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Novel abiotic reactions increase nitrous oxide production during partial nitrification: Modeling and experiments

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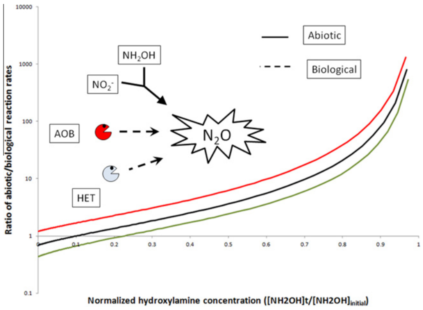
HIGHLIGHTS

- N_2O model incorporating abiotic hybrid reaction was newly developed.
- Abiotic hybrid reaction becomes dominant over N_2O emission when NH_2OH is limited.
- The developed model improves N_2O emissions estimates from partial nitrification.

GRAPHICAL ABSTRACT



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ABSTRACT

This research augmented the detailed Activated Sludge Model (ASM) to include abiotic hybrid nitrous oxide (N_2O) production due to reactions between hydroxylamine (NH_2OH) and nitrite. Model parameters were extracted from a series of abiotic and biological batch tests that showed that abiotic N_2O production rates are 1–3 orders of magnitude greater than biological N_2O production rates under observed experimental conditions. The mass transfer coefficients (k_La) were 19 h^{-1} and 21 h^{-1} for abiotic and biological tests, respectively. Second order reaction rate constants for N_2O indicated that the presence of activated sludge enhanced the rate of abiotic hybrid N_2O production. Experimental findings were supported by standard free energy estimates showing that the hybrid reaction (ΔG° hybrid = −538 kJ/mol) is more thermodynamically favorable than hydroxylamine oxidoreductase (HAO)-mediated oxidation of NH_2OH (i.e. ΔG° −247 kJ/mol). The revised model successfully predicted the ammonia-N removal rate (49 mg N/L-h) as well as the temporal position of the N_2O production observed from independently conducted dynamic loading experiments. Incorporation of abiotic hybrid N_2O production should significantly improve N_2O emissions estimates from partial nitrification systems but it will also alter the material and energy balance for nitrifiers because these abiotic reactions effectively re-route NH_2OH.

1. Introduction

Nitrous oxide (N_2O) emission from wastewater treatment plants is an issue of international concern. N_2O is a colorless gas that is generally not very chemically reactive, but it is a powerful greenhouse gas with radiative impacts that are 300-fold stronger than that of carbon dioxide [1]. Negative impacts are also caused by N_2O reactions with the ozone in the stratosphere, which exposes the earth to more of the ultraviolet radiation being emitted from the sun [1]. Concerns related to N_2O emissions from wastewater treatment systems have been met with a flurry of

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exploratory investigations and quantitative surveys intended to inform policy makers, practitioners, as well as the community at large. These broader concerns have also created the opportunity to discover important fundamental aspects of N_2O formation in wastewater treatment plants.

Previous efforts have established that N_2O emission is associated with biological nitrogen removal (BNR) processes. N_2O is generated by ammonia oxidizing bacteria (AOB), particularly at low dissolved oxygen (DO), or high nitrite or nitrate concentrations [2–6]. N_2O can also be generated by heterotrophic bacteria during denitrification when the reduction of nitrate-N is incomplete. There is a link between N_2O emissions and the DO concentration in wastewater [2,3,6]. These efforts have led to the development of models that have been incorporated in the general ASM model for biological wastewater treatment analysis [7–10]. However, these models are based only on biological reaction mechanisms.

There is evidence that N_2O can be abiotically produced. The earliest evidence was likely that of Anderson 1964, who generated N_2O by rapidly degrading hydroxylamine in the presence of air and low concentrations of copper [11]. Samarkin et al. [12] and Kampschreur et al. [13] both showed that the iron and nitrogen redox chemistries occurring in soil and fully operational bioreactors, respectively, were coupled, and that significant levels of N_2O production can be derived from abiotic interactions. Heil et al. [14] recently collected data suggesting that iron drives abiotic N_2O production in soils. These studies are the most direct evidence for abiotic production of N_2O but there have been others that have implicated abiotic N_2O production. For example, Stuven et al. [15] pointed to the possibility of abiotic N_2O production as a way of closing the mass balance for a nitrification experiment with *Nitrosomonas europaea*. These findings make it clear that abiotic N_2O production is possible, but there is a need to collect more data related to abiotic N_2O production in order to develop models that can eventually be incorporated into the general ASM framework.

