic ammonium/nitrite oxidation and heterotrophic denitrification), together with the associated net N₂O production were designed and executed. The calibrated model predicts the NO and N₂O dynamics at varying ammonium, nitrite and dissolved oxygen levels in the two independent systems.

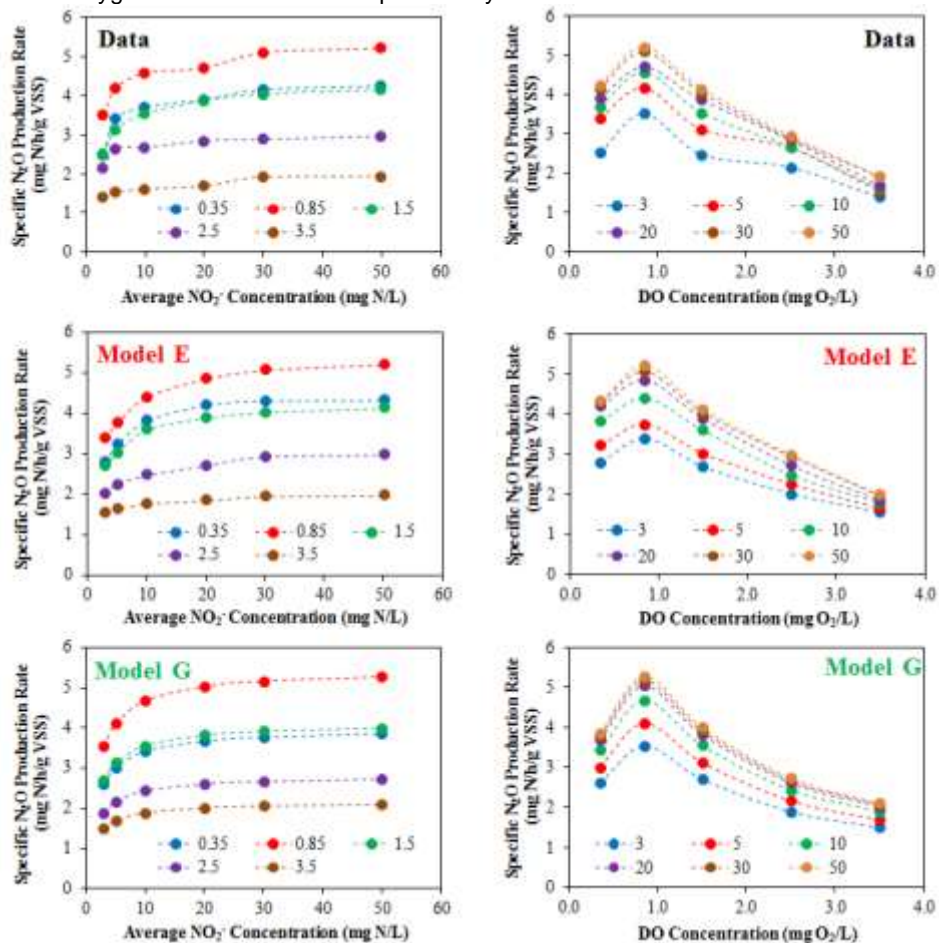


Figure 7.4 Comparison of Model E and Model G predictions (two-pathway models). Experimental and simulated effect of dissolved oxygen and nitrite concentrations on specific N₂O production rate during short-term (batch) experiments (Lang *et al.*, 2017).

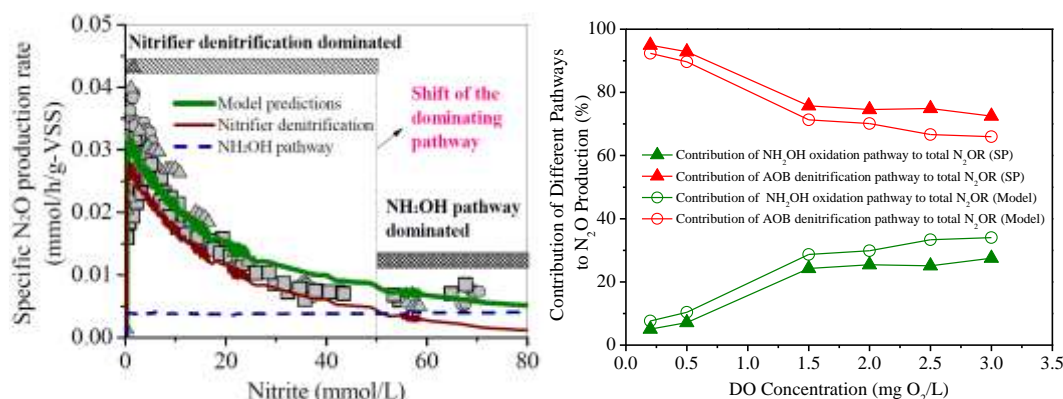


Figure 7.5 Predicted contributions from the nitrifier denitrification pathway and the NH_2OH pathway as well as their shifts using Model E (real data: symbols, model predictions: lines) for a partial nitrification (left panel adapted from Ni *et al.*, 2014) and a full nitrification system (right panel adapted from Peng *et al.*, 2014).

7.3.3 SELECTION OF MODELS FOR N_2O PREDICTION

7.3.3.1 Single-pathway AOB models versus two-pathway AOB models

The model evaluation results strongly suggest that appropriate selection of available N_2O models is important for accurate N_2O prediction in different engineering nitrogen removal systems under different operational conditions. Figure 7.6 presents a possible guideline for model selection in their further applications.

For N_2O production by heterotrophic denitrifiers, Model OHO-A can be used to predict the overall nitrogen and COD removal performance in a wastewater treatment plant, as in most cases the low level accumulation of denitrification intermediates does not significantly affect the overall nitrogen removal rate. However, in the context of predicting the N_2O production by heterotrophic denitrifiers, Model OHO-B is inadequate due to its structural deficiency in describing the electron competition process in denitrification. Model H enhanced our ability to predict N_2O production by heterotrophic denitrifiers and has the potential to describe all N_2O data under different conditions. However, it requires information on both the carbon oxidation reaction kinetics and the nitrogen reduction kinetics.

