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The role of ammonia oxidizing bacteria in Nitrous Oxide (N2O) emissions: a modeling approach for estimating stoichiometric O2 requirements

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**Introduction:**

*Why nitrous oxide is a major concern:*

According to U.S. Environmental Protection Agency (US EPA), wastewater treatment plants (WWTP) and sewage are major sources of greenhouse gases contributing to global warming, including methane and nitrous oxide (N2O) (Foley 2010; U.S.EPA 1996). N2O has a global warming potential equivalent to 310 kg CO2 per kgN2O (Adouani et al. 2010). With a life time that is approximately 120 years (IPCC 2006), the atmospheric N2O concentration is increasing by 0.2 - 0.3% per year (Kishida et al. 2004; Barbaree and Payne 1967). In addition, N2O can be converted to NO by UV light. NO can react with ozone in the stratosphere to form NO2- which creates nitrous acid (HNO2) and contributes to acid rain which causes eutrophication (Aber et al 1989). WWTPs focus on degrading nitrogen compounds via nitrification and denitrification which both contribute to N2O emissions (Foley 2010; Tallec et al. 2006). Despite evidence that these two processes contribute to N2O production, the Intergovernmental Panel on Climate Change (IPCC) does not include N2O when assessing emissions contributing to global warming from WWTPs (IPCC, 2006a). For these reasons, it is paramount that anthropogenic N2O influx is better understood and minimized in future applications.

*Factors influencing N2O emissions:*

Nitrification and denitrification play key roles in the nitrogen cycle. Nitrification produces nitrate (NO3-) which is essential for plants, and denitrification produces nitrogen gas (N2) which does not harm the environment.However, they are major contributors to N2O emissions and often occur in conjunction with one another (Kim 2010; Kampschreur et al. 2009). N2O production from these processes is influenced by several factors including: pH, chemical oxygen demand (COD), C/N ratio, sludge retention time (SRT), temperature, and O2 concentrations (Foley 2010; Park 2000; Khalil 2004).

Nitrification primarily results from the oxidation of ammonia (NH3) or ammonium (NH4+)to other oxidized nitrogen compounds such as nitrite (NO2-) and nitrate. In the first stage of nitrification, NH4+ is oxidized to hydroxylamine (NH2OH) and then oxidized further to nitrite by ammonia oxidizing bacteria (AOB) (Barton and Atwater 2002; Wrage et al. 2001). The oxidation of nitrite by nitrite oxidizing bacteria (NOB) follows as the second step of nitrification, generating nitrate. N2O may be produced as a byproduct during the oxidation of hydroxylamine as a result of dimerization or dehydration of intermediates (See Figure 1). Denitrification primarily results from the reduction of NO3- or NO2- to other reduced nitrogen compounds including NO, N2O, and N2(Metcalf & Eddy Inc 2003). During denitrification, enzymatic reductases function to reduce NO3- to N2 through several intermediate steps (Bhunia et al. 2010; Wrage 2001; Hochstein and Tomlinson 1988). Since N2O is an intermediate of this process, incomplete denitrification may cause N2O emission to the environment (See Figure 2) (Wrage 2001).

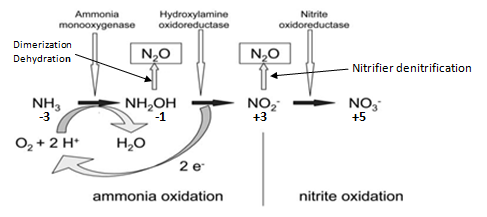


Figure Nitrification pathway (Bhunia et al. 2010)

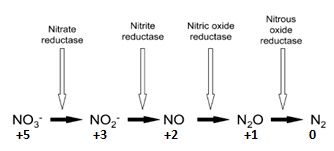


Figure 2 Denitrification pathway (Bhunia et al. 2010)

*N2O properties:*

The solubility of N2O in water is 0.15g/100ml at 15C (IPCS INCHEM 2003). In addition, N2O has a mass transfer coefficient similar to oxygen (Foley 2010). Taking this into consideration, we assume that the proportionate mass transfer emissions of N2O–N and O2, coupled with the solubility of N2O, results in a net contribution of N2O to atmosphere predominantly in the aerated zones in WWTPs.