A most concerning situation for abiotic N_2O production emerges when nitrite concentrations are allowed to reach high levels. Nitrite acts as a weak base and it can participate in chemical reactions as either an oxidizing or reducing agent. Nitrite accumulation has been documented in partial nitrification systems, in which nitrite oxidation is deliberately avoided [16–18]. Nitrite accumulation creates conditions that may promote reactions with other nitrogen intermediates. Recent work by Terada et al. [19] addressed these concerns by carrying out a wide range of experiments to elucidate N_2O production mechanisms under conditions of high nitrite concentration. They found that abiotic N_2O production can occur at pH 7 via a novel hybrid pathway involving the direct participation of hydroxylamine and nitrite: $NH_2OH + HNO_2 \rightarrow N_2O + 2H_2O$ [19]. This reaction, also found in soil but at acidified pH [14], is distinctly different from the abiotic chemistry discussed by previous researchers [13,20,21]. Namely, they described Fe-driven N_2O production as being the result of the oxidation of hydroxylamine (e.g. $4Fe(III) + 2NH_2OH \rightarrow 4Fe(II) + N_2O + H_2O + 4H^+$) producing either N_2O or NO. Thus the abiotic hybrid reaction is new and requires further examination. In light of this new discovery and as well as the considerable evidence supporting abiotic N_2O production, it is now prudent to amend the available process models to include abiotic processes. Such efforts will help avoid underestimating N_2O production.

The objectives of this work are to (1) determine model parameters describing abiotic hybrid N_2O production, (2) use measured data to evaluate model predictions, and (3) determine the effect of abiotic hybrid reaction on N_2O production predictions in biological wastewater treatment systems.

2. Materials and methods

2.1. Model development and simulations

Abiotic hybrid N_2O production is modeled as follows:

$$\partial S_{n2o}/\partial t = k_1\ S_{nh2oh}\ S_{no2} - \mathrm{k}_L\mathrm{a}\ S_{n2o} \tag{1}$$

This expression accounts for two processes: (1) a second order chemical reaction dependent on concentrations of nitrite and hydroxylamine ($\Delta G° = -270$ kJ/mol [22]), and including the second order reaction rate constant k_1 ($M^{-1}\ h^{-1}$), and (2) mass transfer to the gas phase, which requires a volumetric mass transfer coefficient $\mathrm{k}_L\mathrm{a}$ (h^{-1}). The values of k_1 and $\mathrm{k}_L\mathrm{a}$ were retrieved by fitting data that was generated previously [19]. The normalized root mean square error (NRMSE) was calculated for every possible combination of k_1 and $\mathrm{k}_L\mathrm{a}$ values, and the values selected for modeling were those associated with the smallest NRMSE.

The model used to describe abiotic hybrid N_2O production in the presence of biological activity was as follows:

$$\begin{aligned}\partial S_{n2o}/\partial t &= k_1\ S_{nh2oh}\ S_{no2} - \mathrm{k}_L\mathrm{a}\ S_{n2o} \\ &+ k_2\ X_{aob}[S_{no}/(S_{no} + K_{no})][S_{nh2oh}/(S_{nh2oh} + K_{nh2oh})]\end{aligned} \tag{2}$$

Monod-type expressions were incorporated to account for the fact that AOB can reduce NO while oxidizing NH_2OH to produce N_2O. The biological term includes the maximum specific rate of N_2O production (k_2), the AOB biomass concentration (X_{aob}), and half saturation coefficients for nitric oxide (K_{no}) and hydroxylamine (K_{nh2oh}). Parameter values were extracted from batch test data previously reported by Terada et al. [19] by minimizing the NRMSE.

Eq. (1) was then incorporated into a multi-population suspended growth model constructed using MATLAB 8.2 R2013b as the computational platform. The model includes rate expressions describing the action of autotrophic ammonia oxidizing bacteria (AOB), and heterotrophic bacteria (HET, X_{het}). The kinetic and stoichiometric matrices for the mathematical model are provided in Table S1 (Supplemental materials). The parameters used in the model were retrieved from the literature or otherwise determined as shown in Table S2 (Supplemental materials).