For N_2O production by AOB, the single-pathway models (Models A–D) have simpler structures (one single pathway involved) and fewer parameters, which is convenient for model calibration (Table 7.2). This makes their use preferential under certain conditions, even though they may not be able to reproduce all N_2O data. The two-pathway models (Models E–G) have the potential to describe all N_2O data with different operational conditions, but may require more efforts in model calibration because of their larger number of parameters. Specifically (Table 7.2), Models A, A1, B and B1 might be used to describe the regulation of N_2O production by nitrite (or FNA) concentrations. Models C and D might be able to describe N_2O emissions from systems under relatively high DO concentrations and low nitrite accumulation that likely favour the NH_2OH oxidation pathway for N_2O production. In addition, according to the analysis by Peng *et al.* (2015b) (Figure 7.6), for the AOB denitrification model to be used (e.g. Model A) it is preferable that the DO concentration in the system is well controlled at a constant level. NH_2OH oxidation models (e.g. Model D) can be applied under high DO conditions. Under other conditions, the two-pathway models (e.g. Model E or G) should be applied. Model E or Model G could be used under varying DO and NO_2^- concentrations, but stable IC conditions are required, while Model F would be preferable under highly dynamic IC conditions.

Table 7.2 Guideline for model selection for predicting N₂O production by AOB and heterotrophic denitrification

N ₂ O models	Single-pathway models by AOB	Two-pathway models by AOB	N ₂ O models by heterotrophs
Applicable conditions	<ul style="list-style-type: none"> ✓Models A, A1, B and B1 to describe the regulation of N₂O production by nitrite (or FNA) ✓Model A to predict possible DO inhibition on N₂O production at high DO levels ✓Models A1, B and B1 to predict possible pH effect and FA/FNA inhibition on N₂O production ✓Models C and D to describe N₂O emissions at high DO levels and low nitrite accumulation 	<ul style="list-style-type: none"> ✓Model E to predict N₂O production at varying DO and NO₂⁻ with constant IC ✓Model F to describe N₂O production under highly dynamic IC condition ✓Model G to predict NO and N₂O production at varying DO and NO₂⁻ and possible pH and FA/FNA effects on N₂O production 	<ul style="list-style-type: none"> ✓Model OHO-A to predict the overall nitrogen and COD removal performance with low level accumulation of denitrification intermediates ✓Model OHO-B to describe N₂O production under different conditions
Inabilities of the models	<ul style="list-style-type: none"> ✓Model A not to describe the increase of N₂O production with increasing DO ✓Models B and B1 not to predict the N₂O production related to the dynamics of NH₂OH ✓Models C and D not to predict the effect of nitrite accumulation on N₂O production 	<ul style="list-style-type: none"> ✓Model E and G not to describe N₂O production with dynamic IC ✓Model E and F not to describe pH effect on N₂ production ✓Model E and F not to describe NO production 	<ul style="list-style-type: none"> ✓Model OHO-A not to describe N₂O production with electron competition
Key parameters for calibration	<ul style="list-style-type: none"> ✓The half saturation constant for nitrite or FNA ($K_{\text{NO}_2, \text{AOB}}$ or $K_{\text{HNO}_2, \text{AOB}}$ for Models A, A1, B, B1) ✓The reduction factor for N₂O production (η_{AOB}, for all six single-pathway models) 	<ul style="list-style-type: none"> ✓E, F: The affinity constants with respect to electrons (e.g. $K_{\text{Mred},3}$, and $K_{\text{Mred},4}$) ✓E, F: The ratios among the affinity constants to electrons ✓G, H: The reduction factors, affinity constant for HNO₂ and NH₂OH 	<ul style="list-style-type: none"> ✓The N₂O production and reduction rates ✓The relative ratios between electron affinity constants

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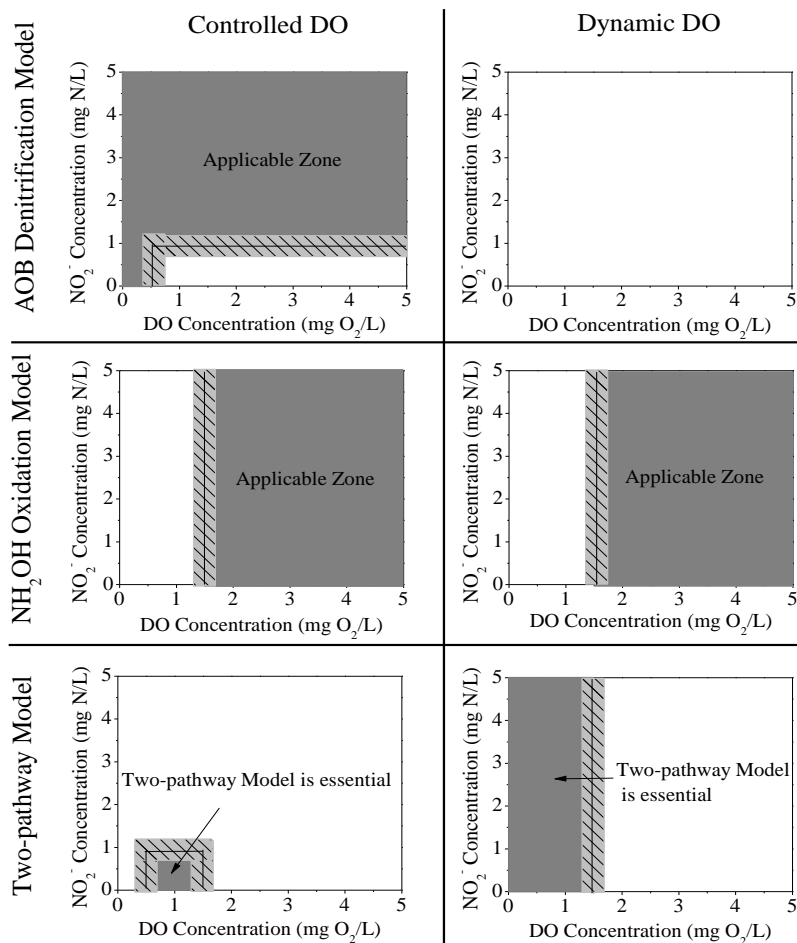