- **During nitrification process**

*Role of O2:*

Nitrification was found to be the predominant source of N2O production along an estuary, with O2 concentration accounting for location and intensity of N2O production (Wilde 2000). For example, O2 levels between 25-100 µmol/l have been observed to generate the most N2O in nitrification processes (Wilde 2000). Khalil also showed that low dissolved O2 (DO) due to low pressure produced the most N2O (2004). Therefore, it is predicted that nitrification may be inhibited by lack of O2 at low DO concentrations (0.5-2.0 mol O2: 1.0 mol NH3) generating N2O.

NH4+ concentrations are a good indicator of N2O concentration in aerated scenarios. The amount of N2O-N produced by nitrification per amount of NH4-N oxidized forms a linear relationship; however, the slope of this relationship is directly related to O2 levels, with lower O2 levels resulting in higher N2O production (Khalil 2004). We hypothesize that NH4+ may be converted to N2O during nitrification because of reactions that occur with intermediates (figures 1 and 5). In addition, NH4+ is only partially oxidized in regions with low O2, which results in reduction via either nitrifier denitrification or is transferred to anaerobic regions where it is reduced to N2O by denitrification (Khalil 2004; Goreau et al. 1980).

**-During denitrification process**

*Role of O2:*

During the denitrification process, N2O is an intermediate thus incomplete denitrification can lead to N2O accumulation (Bhunia et al. 2010; Kampscheur et al. 2009). Usually, N2O reduction is done by enzymes called nitrous oxide reductases; however, O2 inhibits both synthesis and activity of nitrous oxide reductases (Bonin et al. 2002). Importantly, nitrous oxide reductases are inhibited to a greater extent than other denitrification enzymes in the presence of O2 which results in a higher production of N2O than reduction of N2O; resulting in a trend of N2O emissions during denitrification if oxygen is present at low concentrations (Otte et al. 1996). These trends were also observed by Foley who concluded that the largest spikes in N2O production occur at the beginning of the aerobic phase when O2 levels are only slightly above zero (2010). Aerobic denitrification is known to occur in the following organisms which use NO3- as a terminal electron acceptor in the presence of O2: Pseudomonas, Aeromonas, Moraxella, Paracoccus, Microvirgula, and Arthrobacter (Robertson et al. 1989). In addition, aerobic denitrification may occur as a result of autotrophic nitrifying bacteria like Nitrosomonas which can use O2, NO3- and NO2- as terminal electron acceptors (Robertson et al. 1989).

Lack of O2 may limit nitrification and enable NO3- and NO2- to be used as electron acceptors (Tallec et al. 2006; Kampschreur et al. 2008). For example, AOB are generally nitrifying bacteria, but also contain genes responsible for denitrification (nirK, norB, norC) (Cantera 2007; Garbeva 2007). AOB may use nitrite as a terminal electron acceptor to preserve O2 for ammonia oxidation to hydroxylamine in low O2 conditions (Shaw 2006). The denitrification of nitrite by AOB during nitrification process is called nitrifier denitrification (See Figure 3) (Kim 2010; Hanaki et al. 2001; Wrage 2001). By following this pathway, autotrophic AOB can continue growth under low O2 concentrations that are not high enough to maintain their required O2 supply.



Figure 3 Nitrifier denitrification: outline of the pathway and enzymes involved

*Impact of NO2-:*

High concentrations of NO2- resulted in a substantial increase in N2O production when small quantities of O2 were present, indicating that NO2- reduction is a major contributor to N2O production (Foley 2010; Wrage 2001). Therefore, it is presumed that accumulation of NO3- and NO2- coupled with incomplete denitrification may cause an increase N2O concentration. This can be explained by two possible mechanisms. The first one is that microorganisms selectively reduce NO2- rather than N2O because high intracellular level of NO2- is toxic (Ritchie and Nicholas 1972). The second is that NO2- is a more favorable electron acceptor than N2O.