The key AOB processes include (1) ammonia removal, described with a multiplicative Monod-model to account for the impacts of both the oxygen and ammonia concentration; (2) cell growth, described with a multiplicative Monod-terms to account for the concentrations of both hydroxylamine and oxygen; (3) AOB denitrification with nitrite as the terminal electron acceptor; (4) AOB denitrification with N_2O production. The key HET denitrification processes are separated into four distinct steps as described previously [8]. Endogenous decay is also included in metabolisms of AOB and heterotrophic bacteria.

2.2. Experiments

The effects of hydroxylamine concentrations and constituents in synthetic wastewaters on abiotic hybrid N_2O production were investigated. The medium consisted of (in mg/L): $NaNO_2$ (1970), $MgSO_4{\cdot}7H_2O$ (280), KH_2PO_4 (27), $CaCl_2{\cdot}2H_2O$ (120), NaCl (600), $FeSO_4{\cdot}7H_2O$ (3.3), $MnSO_4{\cdot}H_2O$ (3.3), $CuCl_2{\cdot}2H_2O$ (0.8), $ZnSO_4{\cdot}7H_2O$ (1.7), and $NiSO_4{\cdot}6H_2O$ (0.3), modified by elsewhere [23], ensuring NO_2^- concentration of 400 mg N/L. Three hundred milliliter of medium was poured to a cylindrical vessel with a volume of 500 mL, where air was supplied at a flow rate of 1 L/min. For tests on the effect of hydroxylamine concentration on N_2O production, different amount of hydroxylamine was added to ensure the initial concentration of 0.1 and 20 mg N/L with the similar range according to the previous works [24,25]. For the biotic N_2O production test, nitrifying biomass acclimated in a lab-scale activated sludge system was inoculated [4]. During the biotic batch tests, the pH was 7.8 and the DO concentration was 0.5 mg/L. For tests on the effect of the synthetic wastewater constituents on N_2O production, a metal constituent in the medium, i.e., Fe, Mn, Cu, Zn, and Ni, was excluded in each run and abiotic N_2O production was confirmed by spiking 20 mg N/L of hydroxylamine. For these tests, dissolved N_2O was continuously measured by an N_2O microsensor (Unisense, Aarhus, Denmark) at an interval of 1–5 min.

3. Results and discussion

3.1. Modeling abiotic hybrid N_2O production

The data retrieved from Terada et al. [19] showed clearly that N_2O is produced when nitrite and hydroxylamine were mixed at different initial NH_2OH concentrations (Fig. 1). Chemical reduction of nitrite occurred in parallel with the oxidation of hydroxylamine. Using this data set, parameters values associated with Eq. (1) were retrieved as described earlier; the best-fit values were $k_1 = 7\ \mathrm{L\ g\ N^{-1}\ h^{-1}}$ and $k_La = 21\ \mathrm{h^{-1}}$. The N_2O k_La value is close to the values (up to $17\ \mathrm{h^{-1}}$) previously published [26,27]. It should be noted that k_La can vary greatly from one system to another because of differences in the manner and intensity of the mixing occurring in the bulk liquid, which in turn impacts the thickness of the mass transfer boundary layer. Second order rate constants are highly dependent on pH, ionic strength, and temperature and thus wide ranges of values are possible. Second order rate constants for the oxidation of hydroxylamine to N_2O can be found over a broad range (i.e. $0.001–128\ \mathrm{L\ g^{-1}\ h^{-1}}$) with higher values found at $\mathrm{pH} < 2$ [28,29]. Previous research has also suggested that the presence of iron can accelerate the oxidation of hydroxylamine [29,30]. This is notable because previous research has suggested that soluble iron is the catalyst that drives abiotic N_2O production in wastewater [12,13]. In this study, we conducted control experiments that showed that divalent copper ion rather than ferric and/or ferrous ion was involved in the abiotic N_2O production (Fig. S1). Metal catalysts like ferric/ferrous ion or in some cases divalent copper ion can drive abiotic N_2O production.