Figure 7.6 Summary of applicable regions for the AOB denitrification model, the NH_2OH oxidation model and the two-pathway model under various DO and NO_2^- concentrations. The applicable regions were insensitive to the variations of key parameters governing N_2O production by the two-pathway model (Peng *et al.*, 2015b).

7.3.3.2 Two-pathway AOB models: direct versus indirect coupling approach

The two different two-pathway models E and G based on indirect coupling or direct coupling approaches, respectively, were compared by Lang *et al.* (2016). Both were calibrated to describe the experimental N_2O emissions collected from 43 kinetic experiments considering a large range for both DO and nitrite concentrations and three different AOB-enriched cultures (Lang *et al.*, 2017) (Figure 7.4).

Both models enabled the prediction that the increase of DO enhances the hydroxylamine pathway contribution while it reduces the contribution of the AOB denitrification pathway (Figure 7.7). This is related to competition between oxygen and nitrite for electron carriers in Model E, whereas it is described by an inhibition term in the AOB denitrification kinetics in Model G. Regarding the nitrite effect, both concepts similarly describe the shift from the hydroxylamine pathway to the AOB denitrification pathway. Considering FNA in Model G also indirectly enables the pH influence to be described.

The choice between these two concepts will depend on simulation objectives and calibration experience. Regarding the last extended model of Peng *et al.* (2016) which also includes the inorganic carbon effect (Model F), the "indirect coupling" approach is able to reveal some metabolic relations between N_2O production and the cell's anabolism. This mathematical framework constitutes an ideal approach for investigating intracellular and metabolic mechanisms. In comparison, the direct coupling approach is simpler and easily understandable for practitioners as it is based on the conventional ASM approach. Another advantage of Model G is that it considers NO as an external state variable (not an intracellular compound like Model E) which makes it possible to use such data for model calibration (Pocquet *et al.*, 2016).

Finally, actual experience shows that both concepts enable good predictions. Work is now recommended with data from full-scale systems in which the mixed liquor complexity and the combination with other biochemical reactions could reveal stronger differences between these two models.

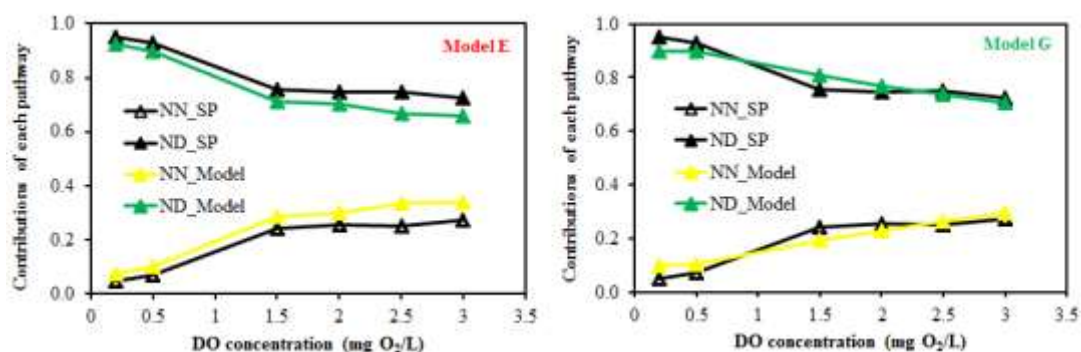


Figure 7.7 Effect of dissolved oxygen on contribution of the two AOB pathways (left: Model E, right: Model G) SP: data from site-preference measurements (Lang *et al.*, 2016).

7.3.4 KEY KINETIC AND STOICHIOMETRIC PARAMETERS FOR CALIBRATION

The calibration approach for N₂O models is based on two successive steps. The first step consists of adjusting the "conventional" parameters (i.e. growth rate, decay rate, substrate removal rate and the affinity constant) to obtain a good prediction of the main substances dynamics: ammonium, nitrite, nitrate, oxygen. In a second step, the specific parameters influencing N₂O emissions are calibrated. A final iteration should be performed if the prediction of the main substances is slightly affected by this second calibration step.

Typical values of the model parameters can be found in literature (Ni and Yuan, 2015). Different sets of parameter values obtained after calibration on different sets of data are provided by Spérandio *et al.* (2016) and Lang *et al.* (2016) for the single-pathway AOB models and the two-pathway AOB models, respectively. Continued testing against more experimental data would delineate a range/pattern in parameter values. It should be noted that these parameters were estimated under different conditions of temperature, sludge retention time and feeding composition, and therefore correction factors must be adjusted by, for example, Arrhenius equations (Snip *et al.*, 2014). Furthermore, the parameter values estimated during batch experiments may not be adequate for continuous processes and may not be compatible with the values of other parameters (Ni *et al.*, 2013a; Spérandio *et al.*, 2016; Snip *et al.*, 2014).

