

Original Research

Theoretical Analysis of Microbial Yield and Pathways Related to N₂O Production in Nitrification

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Abstract

Identifying microbial yield and the pathway relating to nitrous oxide (N₂O) production in nitrification were essential prerequisites for modeling N₂O production and emissions. A thermodynamic method based on standard Gibbs free energy change of bio-chemical reaction was used to evaluate the NH₃-NO, NH₃-NO₂⁻, NH₄⁺-NO, NH₄⁺-NO₂⁻, NH₂OH-NO, and NH₂OH-NO₂⁻ reactions. Meanwhile, the stoichiometric equations of the relevant reactions were built up to ascertain the microbial yield of the microorganisms responsible for N₂O production. The results indicated that: 1) all the above reactions had the possibility of producing N₂O in nitrification as the standard Gibbs free energy changes of all the reactions were negative, 2) all the possible reactions can be used as the pathways to describe N₂O production in nitrification in mathematical models, and 3) theoretically, the yield coefficient of autotrophs relating to N₂O production was in the range of 0.097~0.194 g cell/ g N. Ammonia-oxidizing bacteria were responsible for the production of N₂O in nitrification. In order to develop a unified model for better simulation of N₂O production and emissions in the biological wastewater treatment process, intensive studies should be carried out to reveal undiscovered pathways relating to N₂O production.

Keywords: nitrous oxide, production, nitrification, thermodynamic method

Introduction

As a potent greenhouse gas (GHG), nitrous oxide (N_2O) not only has a strong greenhouse effect (approximately 300-fold stronger than carbon dioxide), but also is an important ozone-depleting substance [1]. Biological wastewater treatment systems had been identified as a source responsible for the increase of N_2O in the atmosphere [2].

In a biological wastewater treatment system, nitrification and denitrification were not only responsible for nitrogen removal, but also for N_2O production and emissions [3-5]. In denitrification, the mechanisms of N_2O production and loss are quite clear and uncontested. N_2O is produced in nitric oxide (NO) reduction as an intermediate, and finally is reduced to N_2 by heterotrophic denitrifiers. However, mechanisms for N_2O production in nitrification are complicated.

In nitrification, ammonia (NH_3) is converted to nitrate (NO_3^-) via hydroxylamine (NH_2OH) and nitrite (NO_2^-) by autotrophs. NH_3 is oxidized to NH_2OH catalyzed by ammonia monooxygenase (AMO). Thereafter, NH_2OH is oxidized to NO_2^- catalyzed by hydroxylamine oxidoreductase (HAO). During these processes, N_2O can be produced by chemical breakdown of the unstable nitrosyl radical (NOH) during the oxidation of NH_2OH [6-7] as path 1 in Fig. 1. Meanwhile, reduction of NO produced from the oxidation of NH_2OH can also produce N_2O , as path 2 in Fig. 1. In addition, nitrifier denitrification, namely ammonia-oxidizing bacteria, can also lead to the production of N_2O [8-10], as shown in path 3 in Fig. 1. Besides, as NH_2OH had extremely reactive properties [11], other chemical reactions NH_2OH participated also had the possibility of improving N_2O production in the biological wastewater treatment process [12].

The mathematical model had already been a powerful tool for researchers working in biological wastewater treatment processes. However, accurate microbiological modeling required the impact of biodegradation reactions

on all important chemical and biological species in the system [14]. As illustrated in Fig. 1, there were different pathways responsible for the production and consumption of N_2O in nitrification, and there were many debates about them [15], which made a great uncertainty for simulation of N_2O production in the nitrification process. Consequently, simulation of N_2O production and loss by mathematical models were quite different [8, 16-19]. Besides, NH_2OH , N_2O , and NO in biological wastewater treatment processes were not easy to determine, and parameters relating to these matters were difficult to ascertain. To sort out these problems, the thermodynamic analysis of the biological synthesis in activated sludge could be performed and the stoichiometrics could be more accurately estimated with the cell yield derived from thermodynamic considerations of the flows of energy and electrons in the catabolic and anabolic pathways [20].

In this study, in order to determine possible pathways of N_2O production in nitrification and the microbial yield relating to N_2O production, a thermodynamic method based on standard Gibbs free energy change of bio-chemical reaction was used to evaluate possible reactions relating to N_2O production in nitrification. Meanwhile, the stoichiometric equations of the relevant reactions were proposed to ascertain the microbial yields of the microorganisms responsible for N_2O production in nitrification.