The experiments reported by Terada et al. [19] also included N_2O generation in the presence of activated sludge (i.e. abiotic/biological tests), and these tests generated higher concentrations of N_2O than the abiotic tests. For example, when 20 mg N/L hydroxylamine was added to 400 mg N/L of nitrite, soluble N_2O concentrations of up to 5 mg N/L were observed, which is significantly higher than the 1.47 mg N/L that was measured during the abiotic test (Fig. 2). Similar results were observed when lower concentrations of hydroxylamine were introduced. The presence of activated sludge appeared to cause higher N_2O production (Fig. 2). These biological test data were used for model fitting to determine the parameters featured in Eq. (2). The parameter values for the abiotic/biological model were $k_1 = 16\ \mathrm{L\ g\ N^{-1}\ h^{-1}}$, $k_La = 19\ \mathrm{h^{-1}}$, $K_{nh2oh} = 19.5$ mg N/L, $K_{no} = 24.6$ mg N/L, and $k_2 = 1.1$ mg N/mg VSS/h. The k_1 value is approximately 2.3 times greater than the k_1 value associated with the abiotic experiments. This shows that activated sludge accelerated the abiotic hybrid N_2O formation rate. Kampschreur et al. [13] made a similar suggestion when they correlated N_2O production with the appearance of iron precipitates. The k_La for the abiotic/biological tests is in good agreement with the previous k_La determined from the abiotic tests and with previously published values from Foley et al. [27]. The half saturation coefficients for NH_2OH and NO are *extant* (not intrinsic) values, and they are therefore not related to the intrinsic biological affinity of these substrates but instead they are a reflection of the effects of diffusion on N_2O process kinetics; a similar observation was made during a previous study of denitrification kinetics [31]. The maximum biological rate of N_2O production (k_2) has a value that is

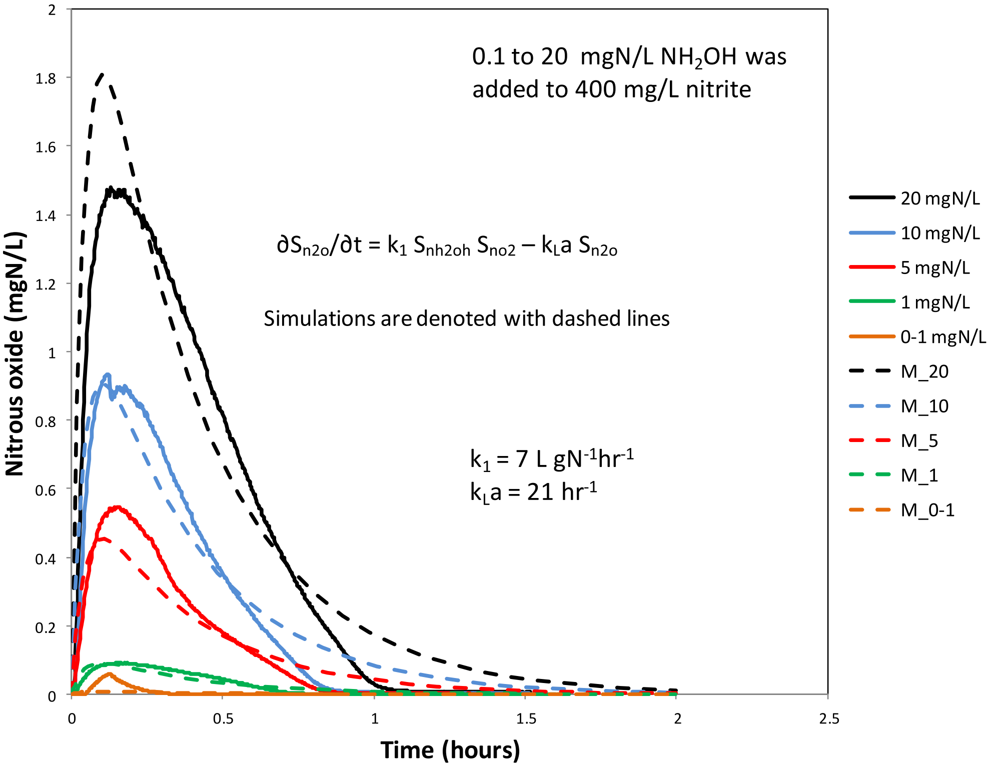


Fig. 1. Abiotic nitrous oxide production, hydroxylamine reacting with 400 mg N/L nitrite.