Regarding the ASM-ICE of the heterotrophic denitrifiers (Model OHO-B in Table S2), information on both the carbon oxidation reaction kinetics and the nitrogen reduction kinetics is required for its calibration and application (Table 7.2). Due to the lack of understanding of the electron competition process in most of the previous studies, the respective reaction kinetics of the carbon oxidation and nitrogen reduction processes were not well established. For instance, the maximum carbon source oxidation rate ($r_{COD,max}$), which is the key parameter to restrict the overall model predictions of the carbon oxidation (electron supply) rate, is not available in literature and thus needs to be measured or estimated (Pan *et al.*, 2015). Similar to the two-pathway models of AOB, the relative ratios between electron affinity constants ($K_{Mred,1}$, $K_{Mred,2}$, $K_{Mred,3}$, and $K_{Mred,4}$) rather than their absolute values are important for the reaction rate. Therefore, increased efforts are needed to provide more information on these key parameters of the ASM-ICE model for its further implementation (Table 7.2).

For the six single-pathway AOB models (Models A–D in Table S3), the model parameters were obtained after significant calibration efforts, and thus some of the parameters showed wide variation (more than 100%) among case studies during model evaluations (Ni *et al.*, 2011; Ni *et al.*, 2013a; Spérandio *et al.*, 2016). Among them, the half saturation constant for nitrite or FNA ($K_{NO_2,AOB}$ or $K_{HNO_2,AOB}$ for Models A, A1, B, B1) and the reduction factor for N₂O production (η_{AOB} , for all six single-pathway models) were most variable and very influential on N₂O emissions (Spérandio *et al.*, 2016). Regarding the models based on the AOB denitrification pathway (e.g. Models A, A1, B and B1), the large variation of these two key parameters was related to the range of nitrite (or FNA) concentrations observed in each system (Spérandio *et al.*, 2016), likely due to the adaptation of enzymatic activity (NirK). Regarding the models based on the NH₂OH oxidation pathway (e.g. Models C and D) the large variation of η_{AOB} might be dependent on the possible NO accumulation in each system. High NO accumulation would lead to a low value for η_{AOB} (Spérandio *et al.*, 2016). Thus, calibration will be required for the application of the single-pathway models regarding these key parameters (Table 7.2).

For the electron balance-based two-pathway AOB models (Models E and F in Table S4), the affinity constants with respect to electrons (e.g. $K_{Mred,3}$, and $K_{Mred,4}$) are unique to the two-pathway models and the key parameters governing the N₂O production via the two pathways. The values represent the affinity of the corresponding reduction reaction to electrons, with lower values indicating a higher affinity and thus a higher ability to compete for electrons. For example, the estimated $K_{Mred,3}$ has a value that is about one magnitude smaller than $K_{Mred,4}$ (Ni *et al.*, 2014), indicating that O₂ reduction has a higher ability to compete for electrons than the main electron acceptor during NH₂OH oxidation. Ni *et al.* (2014) revealed that the absolute value of C_{tot} is not critical for model calibration and predictions, and it is the ratios between parameters K_{Mox} , $K_{Mred,1}$, $K_{Mred,2}$, $K_{Mred,3}$, and $K_{Mred,4}$ and parameter C_{tot} that affect the model output. Therefore, attention should be paid to these ratios for the calibration and application of the two-pathway models (see Table 7.2).

For the two-pathway model G, the reduction factor for both AOB denitrification and hydroxylamine pathway ($\eta_{\text{AOB_ND}}$, $\eta_{\text{AOB_NN}}$) are the two major influential parameters which control the maximal specific N_2O production rates of each pathway (Lang *et al.*, 2017). The affinity constant for FNA also has to be calibrated from one culture to another, especially when working at very different nitrite concentrations. The hydroxylamine pathway contribution is also sensitive to the affinity constant for nitric oxide as determined by NO measurements (Pocquet *et al.*, 2016). In parallel, the AOB denitrification contribution is influenced by the inhibition constant for oxygen which is a key parameter for predicting the effect of lowering aeration on N_2O emissions (Lang *et al.*, 2017).

Model H was calibrated following a global sensitivity analysis and an information-based parameter selection procedure (Domingo-Félez *et al.*, 2017; Domingo-Félez and Smets, 2020b). First, parameters associated with heterotrophic denitrification were fitted. Then, parameters associated with aerobic nitrite and ammonia oxidation were sequentially fitted to DO consumption profiles by isolating individual processes, followed by N_2O production profiles. In the AOB-enriched biomass the reduction factors for the NN pathway and the N_2O -production process were estimated together with the HNO_2 and NH_2OH affinity constants. In the activated sludge mixed liquor biomass three reduction factors were estimated: NO-producing NN and ND pathways, and N_2O production processes. The pH-dependency of AOB-driven N_2O production and heterotrophic N_2O consumption was also described (Su *et al.*, 2019; Domingo-Félez and Smets, 2020b). The uncertainty associated with parameter estimation results was propagated to validate the model response, and is recommended to be included with best-fit simulations.

7.3.5 APPLICATION OF N_2O MODELS IN BIOFILM SYSTEMS

The previous sections provide a basis for modelling the formation and consumption of N_2O by AOB and heterotrophic denitrifying bacteria. In this section, we discuss how these kinetics are applied to biofilm processes. Biofilm treatment processes, such as the moving bed biofilm reactor (MBBR), integrated fixed-film activated sludge (IFAS), biological aerated filters (BAF), denitrifying filters, and granular sludge, are becoming increasingly popular for wastewater treatment. Due to substrate gradients and microbial stratification, the behaviour of biofilms is typically different from suspended growth processes, and this may be especially true for N_2O production and emissions (Sutka *et al.*, 2006; Schreiber *et al.*, 2009; Law *et al.*, 2012). These systems appear to have among the highest N_2O emission rates (e.g. Bollon *et al.*, 2016).

A schematic of a biofilm, with diffusion of substrates and products, is shown in Figure 7.8. In conventional biofilms, both the electron donor and acceptor substrates diffuse from the bulk liquid into the biofilm. Substrates penetrate into the biofilm by diffusion and are consumed within the biofilm by microbially catalysed reactions. Substrates diffusing into the biofilm from the bulk liquid side first pass the liquid diffusion (or boundary) layer. The liquid diffusion layer adds diffusive resistance to substrate transport into the biofilm, decreasing the substrate concentration at the biofilm surface with respect to the bulk liquid.