Materials and Methods

The Thermodynamic Method

The thermodynamic method that was based on electron equivalents and differentiated the energy portion of an overall biological reaction and the synthesis portion was employed to determine energy changes and cell yields of different biological reactions [21-24].

Microorganisms must use an electron-donor substrate for synthesis in a bio-chemical reaction. A portion of

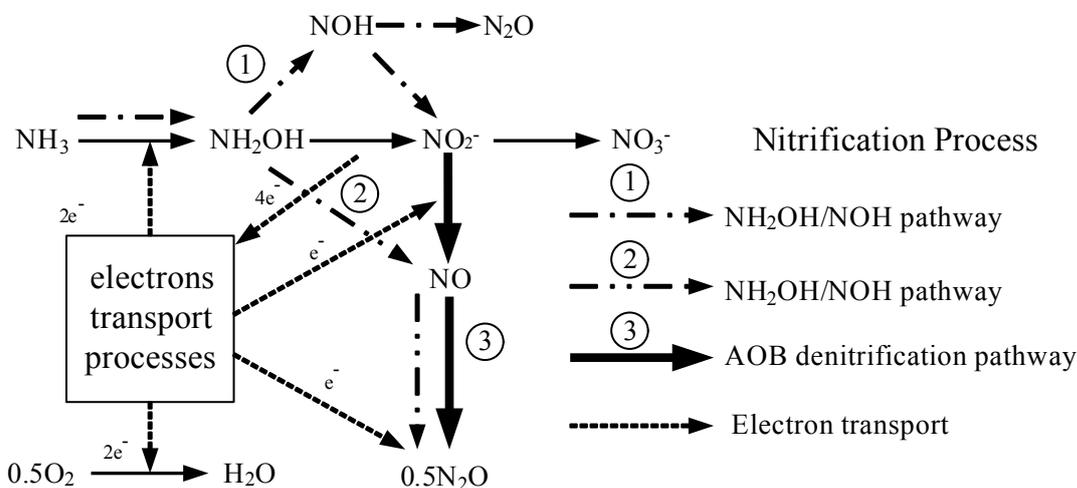


Fig. 1. N_2O production pathways during nitrification [7, 13].

electrons (f_e) provided by the electron-donor was initially transferred to the electron acceptor to provide energy for conversion of the other portion of the electrons into microbial cells (f_s). The sum of f_e and f_s was 1. The overall reaction for biological growth can be determined by an energy reaction (R_e) and a synthesis reaction (R_s) combined with f_e and f_s . Detailed information of the method is illustrated from Equation (1) to Equation (8).

The energy reaction (R_e) can be calculated by:

$$R_e = R_a + R_d \quad (1)$$

...where R_a was the acceptor half-reaction and R_d was the donor half-reaction.

The synthesis reaction (R_s) can be calculated by:

$$R_s = R_c + R_d \quad (2)$$

...where R_c was the half-reaction for synthesis.

Eventually, the overall reaction for net synthesis of bacteria (R) was calculated by:

$$R = f_e \cdot R_e + f_s \cdot R_s = f_e \cdot R_a + f_s \cdot R_c + R_d \quad (3)$$

f_s and f_e can be computed by:

$$f_s = \frac{1}{1+A} \text{ and } f_e = 1 - f_s = \frac{A}{1+A} \quad (4)$$

At steady state, the energy supplied and consumed must balance, and the following equation can be achieved:

$$A \cdot \varepsilon \cdot \Delta G_r + \Delta G_s = 0 \quad (5)$$

A equaled to:

$$A = -\frac{\Delta G_s}{\varepsilon \cdot \Delta G_r} \quad (6)$$

...where ΔG_s was the energy required to synthesize one equivalent of cells for a given electron donor, and was calculated by:

$$\Delta G_s = \frac{\Delta G_p}{\varepsilon^n} + \frac{\Delta G_p}{\varepsilon} \quad (7)$$