*Other factors:*

Nitrous oxide reductase is inhibited by lower pH (below 5.5) (Thron and Sorensson 1996), and high hydrogen sulfide concentration (Schoharting et al. 1998). Also, N2O emission occurs in denitrification process when the availability of electron donors is insufficient to fully reduce NOx compounds via heterotrophic denitrification (Bhunia et al. 2010; Kampscheur et al. 2009). According to a study about N2O emission with respect to COD/N (as NO3-), N2O was not detected when COD/N is greater than 4, and N2O emissions were maximized at N2O: COD/N = 1.5 (Hanaki et al. 1992). Figure 4 shows a review of the factors that contribute to N2O emission from nitrification and denitrification (Kampschereur et al. 2008).

Overall, N2O emission is related to both the nitrification and denitrification processes. N2O production seems to be related to both nitrifier denitrification in low O2 concentration (NO2- 🡪 N2O) (Khalil 2004) and incomplete denitrification (Bhunia et al. 2010; Kampscheur et al. 2009). However, Ahn *et al*, during an extensive study of WWTPs across America, discovered that most N2O production occurred in the aerobic stage (2010). From this evidence, we hypothesize that most N2O emissions result as a byproduct from AOB during nitrification at WWTPs. The impact of heterotrophic nitrifying bacteria genera on N2O emission is unclear and will not be considered in this paper (Zhou et al 2008). Nevertheless, in this paper, we postulate that N2O production occurs under low O2 conditions from either nitrification as the result of dimerization and dehydration occurring with the intermediates HNO and HN(OH)2, or from nitrifier denitrification which occurs when AOB substitute NO2- as their terminal electron acceptor.

Low O2 High nitrite

High O2 High nitrate Low COD/N

- Insufficient aeration

- High organic loading (combined with insufficient aeration)

- Insufficient aeration

- Low SRT

- Toxic compounds

- Low temperature

- High ammonium conc.

- over-aeration :

nitrifying stage.

- COD limitation

- Nitrite transfer from nitrification stage

- Influent characteristics

- Too efficient pre-sediment

N2O

Nitrification Stage

Denitrification Stage

Figure 4 Summary of conditions which enhance N2O emission (Kampschereur et al. 2008).

‘Low’ or ‘High’ concentrations are in relation to optimal concentrations for both nitrification and denitrification.

*Objectives:*

The main objective of this paper is to estimate N2O emissions resulting from AOB during nitrification. AOB are proposed to be the main contributing organisms for N2O production since N2O emissions rates are often higher in aerated zones than they are in anoxic zones of WWTPs (Ahn *et al* 2010). We assume that the primary factor for N2O emission is O2 concentration, with low O2 concentrations being the main contributing factor. O2 concentrations are assumed to play a critical role for two reasons:   
1. Low O2 concentrations will inhibit nitrification, resulting in the dimerization and dehydration of intermediates.  
2. Under low O2 concentrations, AOB will replace O2 with NO2- as the terminal electron acceptor resulting in nitrifier denitrification (Kim 2010; Hanaki et al. 2001; Wrage 2001).  
Taking this these assumptions into consideration, we have developed a stoichiometric model that predicts N2O emissions based on a mol O2: mol NH3 ratio. Our model reveals that factors contributing to N2O emissions such as ammonia and nitrite concentrations are highly linked to O2 concentrations. This model was constructed by extrapolating many possible pathways for N2O production including the proposal of new pathways involving dimerization and dehydration of intermediates.

**Results and Discussion**

*1. Ammonium oxidation pathways by AOB:*Typically, ammonium in liquid is oxidized by mono oxygenation which is;

NH3 + 0.5O2 🡪 NH2OH (1)

As a result, one mole of hydroxylamine is generated. Hydroxylamine oxidation is done by a specific enzyme called hydroxylamine oxidoreductase (HAO) (Kim et al. 2010). Depending on HAO’s molecular structure and properties (especially heme cofactors), which will be discussed later, hydroxylamine may pass two different pathways. The overall reaction is the oxidation of hydroxylamine to nitrite;

NH2OH + O2 🡪 NO2- + H2O + H+ (hydroxylamine oxidoreductase) (Kurnikov 2005) (2)

In this reaction, four electrons are transferred from one mole of hydroxylamine to one mole of oxygen. However, simultaneously transferring four electrons is not likely because of the limited reduction capability of HAO (Kurnikov 2005). Therefore, only two electrons are available to transfer during each step. Nitroxyl (HNO) can be formed as an intermediate species and can be further oxidized to nitrite after donating two additional electrons. Likewise, oxygen plays a significant role as an electron acceptor in each step.