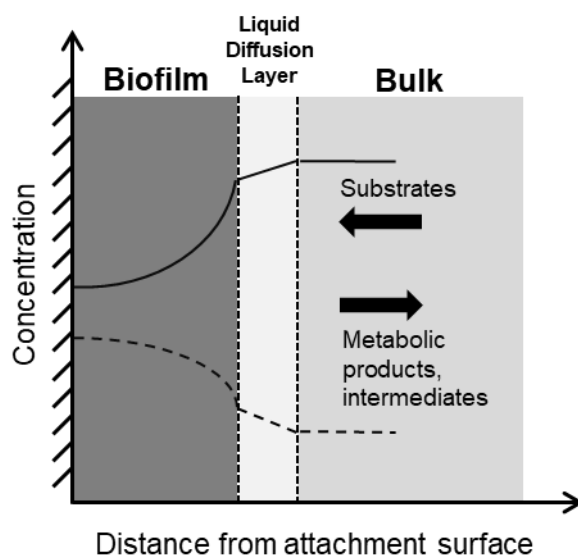


Figure 7.8 Schematic of a biofilm with substrate penetration and metabolic products/intermediates formation.

When modelling N_2O emissions from biofilms, the underlying rate expressions are the same as those described for the suspended growth processes. However, diffusion and microbial stratification within the biofilm can change the observed behaviour. For example, suspended growth bacteria in an aerobic zone of a treatment process are unlikely to have appreciable denitrification. However, biofilms in aerobic zones of a treatment process may have

anoxic zones in their interior. This can allow heterotrophic denitrification, including formation and consumption of N_2O and AOB denitrification.

Sharp gradients of O_2 and other substrates within a biofilm, combined with different microbial species in close proximity, allow the diffusion of intermediates to different redox environments or zones with different microbial metabolisms. For example, NH_2OH can be produced by AOB in the outer, aerobic zones of a biofilm, and consumed in inner, anoxic zones where it leads to peaks in N_2O formation due to AOB denitrification. Nitrite oxidizing bacteria (NOB) can enhance this effect by increasing the O_2 gradients within the biofilm (Sabba *et al.* submitted).

The importance of intermediate diffusion is illustrated in Figure 7.9, which shows N_2O emissions from a pilot-scale granular sludge reactor for side-stream nitrification (Pijuan *et al.*, 2014). When modelled with very limited NH_2OH diffusion (1% of the actual value), the model could not capture the actual N_2O emissions (Figure 7.9). However, when NH_2OH diffusion was included in the model, the model provided an excellent fit to the data (Sabba *et al.*, 2015).

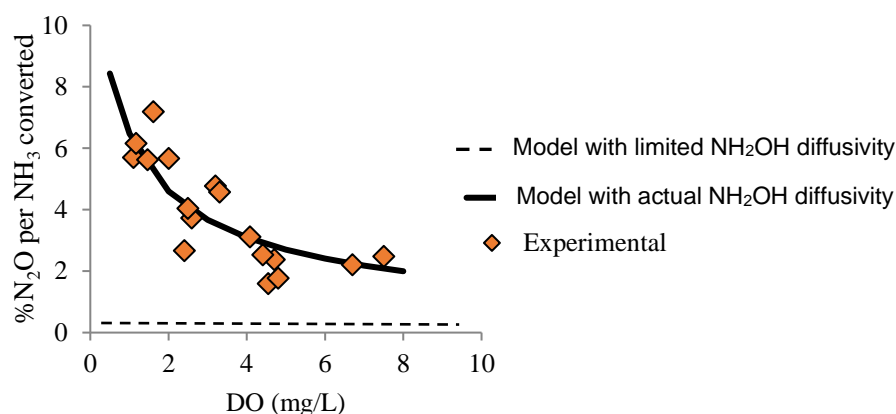


Figure 7.9 Comparison between measured $\% \text{N}_2\text{O}$ from NH_3 converted and model-calculated values in steady-state conditions (Sabba *et al.*, 2015).

Another example of interactions within a biofilm is the scavenging of N_2O formed in the outer zone of a biofilm, for example by AOB, by heterotrophic denitrifiers in the deeper, anoxic zones of the biofilm. This can lead to lower net N_2O emissions, although the complexity of biofilms makes this highly dependent on the specific reactor conditions.

Biofilm processes can be more challenging to calibrate than suspended growth processes. Information is needed about the biofilm thickness, density, substrate diffusivities, and microbial community structure. In most cases, a one-dimensional model can capture biofilm behaviour. Special care should be taken when analysing putative suspended-growth processes that may actually display biofilm behaviour. This may be true for processes with large flocs. It also may be true for bench- or pilot-scale systems, as reactor wall area is more significant, relative to reactor volume. This can lead to a greater impact of biofilms growing on walls than in a full-scale system.

Recently the two pathway AOB model (G) and the multiple step denitrification model (OHO-A) were combined for describing N_2O emissions from a nitrifying biofilter and denitrifying biofilter (Fiat *et al.*, 2019; Zhu *et al.*, 2019) as well as a granular sludge system (Lang *et al.*, 2019). Biofilm structure was described by a one-dimensional model. For a granular sludge partial nitrification anammox (PNA) system, the model was successfully calibrated to experimental data by adjusting the affinity constant for hydroxylamine mainly (Lang *et al.*, 2019). The effect of varying nitrite concentration and air flow rate was correctly predicted. Simulation demonstrated that a part of the N_2O produced by AOB was consumed by heterotrophic denitrification in the biofilm, and N_2O emission was highly influenced by the level of NOB repression. This work clarified the behaviour of N_2O emission under very low oxygen concentration, indicating that nitrifier denitrification was the major contributing pathway.