...where ΔG_p was equal to 113.8 kJ/e⁻ eq under autotrophic conditions [24] and ε was the energy-transfer efficiency. Under optimum conditions, transfer efficiencies of 55-70% were typical [24]. To autotrophs, the conversion of NH_3 to NO_3^- via NO_2^- was the dominating process. However, to N_2O production, the energy-transfer efficiency probably was not under optimum conditions, and ε in this study was supposed to be 27.5% (half of the lower limit of the transfer efficiency under optimum conditions); ΔG_{pc} was the energy required for converting pyruvate carbon

to cellular carbon and was estimated to equal 3.33kJ per gram cells. As the empirical formula of bacterial cells was $\text{C}_5\text{H}_7\text{O}_2\text{N}$, ΔG_{pc} was 18.8kJ/e⁻ eq; exponent n indicated energy produced or energy required in the conversion of external carbon source to pyruvate. When energy was released, n was -1, and when energy was required, n was 1; ΔG_p was the energy required to convert the carbon source; ΔG_r was the energy that can be released by oxidizing per equivalent of electron donor for energy generation, and was calculated by:

$$\Delta G_r = \Delta G_a^0 + \Delta G_d^0 \quad (8)$$

...where ΔG_a^0 and ΔG_d^0 were standard Gibbs free energy changes of the acceptor half-reaction and the donor half-reaction, kJ/e⁻ eq.

Eventually, A , f_e , f_s , and the overall reaction for net synthesis of bacteria can be determined and the stoichiometric equation of a biochemical reaction also can be constructed.

Half-reactions Relating to N_2O Production

Except for NH_3 and NO_2^- , there are three intermediates produced in nitrification, including NH_2OH , nitric oxide (NO), and N_2O . Chemical valances of nitrogen in NH_3 , NH_4^+ , NH_2OH , N_2O , NO, and NO_2^- are -3, -3, -1, +1, +2, and +3, respectively. From the viewpoint of chemicals, NH_3 -NO, NH_3 - NO_2^- , NH_4^+ -NO, and NH_4^+ - NO_2^- reactions, NH_2OH -NO and NH_2OH - NO_2^- reactions have the possibility to produce N_2O in nitrification. The acceptor half-reactions, donor half-reactions, and cell synthesis reaction that can comprise overall reactions of N_2O production are shown in Table 1. Nitrogen was an essential element for growth of microorganisms. Generally, ammonia nitrogen was a preferred nitrogen source in cell synthesis [25]. Consequently, ammonia nitrogen was selected as the nitrogen source in this study. All formations are supposed to be reacted to at pH 7.0 and 25°C. Table 2 listed the standard Gibbs free energy formations under the standard condition used for calculating the standard Gibbs free energy changes of the reactions.

Results and Discussion

The Yield Coefficient of Microbes Relating to N_2O Production

The standard Gibbs free energy changes (ΔG_r) and f_e as well as f_s of the six reactions relating to N_2O production were shown in Table 3. The overall reactions and the yield coefficient of microbes relating to N_2O production in nitrification were shown in Table 4.

In Table 3, standard Gibbs free energy changes of reactions relating to N_2O production in nitrification were negative. From the viewpoint of thermodynamics, all reactions listed in Table 3 can proceed spontaneously. The

Table 1. Donor half-reactions, acceptor half-reactions, and cell synthesis reaction.

Reactions	Half-reactions
D-1	$\frac{1}{4}\text{NH}_3(aq) + \frac{1}{8}\text{H}_2\text{O}(l) \rightarrow \frac{1}{8}\text{N}_2\text{O}(g) + \text{H}^+(aq) + e^-$
D-2	$\frac{1}{4}\text{NH}_4^+(aq) + \frac{1}{8}\text{H}_2\text{O}(l) \rightarrow \frac{1}{8}\text{N}_2\text{O}(g) + \frac{5}{4}\text{H}^+(aq) + e^-$
D-3	$\frac{1}{2}\text{NH}_2\text{OH}(aq) \rightarrow \frac{1}{4}\text{N}_2\text{O}(g) + \frac{1}{4}\text{H}_2\text{O}(l) + \text{H}^+(aq) + e^-$
A-1	$\text{NO}(g) + \text{H}^+(aq) + e^- \rightarrow \frac{1}{2}\text{N}_2\text{O}(g) + \frac{1}{2}\text{H}_2\text{O}(l)$
A-2	$\frac{1}{2}\text{NO}_2^-(aq) + \frac{3}{2}\text{H}^+(aq) + e^- \rightarrow \frac{1}{4}\text{N}_2\text{O}(g) + \frac{3}{4}\text{H}_2\text{O}(l)$
Cell synthesis	$\frac{1}{5}\text{CO}_2(g) + \frac{1}{20}\text{HCO}_3^-(aq) + \frac{1}{20}\text{NH}_4^+(aq) + \text{H}^+(aq) + e^- \rightarrow \frac{1}{20}\text{C}_5\text{H}_7\text{O}_2\text{N}(s) + \frac{9}{20}\text{H}_2\text{O}(l)$

D and A represented the donor half-reaction and the acceptor half-reaction, respectively. Physical states of formations in Table 1 were expressed as *l* (liquid), *g* (gas), *aq* (water solution), and *s* (solid).

values of f_s were in the range of 0.07 to 0.16, while the values of f_e were in the range of 0.84 to 0.93. When the electron donor and the electron acceptor were NH_2OH and NO , respectively, the portion of electrons used by microbes for cell synthesis was the maximum. Contrarily, when the electron donor and the electron acceptor were NH_4^+ and NO_2^- , respectively, the portion of electrons used by microbes for cell synthesis was the minimum.