NH2OH + 0.5O2🡪 HNO + H2O (3)

HNO +0.5O2 🡪 NO2- + H+ (4)

However, nitroxyl is very reactive towards nucleophiles and quickly dimerizes to hyponitrous acid, H2N2O2 which is then dehydrated to nitrous oxide and water.

2HNO 🡪 H2N2O2 🡪 N2O +H2O (Rapid dimerization) (Farmer 2005) (5)

We also propose an alternate pathway for the two-electron transfer from hydroxylamine to the intermediate species HN(OH)2. This pathway is based on a predicted intermediate which contains no double bonds, has an oxidation state of +1, and can dehydrate to form N2O;

NH2OH + 0.5O2 🡪 HN(OH)2 (6)

HN(OH)2 + 0.5O2 🡪 NO2- + H2O + H+ (7)

The intermediate species, N(OH)2H, may generate nitrous oxide via a dehydration pathway;

2HN(OH)2 🡪 N2H2O2 + 2H2O 🡪 N2O + H2O (8)

The key difference between HNO and HN(OH)2 seems to be the molecular structure of each. While a single molecule of HNO has a single covalent bond with hydrogen and a double bond with an oxygen molecule, HN(OH)2 has three single covalent bonds with hydrogen and hydroxyl ions. Therefore, HN(OH)2 seems to have more stable structure in terms of reactivity. Therefore it is likely that HN(OH)2 will tend to react predominately towards the nitrite generation pathway (7) rather than the dimerization pathway (8) if O2 is not limiting.

For nitrifier denitrification, under the low O2 conditions, nitrite may substitute as the terminal electron acceptor, resulting in its reduction to nitrous oxide;

NO2- + e- + H+ 🡪 NO + OH- (nitrate reductase: nirk) (Cantera 2007)(9)

NO + e- + H+ 🡪 0.5N2O +0.5H2O (nitric oxide reductase: norB or norC) (Garbeva 2007) (10)

However, it is possible that once N2O is generated, N2O will be further oxidized to NO2- donating two electrons to AOB if it remains dissolved in the liquid;

0.5N2O +0.5H2O 🡪 NO + e- + H+ (11)

NO + OH- 🡪 NO2- + e- + H+ (12)

Nevertheless, due to the low solubility of N2O, which is 1.5g/l at 15C (IPCC 2003), N2O is likely to leave the liquid phase quickly. However, most of NO2- is likely to remain dissolved in the liquid due to its high solubility of 815g/l at 15C (Sodium nitrate 2009). For this reason, reactions (11) and (12) are more difficult to observe than reactions (9) and (10), especially under the low O2 conditions.

Figure 5 shows possible pathways by which one mole of ammonia is oxidized to nitrous oxide by ARB.

(4)

H2O

0.5O2

H+

**HNO**

(5)

**HNO**

**Nitroxyl**

**H2N2O2**

**Dimer**

0.5O2

(3)

H2O

(12)

(11)

(2); Not likely

(1)

**NH2OH**

**Hydroxylamine**

**NH3**

**Ammonia**

**N2O**

**Nitrous Oxide**

**NO**

**Nitric Oxide**

**NO2-**

**Nitrite**

(9)

(10)

0.5O2

(6)

(8)

0.5O2

H2O + H+

**H2N2O2**

**Dimer**

**HN(OH)2**

**HN(OH)2**

0.5O2

2H2O

(7)

H2O

**+2**

**+1**

**-1**

**-3**

**+3**

**Figure 5 Ammonium Oxidation pathways by Ammonia oxidizing bacteria (AOB)**

**Note that the nitrite reduction to nitrous oxide is possible only when there is not enough oxygen. Under the conditions of enough conditions, nitrite will be further oxidized to nitrate by Nitrite oxidizing bacteria (NOB). Each number represents specific reaction suggested above.**