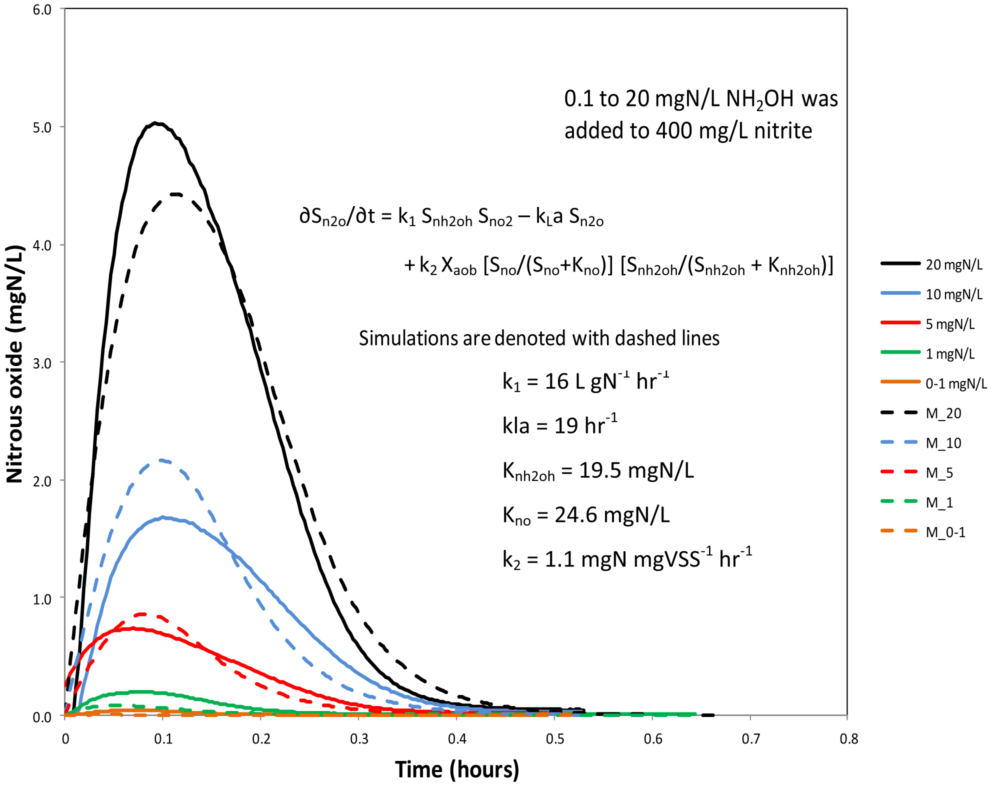


Fig. 2. Nitrous oxide production in the presence of activated sludge.

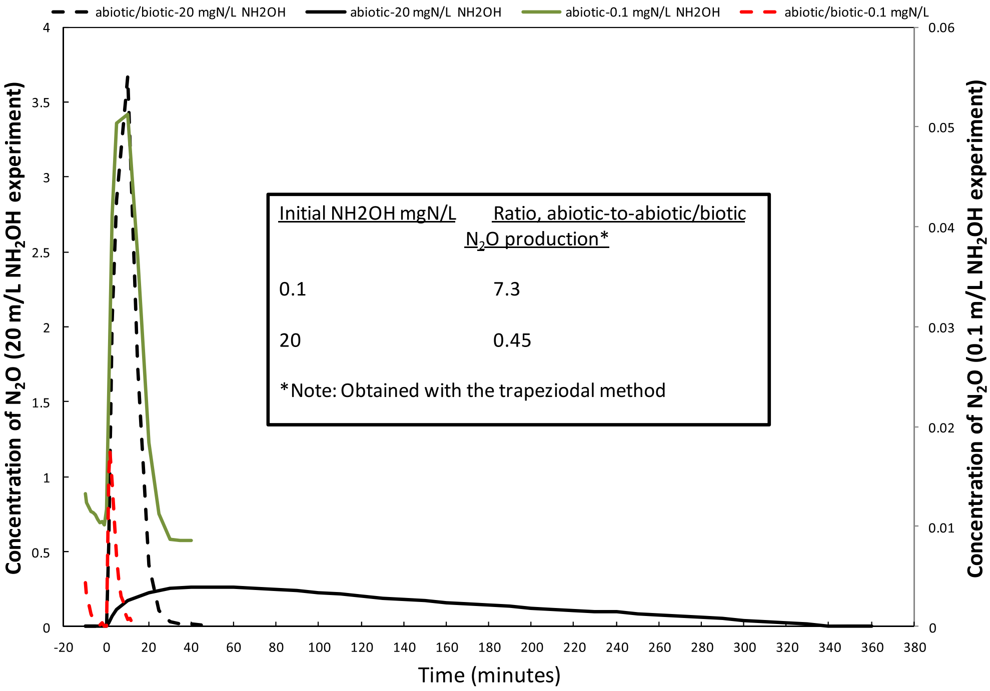
two orders of magnitude greater than what would be expected from nitrifier denitrification pathways, 0.28 mg N_2O–N/mg VSS/h [8]. Thus k_2 value may be impacted indirectly by the Cu-related catalysis mentioned earlier (i.e. Fig. S1) or there may be undiscovered or newly emergent biological mechanisms that are responsible for N_2O production in this data set. For example, Stieglmeier et al. [32] recently demonstrated that ammonia-oxidizing archaea aerobically generate N_2O using both ammonium and nitrite in a 1:1 M ratio. Such mechanism may be present in AOB, which warrants future investigation [32].