Regarding the biofilters systems, the N_2O models (A and G) were used in successive reactors to describe longitudinal heterogeneity in the biofilter. The simulations were compared to data monitored on full scale installations (results are described in the next paragraph).

In summary, biofilm processes are significantly more complex than suspended growth processes, and modelling can be a critical tool to understand the mechanisms and predict the N_2O formation and emissions.

7.3.6 APPLICATION OF N_2O MODELS IN FULL-SCALE WWTPS

Mathematical modelling of N_2O emissions from full-scale WWTPs was first conducted successfully by using ASM-type models that combine one of the single-pathway models of AOB with ASMN of heterotrophic denitrifiers (Ni *et al.*, 2013b). Ni *et al.* (2013b) applied a model based on the NH_2OH pathway model of AOB (Model D, Table 7.1) and ASMN (Model OHO-A, Table 7.1) to describe the N_2O emissions from full-scale WWTPs. The model described well the dynamic ammonium, nitrite, nitrate, DO and N_2O data collected from both an open oxidation ditch (OD) system with surface aerators and a sequencing batch reactor (SBR) system with bubbling aeration. Ni *et al.* (2013b) also performed additional evaluations on the other three single-pathway N_2O models of AOB (Model A, Model B and Model C in Table 7.1) to evaluate the experimentally observed N_2O data from the two full-scale WWTPs. The

results indicated that Model A could not predict the N₂O data from either WWTP (Ni *et al.*, 2013b; Spérandio *et al.*, 2016). Models B and C, on the contrary, obtained very similar good fits between the model-predicted and experimentally observed N₂O data (Ni *et al.*, 2013b, Spérandio *et al.*, 2016).

Dynamic simulations were also confronted to the data collected on the UCT (University Cape Town configuration) process of the Eindhoven plant by using ASM-type models that combine one of the single-pathway models of AOB with ASMN of heterotrophic denitrifiers (Guo and Vanrolleghem, 2014; Spérandio *et al.*, 2016). Model A1 + Model OHO-A, Model B1 + Model OHO-A and Model D + Model OHO-A were all implemented for this plant and calibrated using data collected in a 1-month measurement campaign. The conclusion was that all these models could be calibrated to the same level of fit (Spérandio *et al.*, 2016). They had similar performance and could follow the dynamic variations in the measured N₂O data (see Figure 7.10). In addition, the results showed that there was less N₂O emission under wet-weather conditions compared to dry-weather conditions and all three models showed better simulation performance under dry-weather conditions than wet-weather conditions (Spérandio *et al.*, 2016).

Mathematical modelling of N₂O emissions from full-scale WWTPs was then conducted successfully by using electron balance-based models that integrate the two-pathway model of AOB and the ASMN of heterotrophic denitrifiers (Ni *et al.*, 2015). Ni *et al.* (2015) applied an integrated model incorporating the electron balance-based two-pathway model of AOB (Model E, Table 7.1) and the ASMN of heterotrophic denitrifiers (Model OHO-A, Table 7.1) to describe N₂O emissions from a step-feed full-scale WWTP. The model described well all dynamic ammonium, nitrite, nitrate, DO and N₂O emission data. Modelling results revealed that the AOB denitrification rate decreased and the NH₂OH oxidation rate increased along the path of both steps, with the second step of the full-scale WWTP having much higher N₂O emission than the first step. The integrated N₂O model captured all these trends regarding the shifting/distribution between the different N₂O pathways observed in this full-scale WWTP (see Figure 7.11). A potential strategy to mitigate N₂O emission from this plant was also evaluated using the model. The overall N₂O emission from the step-feed WWTP would be largely mitigated if 30% of the returned activated sludge was returned to the second step with the remaining 70% returning to the first step.

More recently, the electron balance-based model (Model F) has been successfully applied to guide a full-scale N₂O mitigation study (Duan *et al.*, 2020). Full-scale treatment plants have inevitably more complexities than laboratory reactor operations. It was inconclusive whether the model predictions can be reliably applied to guide full-scale mitigations. In this recent work, Model F was calibrated and validated against full-scale results, before being applied to predict the N₂O emissions and nutrient removal performances with different N₂O mitigation measures. The close agreement between the measured emission factor (EF) (0.58 ± 0.06%) after the implementation of the proposed mitigation strategy, and the EF predicted by the mathematical model (0.55%), showed that the N₂O mathematical model is indeed a useful tool to evaluate N₂O mitigation strategies at full-scale. The model can be a powerful tool for the prediction of N₂O emissions from full-scale WWTPs and development of effective mitigation strategies, although it may require more efforts on model calibration.

Regarding biofilm systems, only a few studies are currently available at full scale. In the work of Fiat *et al.* (2019) and Zhu *et al.* (2019) tertiary nitrifying and denitrifying biofilters were modelled including the main N₂O biological pathways. Simulations were confronted to full-scale data from Seine Aval, the largest wastewater resource recovery facility in Europe. Zhu *et al.* (2019) obtained a satisfying prediction of the emission factor which was higher in the winter period (5.9%) than the value obtained in the summer period (2.9%), in accordance with experimental observations. Fiat *et al.* (2019) demonstrated that the model should include a mass balance on the gaseous phase in each reactor compartment of the BAF in order to correctly describe the N₂O gas-liquid partition and N₂O emissions. Preliminary modifications of the model structure were made to include the gas phase as a compartment of the model, which significantly affected the prediction of nitrification. In particular, considering gas hold-up influenced the prediction of the hydraulic retention time, and thus nitrification performances. Finally, the value of the volumetric oxygen transfer coefficient was adjusted to successfully predict both nitrification and N₂O emissions.