*The role of hydroxylamine oxidoreductase (HAO):*

As can be seen in the proposed stoichiometric pathways of figure 5, N2O emissions are a direct consequence of AOB under low O2 conditions. The two proposed mechanisms for N2O production are: 1. low oxygen levels result in respiration of NO2- by nitrifier denititrification, and 2. an intermediate develops during the four electron transfer associated with the reduction of NH2OH to NO2- that spontaneously dimerizes and dehydrates. Scenario number 1 involves AOB which become depleted of oxygen as an electron acceptor (equations 9 and 10). A low O2 environment initiates the transcription of genes responsible for the respiration of NO2- including nitrite reductase and nitric oxide reductase (Cantera 2007; Garbeva 2007). Since nitrous oxide reductase is more sensitive to O2 than nitrite reductase and nitric oxide reductase, a net accumulation of N2O develops (Shaw 2006).

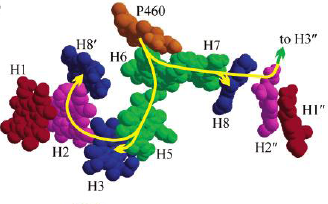
Scenario 2, however, does not involve the activation of reductive genes in AOB. Scenario 2 involves a very elaborate understanding of hydroxylamine oxidoreductase (HAO), the enzyme responsible for the oxidation of NH2OH. HAO is responsible for the four electron transfer which occurs during the oxidation of NH2OH to NO2- as was shown in equation 2.

HAO is part of a class of rare, yet not uncommon multiheme enzymes capable of multielectron redox processes (Kurnikov 2005). A heme is a porphyrin ring which is associated with an iron atom at its center; a multiheme protein associates more than one heme into its structure. HAO is a homotrimer, meaning that is consists of three monomers which are identical. These three monomers are composed of seven c-type hemes and the unique heme P460 (Kurnikov 2005). P460 contains a vacant coordination site at its iron center which allows hydroxylamine to bind and become oxidized (Kurnikov 2005). The hemes are aligned close together, allowing for the rapid transport of electrons to the iron centers of adjacent hemes. The hemes are also coordinated in a branched fashion, allowing for the electrons accepted at P460 to be quickly transported over large distances (Kurnikov 2005). Once hydroxylamine has associated with P460,two electrons at a time are transferred from hydroxylamine:

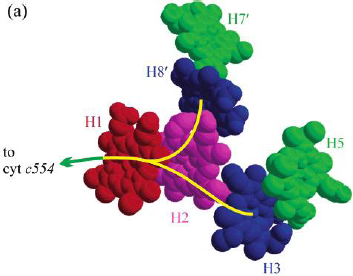
NH2OH 🡪 HNO + 2e- + 2H+ (hemes 3 and 8 accept the first two electrons) (13) -1 +1

HNO + H2O 🡪 NO2- + 2e- + 3H+ (Cytochrome C554 accepts the second two electrons) (14) +1 +3

The path which the electrons take within HAO is contingent upon the potentials of the various hemes (cofactors) within the enzyme. These potentials can be quite different from heme to heme and are susceptible to potential fluctuations caused by pH (Kurnikov 2005).

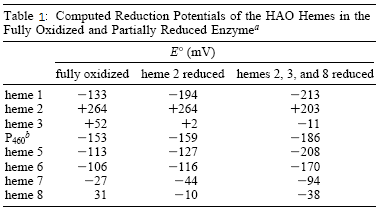
  
**Figure 6: Pathway of first two electrons from hydroxylamine through hydroxylamine oxidoreductase (Kurnikov 2005)**

The path of electrons through HAO is determined by a combination of spatial and electrostatic observations. Electrons from hydroxylamine are transferred into HAO via P460. As shown in figure 6, electrons first travel to hemes 3 and 8 because they have the highest potentials (table 1). After the first two electrons are transferred into the system, the potentials of heme 3 and 8 become too low for further reduction and additional electrons may be transferred to adjacent hemes 3’ or 8’ (table 1).