The aforementioned model parameters were used to calculate the abiotic (i.e. k_1 S_{nh2oh} S_{no2}) and biological (i.e. k_2 X $[S_{no}/(S_{no} + K_{no})]$ $[S_{nh2oh}/(S_{nh2oh} + K_{nh2oh})]$) N_2O production rates; Fig. S2 (Supplemental materials) shows the ratio of these two rates as a function of the normalized hydroxylamine concentration (i.e. $NH_2OH_{(t)}/NH_2OH_{initial}$). The ratio of these two rates as a function of time is also provided (Fig. S3).The production rate ratio was much greater than 1 for most of the experimental conditions, which indicates that the abiotic production rate was the primary driver for N_2O production in the batch experiments. This modeling result is consistent with experimental findings showing that the initial NH_2OH concentration determined the degree of the abiotic contribution to N_2O production (Fig. 3). A smaller amount of NH_2OH (0.1 mg N/L at the beginning) increased the relative contribution of abiotic N_2O reaction. The reason for this is because the abiotic term depends on the nitrite and hydroxylamine concentrations, while the biological term depends on the hydroxylamine concentration and the *nitric oxide* concentration, which is initially zero and must be biologically produced from the oxidation of hydroxylamine under, in this case, an aerobic condition before the biological N_2O production rate can increase [33]. This explanation is illustrated in Fig. S4 (Supplemental materials), which shows the relationship between the abiotic/biological N_2O production rate ratio and the nitric oxide concentration. N_2O production eventually becomes biologically-dominated when 20 or 10 mg N/L hydroxylamine is added because of the NO levels associated with these trials. Hydroxylamine is highly reactive [23] so that only relatively low (i.e. ppb or low ppm) levels are expected in bioreactors; the current findings show that N_2O production under such conditions, e.g. in a partial nitrifying bioreactor, is abiotically-dominated. In general, abiotic hybrid N_2O production would likely occur concomitant with the other reported N_2O production pathways [7,34–36].

It is important to determine whether the observations documented in this study are supported by the thermodynamic characteristics of the abiotic and biological pathways that compete for NH_2OH. To this end, the thermodynamic favorability of the hybrid reaction can be compared to that of the HAO-mediated oxidation of NH_2OH, well-known to be −247 kJ/mol [37]. Using literature-based estimates for the standard free energy (ΔG°) of formation (i.e. $\Delta G^{\circ f}$) of the dissolved species of HNO_2, NH_2OH, and N_2O, the standard ΔG° of formation was estimated as follows:

$$\begin{aligned}\Delta G^\circ_{hybrid} &= 2^*\Delta G^{\circ f}_{H_2O} + \Delta G^{\circ f}_{N_2O} - \left(\Delta G^{\circ f}_{HNO_2} + \Delta G^{\circ f}_{NH_2OH}\right)\\ &= 2 * (-237\ \text{kJ/mol}) + 100.8\ \text{kJ/mol}\\ &\quad -(-55.6\ \text{kJ/mol} + 221\ \text{kJ/mol}) = -538\ \text{kJ/mol}\end{aligned}$$

where $\Delta G^{\circ f}_{H_2O} = -237$ kJ/mol [38], $\Delta G^{\circ f}_{N_2O} = 100.8$ kJ/mol, [39], $\Delta G^{\circ f}_{HNO_2} = -55.6$ kJ/mol [40], and $\Delta G^{\circ f}_{NH_2OH} = 221$ kJ/mol (calculated from data shown in [41]). The ΔG° associated with the abiotic hybrid reaction ($\Delta G^\circ_{hybrid} = -538$ kJ/mol) is more favorable than the ΔG° associated with HAO-mediated oxidation of NH_2OH (i.e. −247 kJ/mol, [37]). This ΔG°_{hybrid} estimate must be regarded as tentative because the appropriate $\Delta G^{\circ f}$ values for the dissolved form of the key nitrogenous species (i.e. HNO_2, NH_2OH, and N_2O) have not been formally tabulated (e.g. [38]) which indicates that consensus