It should be noted that there are still only a limited number of studies presented in literature regarding the real application of N₂O models at full-scale WWTPs although many full-scale measurement campaigns have been performed in different places during recent years. More full-scale applications of the models using these full-scale N₂O data are still needed for the models to be developed into a useful tool for practical applications. In addition, the requirement for good fundamental knowledge on N₂O emission by the modeller/engineer might also hinder the N₂O model applications due to the complicated procedure for model selection and calibration, which consequently limit the development of effective mitigation strategies. Hopefully this chapter will facilitate the selection of suitable N₂O models, the estimation of site-specific N₂O emissions and the development of mitigation strategies for wastewater treatment plants taking into account the specific design and operational conditions of the plant.

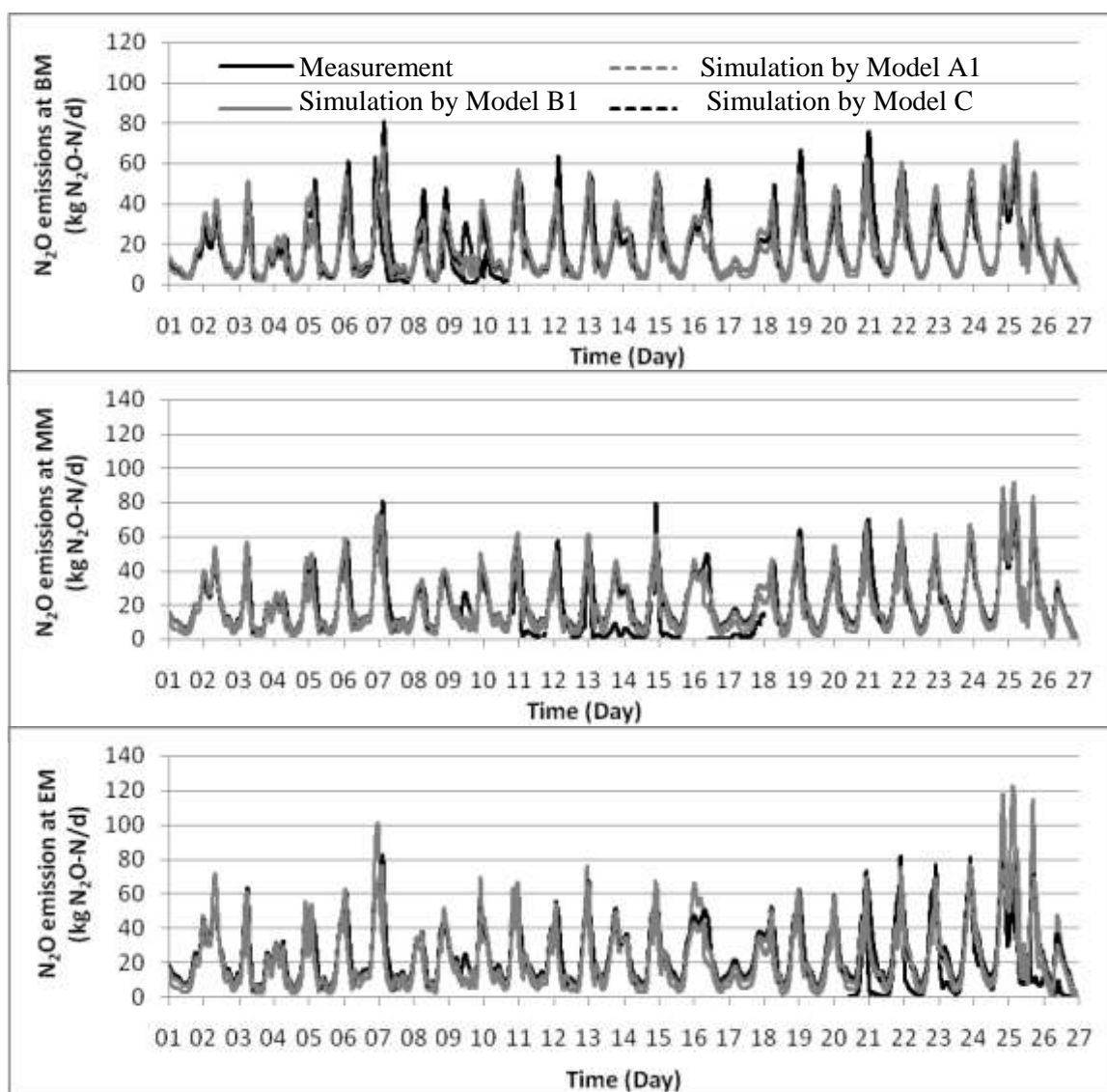


Figure 7.10 Model evaluation results for N_2O emissions using the measurement results at the beginning (BM) (upper panel), the middle (MM) (middle panel) and the end section (EM) (bottom panel) of the summer aeration package on the UCT process at the Eindhoven treatment plant by using ASM-type models that combine one of the single-pathway models of AOB (Models A1, B1 and C) with the ASMN (Model OHO-A) of heterotrophic denitrifiers (Spérandio *et al.*, 2016).