**Figure 7: Pathway of second two electrons from hydroxylamine through hydroxylamine oxidoreductase (Kurnikov 2005)**

However, if all of hemes 3 and 8 become reduced, the enzyme will have an overall negative potential which creates a bottleneck for electron transfer. Figure seven shows that, to accommodate for this, Cytochrome C554 binds to heme 1 which, due to its high potential, acts as an electron acceptor for the second set of electrons. Cytochrome C554 is then oxidized by O2 which reestablishes its high oxidative potential, enabling the enzyme to continue oxidizing hydroxylamine. However, if O2 is not present, then the potential of Cytochrome C554 will remain too low for the complete oxidation of hydroxylamine. This will lead to a buildup of HNO.



**Table 1: reduction potentials for hemes associated with HAO (Kurnikov 2005)**

The chemical properties of HNO were studied extensively by Farmer in 2005. This intermediate is highly reactive and seldom observed due to its relatively short lifespan; it dimerizes to N2O at a rate of 8 x 106/Ms (Farmer 2005; Yi 2008; Dannis *et al*. 1985). Therefore, conditions in which HNO accumulates within the cell will lead to net production of N2O as was shown in equation 5.

These data have attempted to explain nitrous oxide production from AOB as a result of low O2 at a very fundamental physiological level. It is proposed that lack of oxygen will deplete HAO of an oxidizing agent for Cytochrome C554. This will leave hydroxylamine only partially oxidized because electron transfer will become inhibited by low intercellular enzyme potentials. Since hydroxylamine will not be able to transfer four electrons into HAO, an accumulation of the intermediate HNO or HN(OH)2 may occur, resulting in quick dimerization into N2O causing an increase in N2O production levels. In addition, low O2 will leave HAO in a highly electronegative state, resulting in AOB with large quantities of electrons. In this scenario, we also predict that the AOB may substitute NO2- as an electron acceptor resulting in nitrifier denitrification to deplete HAO of its electrons.

*2. Modeling for N2O emission in terms of O2 and NH3:*

To find out how N2O emission is related to O2 concentration, we set up a theoretical situation. We assumed that all reactions occur in a batch system with limited concentrations of NH3, O2, and carbon source. By establishing these conditions, we can determine how N2O generation is related to initial O2 and NH3 concentrations based on figure 5 and equations 1-10. For the 0.5 mol O2: 1.0 mol NH3, only reaction (1) will happen and no N­2O will be produced. When the O2 ratio is increased to 1.0 mol O2/: 1.0mol NH3, reactions (1), (3), and (6) will happen and will proceed to reactions (5) and (8).When O2 ratio is increased to 1.5 mol O2: mol NH3, reactions (4) and (7) will be possible. Figure 8 predicts that N2O production as a result of intermediate HNO or HN(OH)2 dimerization and dehydration will be optimized when ammonia concentrations reach 1.0 mol O2: 1.0 mol NH3 since there is only enough O2 for reactions 1, 3, and 6 to occur.

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**Figure 8: Hypothesized N2O from Dimerization**

Figure 9 predicts that N2O production as a result of nitrifier denitrification will be optimized when ammonia concentrations reach 1.5 mol O2: 1.0 mol NH3. This is because oxygen concentrations will be diminished once nitrite is formed and research suggests that AOB initiate nitrifier denitrification under low O2 concentrations (Kim 2010; Hanaki et al. 2001; Wrage 2001; Cantera 2007; Garbeva 2007). The production of N2O is also increased by low O2 since nitrous oxide reductase is very sensitive to O2 and can be inhibited by very low concentrations (Bonin et al. 2002).

**Figure 9: Hypothesized N2O from Nitrifier Denitrification**

Overlaying the two graphs reveals that N2O production may be catalyzed by low O2 concentrations ranging from 0.5-2.0 mol O2: 1.0 mol NH3. O2 levels between 1.0 – 1.5 mol O2: mol NH3 are hypothesized to optimize N2O production during nitrification processes. At 0 mol O2, nitrification is inhibited; at or above 2 mol O2: mol NH3, nitrification often results in the production of nitrate by NOB.