Initial NH2OH mgN/L	Ratio, abiotic-to-abiotic/biotic N_2O production*
0.1	7.3
20	0.45

*Note: Obtained with the trapeziodal method

Fig. 3. The effect of the initial hydroxylamine concentration on abiotic and biological N_2O production.

is not yet established. The observed ΔG values will, of course, also depend on the intra- and extra- cellular concentrations of the reacting constituents and on temperature. However, allowing for the aforementioned stipulations, the abiotic hybrid reaction appears to be more thermodynamically favorable than HAO-mediated oxidation of NH_2OH, which is in keeping with the findings of this study (i.e. Figs. 1–3, S2–S3).

3.2. Comparing model predications to batch test data

A revised model (i.e. Harper et al., model) was constructed by incorporating Eq. (1) into the general ASM model presented by Ni et al. [8] and then further expanded to include N_2O mass transfer to the gas phase. Model predictions were then compared to data retrieved from independently conducted batch tests in order to evaluate the model's capability to predict dynamic behavior. Fig. 4 shows predictions generated from both the revised and the unmodified (i.e. Ni et al. [8]) models. Because nitrate was not detected at all, it is not shown in this figure. The revised model captured the primary features shown by the nitrogen transformations, including ammonia removal, nitrite production, and biotic/abiotic N_2O production [16]. The initial ammonia-N uptake rate (49 mg N/L-h) was predicted well by both models, but after 1 h the measured ammonia N concentrations were noticeably higher than predicted by the model, perhaps because of a high abundance of AOB with a higher ammonia half saturation coefficient than the value (K_{nh4} = 2.4 mg N/L) used in the model; Terada et al. [4] previously found that AOB enriched from SBRs had a relatively high half saturation coefficient of 28.9 mg N/L, which is close to the value for the *Nitrosmonas europaea* culture (26.6 mg N/L) [42]. The nitrite profile generated by the current model captured well the overall trends reflected in the measured data. The initial nitrite production rate agreed with that of the current model but after 1 h differences were again observed possibly because of dispersion and difference in affinity of ammonia. The measured N_2O profiles showed three distinct peaks at times of 2, 14, and 17 h. The current model successfully predicted the temporal position of these peaks, however the maximum N_2O concentrations that were 2–5 times larger than the measured values; this discrepancy between the model and measured values is likely because the injection of NH_2OH increased hydraulic turbulence, decreasing mass transfer resistance and causing elevated evaporation of newly-formed N_2O. The Ni et al. [8] model overestimated the nitrite levels and failed to predict the position or magnitude of the N_2O signals. The reason for this is DO, which was between 0.79 and 4.30 mg/L during the batch test. The Ni et al. [8] model predicts negligible N_2O production under such conditions because of the inhibiting effect of DO (Processes 3 and 4 in Table S1). The revised model captures these N_2O peaks via abiotic hybrid mechanisms, as listed in Process 12 in Table S1, despite discrepancies in terms of the peak N_2O concentration. Nitrite injection at 9 h had no effect on N_2O production as expected, while hydroxylamine injections induced N_2O production consistent with biological and abiotic hybrid N_2O production mechanisms. These results were confirmed with a second batch test (Fig. S5). This observation implies noticeable involvement of hybrid reactions with two different nitrogen constituents.

3.3. Process implications

These results have clear implications on N_2O emissions estimates, but there are also direct and significant implications for the analysis and design of partial nitrification systems because abiotic hybrid reactions alter the flux of NH_2OH, a central intermediate observed during nitrification. NH_2OH is produced by the membrane-bound enzyme ammonium-monooxygenase (AMO) by the incorporation of oxygen into NH_4^+, and it is consumed by the periplasmic enzyme hydroxylamine oxidoreductase (HAO) to produce energy (i.e. ATP) and as well as the reducing equivalents (i.e. NADH) that are required to construct biomass and to drive additional NH_4^+ oxidation [43]. The well-established conceptual and mathematical models for nitrification are based upon the assumption that NH_2OH is available to participate in these enzymatically-mediated reactions but this notion is not necessarily applicable to a partial nitrification system. Given that NH_2OH oxidation by AOB produces electrons to be distributed for ammonia