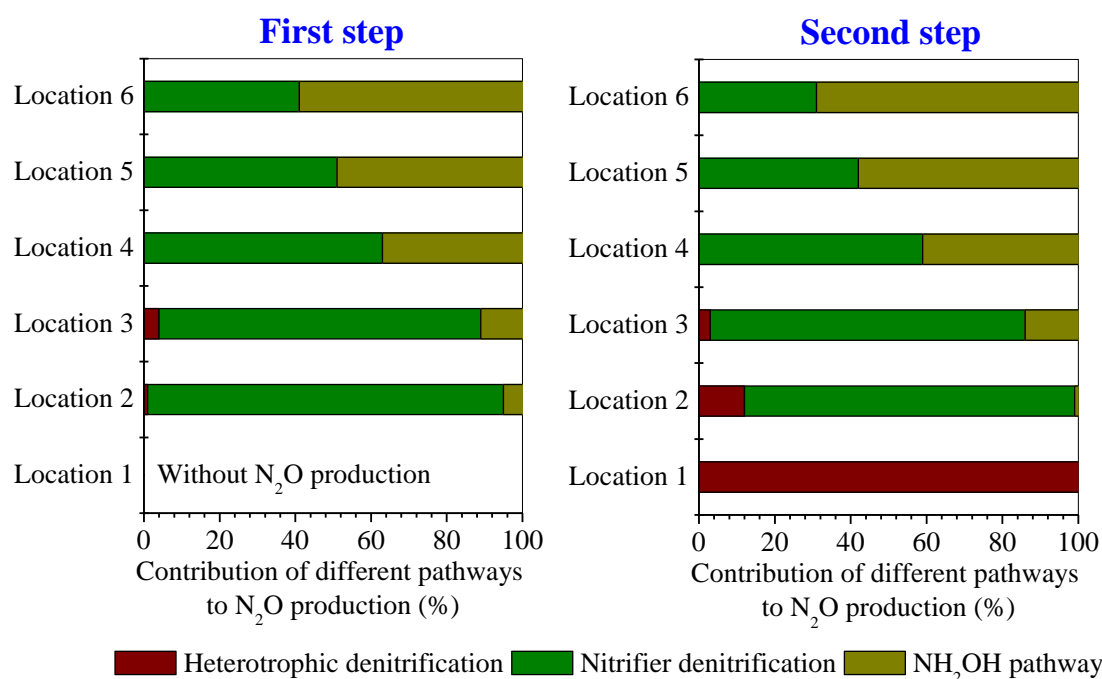


Figure 7.11 Model predicted percentage contributions from the three N₂O pathways to total N₂O productions at six different locations of the first step (left panel) and the second step (right panel) in the step-feed full-scale WWTP, i.e. the nitrifier denitrification pathway, the NH₂OH pathway and the heterotrophic denitrification pathway (Ni *et al.*, 2015).

7.4 CONCLUSIONS AND PERSPECTIVES

In this chapter, the existing N₂O models available in literature based on the three major N₂O production pathways were reviewed and compared to illuminate their structural differences, their capabilities and inabilities in describing experimental data, and their potential range of applications. The key conclusions are:

- Our understanding of fundamental mechanisms related to N₂O production and consumption has strongly progressed in recent decades, leading to the development of N₂O models with different mathematical structures but relatively similar metabolic pathways.
- For AOB, the two-pathway models have the potential to describe most of the N₂O data. The combined effect of DO and nitrite is described well by these models, either based on the direct or indirect coupling approach. In comparison, the single-pathway models can be used under several particular conditions depending on the concentrations of oxygen and nitrite which determine the dominating pathway. Despite calibration works still being necessary, recent studies have demonstrated good prediction capabilities for both lab-scale and full-scale observations. The uncertainties around parameters and their propagation on prediction should be considered appropriately.
- For heterotrophic denitrifiers, the ASMN-type model was the most used model for predicting the overall nitrogen and COD removal performance in the case where there is only low accumulation of intermediates, whereas new alternatives have been proposed recently and should be considered in the future. In a heterotrophic denitrifier biofilm, the potential for N₂O accumulation together with its consumption in the inner biofilm should be taken into account. The ASM-ICE type model has the potential to describe all N₂O data, but requires more information on reaction kinetics. Full-scale data sets still need to be properly consolidated by adding highly different reactor set-ups, measurement methods, culture history, documentation, and/or interpretations, which would limit the failure of model predictions. Numerous full-scale data sets are starting to be available for suspended growth systems. However, very few studies have identified emissions from biofilm systems.
- Future efforts should be devoted to comparing the multiple pathway models to data from real WWTPs to observe the key differences and to enhance their practical applications. Ideally, more information on pathway contributions should be collected in such systems (by means of isotope techniques, or NO:N₂O ratio variations).
- Although suspended growth models seem to capture N₂O emissions efficiently, biofilm mechanisms of N₂O production need further investigation. Only a few experiences with mathematical modelling of N₂O emission from biofilm systems have been reported and this should also be conducted using more monitoring data from such systems.
- Mathematical modelling of N₂O production has reached a maturity that facilitates the estimation of site-specific N₂O emissions and the development of mitigation strategies. Although existing models still have limitations, their application will undoubtedly increase in the near future. Their confrontation to full-scale data should improve the robustness of the parameters and would certainly suggest further model improvement. For

instance the coupling of an N₂O model with more detailed description of hydrodynamics and heterogeneities is probably a future need.

- Integration of N₂O models with the models describing other sections of the WWTPs into a plant-wide model could be a powerful tool for future optimization works.

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Nomenclature

ADP	Adenosine diphosphate	IC	Inorganic carbon
AMO	Ammonia monooxygenase	Mox	Electron carriers in oxidized form
AOB	Ammonia-oxidizing bacteria	Mred	Electron carriers in reduced form
ASM	Activated sludge model	N ₂ OR	N ₂ O reductase
ASM-EC	Activated sludge model – electron competition	Nar	Nitrate reductase
ASM-ICE	Activated sludge model with indirect coupling of electrons	ND	Nitrifiers denitrification
ASMN	Activated sludge model for nitrogen	Nir	Nitrite/nitric oxide oxidoreductase
ATP	Adenosine triphosphate	NirK	Nitrite reductase
COD	Chemical oxygen demand	NN	Hydroxylamine pathway
DO	Dissolved oxygen	NOB	Nitrite oxidizing bacteria
EF	Emission factor	NOR	NO reductase
FA	Free ammonia	OHO	Ordinary heterotrophic organisms
FNA	Free nitrous acid	SP	Site-preference Haem–copper nitric oxide reductase
HAO	Hydroxylamine oxidoreductase	sNOR	
		WWTP	Wastewater treatment plant