**Figure 10: Hypothesized N2O production Combined effect of dimerization and nitrifier denitrification**

*3. N2O modeling limitations:*

As can be predicted from our model, N2O production will occur under conditions of 0.5-2.0 mol O2: mol NH3 with the greatest amount occurring between 1.0-1.5 mol O2: mol NH3. However, these results will only occur if the assumptions of our model are found to be true; specifically, that each reaction towards the production of N2O will happen as quickly as possible and consume as much O2 as is necessary for the reaction to occur. These hypotheses have been incorporated in our model because we believe that it is reasonable to assume that spontaneous reactions will occur as quickly as possible and consume as many moles O2 as is necessary for the reaction to occur. However, actual conditions may not reflect this assumption and thus N2O production rates will not be those predicted by our model; intermediate stages may use oxygen at disparate rates. For example, our model predicts that 1.0mol O2: 1.0 mol NH3 will produce the most N2O from dimerization or dehydration of intermediates because we assume that equations (1) and (3) or (6) in the nitrification process will occur in equal amounts leading to the net accumulation of HNO or HN(OH)2. However, the case may be that equation (1) occurs at a much greater rate than equations (3) or(6), and that 1.0 mol O2: 1.0 mol NH3 will produce a net accumulation of the intermediate NH2OH, inhibiting dimerization and dehydration due to lack of production of intermediates.

In addition, our model predicts that N2O production from nitrifier denitrification will be optimized at 1.5 mol O2: 1.0 mol NH3 because of our assumption that these concentrations will lead to NO2- accumulation that is susceptible to reduction by O2 depleted AOB. Nitrifier denitrification can only occur if NO2- is present in the media, thus our model predicts that nitrifier denitrification can only occur if there is enough O2 to produce NO2-. AOB rich in electrons as a result of electron storage in enzymes may then respire NO2-. This means that in our model, we predict that NH3 becomes an indirect electron donor for reduction of NO2- only if O2 concentrations are not high enough, above 2.0 mol O2: 1.0 mol NH3, for complete nitrification to NO3- by NOB. This may result from accumulation of electrons in intracellular enzymes, such as HAO, and activation of nitrite reducing genes, such as nirK, norB, and norC under low O2 conditions. It is predicted that nitrifier denitrification may occur under O2 conditions lower than 1.5 mol O2: 1.0 mol NH3 in systems which already have NO2- present; however, we have excluded this from the model because we assume that only NH3, O2, and a carbon source are present during startup of our system.

Lastly, our model neglects effects that may be caused by pH and temperature may have on N2O production. High or low temperature will influence enzyme kinetics, potentially altering the favorability of certain steps. For example, high or low temperatures may favor dimerization; however, they may also inhibit enzyme function such as HAO which will prevent equations (3) and (6). Enzymes within living systems often have a very narrow temperature range within which they are most efficient. In addition, many of the intermediate steps involve the production of either H+ or OH- ions which will have a dramatic impact on the pH of the system. For example, the oxidation of hydroxylamine to nitrite results in a net increase of 1.0 mol H+ ions into the media. This will result in a much lower pH that may alter enzyme function, make hydroxylamine synthesis unfavorable, and result in the protonation of intermediates such as nitrite to form nitrous acid.

**Conclusion:**

In this paper, we have attempted to represent the pathways responsible for N2O accumulation from AOB during the nitrification process at WWTPs. To accomplish this, we have developed a theoretical stoichiometric model which predicts N2O production as a direct consequence of the ratio of mol O2: mol NH3. In this model, we have predicted that N2O production occurs under oxygen limited conditions, between 0.5 and 2.0 mol O2: mol NH3, which cause dimerization and dehydration of intermediates. We have also predicted that N2O production is optimized between 1.0-1.5 mol O2: mol NH3.

Lastly, we also attempted to incorporate the concept of nitrifier denitrification from AOB into our model. Nitrifier denitrification is hypothesized to occur under low O2 concentration, leaving NO2- as an alternative electron acceptor for respiration. This concept has been supported by the activation of nitrite reductases in AOB under low O2 conditions. We predict that AOB will retain reductive power from the buildup of electrons in multiheme enzymes and pass these electrons to NO2- resulting in the production of N2O.

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