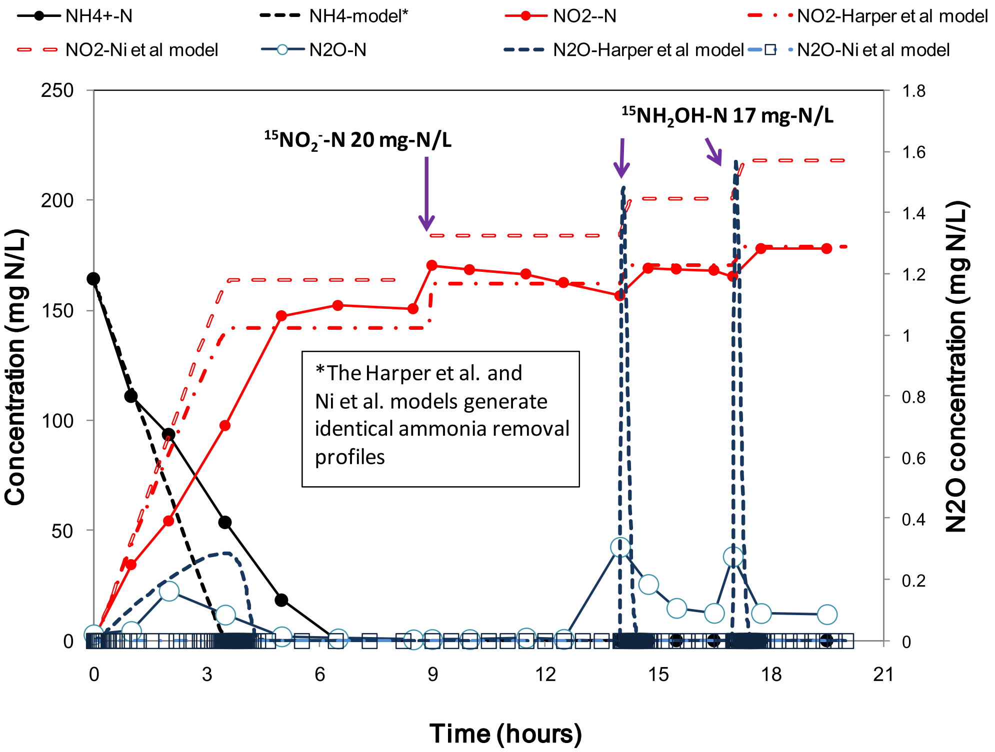


Fig. 4. Nitrogen transformations during partial nitrification, measured data and model simulations.

oxidation and consecutive denitrification steps, the model incorporating such electron transport processes has been developed [10]. On the contrary, the current work shows that NH_2OH can be abiotically removed to generate N_2O, which means that some fraction of NH_2OH will not be available for metabolic pathways involving the production of NADH and ATP. Removal of NH_2OH via abiotic reactions will therefore diminish the synthesis of AOB. When partial nitrification systems are being designed, practitioners should account for abiotic removal of NH_2OH. This correction will not only improve N_2O estimates (as demonstrated in this article), but it will also impact the determination of design parameters that depend on biomass yield (e.g. sludge production, aeration and nutrient requirements, clarifier loading rates). Abiotic hybrid reactions may also impact biological treatment in ways that are not yet understood. Future efforts should address this issue by investigating the effect of abiotic hybrid reactions on the mass, energy, and reducing equivalent balances for various strains of AOB. Such studies can be carried out using a combination of isotopic experiments (e.g. [19]) and metabolic flux analysis [44].

4. Conclusions

This research analyzed batch data taken during partial nitrification to retrieve the parameters needed to model abiotic N_2O production due to reactions between hydroxylamine and nitrite occurring at circum-neutral pH. Analysis of batch data showed that the rates of abiotic N_2O generation were significantly higher than the biological contribution, and that the relative contribution from abiotic processes increased as the hydroxylamine concentration decreased. The revised form of the detailed ASM successfully predicted the rates of ammonia-N removal and nitrite-N production as well as the temporal position of the N_2O emissions produced from independently conducted dynamic loading experiments with nitrifying biomass. These results are particularly notable because they demonstrate that N_2O can be correctly predicted when DO is between 0.8 and 4.3 mg/L, an important and operationally relevant DO range. The revised model is expected to significantly improve N_2O emissions estimates from partial nitrification systems in which nitrite is forced to accumulate.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2015.06.109.

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