The reported value of the yield of autotrophs was in the range of 0.07~0.28 g cell COD/ g N, and the default value was 0.24 g cell COD/g N in activated sludge models (ASMs) [27]. The yield of autotrophs was adjusted to 0.049~0.197 g cell / g N using a conversion ratio of 1.42 g COD/g cell. In Table 4, the microbial yields of S-1, S-2, S-3, S-4, S-5, and S-6 were 0.194 g cell/g N, 0.194 g cell/g N, 0.129 g cell/g N, 0.097 g cell/g N, 0.129 g cell/g N, and 0.113 g cell/g N, respectively. Microbial yield was an important parameter reflecting the

amount of new cells synthesized by utilizing unit mass of substrate. The yields obtained from the stoichiometric equations in Table 4 were in the range of 0.097~0.194 g cell/g N and in the range of autotrophs in ASMs. Theoretically, when simulating N_2O production in nitrification by a mathematical model, the nitrifier can be selected as the functional microbes because of the microbial yield and the reaction proceeded. Furthermore, ammonia-oxidizing bacteria (AOB) were responsible for the conversion of NH_3 to NO_2^- , while nitrite oxidation bacteria (NOB) were responsible for the conversion of NO_2^- to NO_3^- . From the perspective of chemical balance, the valence of nitrogen in N_2O is lower than that in NO_2^- . N_2O was produced in the conversion of NH_3 to NO_2^- via different pathways, which meant that NOB had nothing to do with the N_2O production. The functional microorganism responsible for N_2O production was AOB.

The basic value of f_s of AOB was 0.14 [24]. f_s obtained in this study was in the range of 0.07~0.16. A great number of the electrons provided by the donor were used to supply energy for cell synthesis in the six reactions (Table 3), and meanwhile the yield of microbes relating to N_2O production was small, which meant microbes cannot grow fast through the reactions in Table 4. Values of f_s and Y were deeply affected by the energy-transfer efficiency, ε . A big ε resulted in big f_s and Y .

In this study, the values of ε in all reactions were supposed to be the same and equaled 27.5%. Although actual values of ε in different reactions were probably not equal to 27.5%, according to the reported low N_2O emission factors which were only from 0.01% to 1.8% of influent total nitrogen [3], N_2O production in nitrification cannot be the dominant process under normal conditions. In the AOB denitrification pathway, N_2O was an intermediate produced during the reduction of NO . However, in other metabolic pathways of nitrogen

Table 2. Standard Gibbs free energy of formations.

Number	Substance	State	$\Delta G^0(25^\circ\text{C})$	References
1	$\text{H}^+(10^{-7})$	<i>aq</i>	-39.870	[26]
2	NH_3	<i>aq</i>	-26.570	
3	NH_4^+	<i>aq</i>	-79.370	
4	N_2O	<i>g</i>	-104.180	
5	NO	<i>g</i>	86.570	
6	NO_2^-	<i>aq</i>	-37.200	
7	H_2O	<i>l</i>	-237.178	
8	HCO_3^-	<i>aq</i>	-586.650	
9	CO_2	<i>g</i>	-394.359	
10	NH_2OH	<i>aq</i>	-43.670	[6]

Table 3. ΔG_r , f_e , and f_s of different reactions.

Reaction	Energy Reactions	ΔG_r (kJ/e ⁻ eq)	f_e	f_s
R-1	$\frac{1}{4}\text{NH}_3(\text{aq}) + \text{NO}(\text{g}) \rightarrow \frac{5}{8}\text{N}_2\text{O}(\text{g}) + \frac{3}{8}\text{H}_2\text{O}(\text{l})$	-233.98	0.88	0.12
R-2	$\frac{1}{4}\text{NH}_4^+(\text{aq}) + \text{NO}(\text{g}) \rightarrow \frac{5}{8}\text{N}_2\text{O}(\text{g}) + \frac{3}{8}\text{H}_2\text{O}(\text{l}) + \frac{1}{4}\text{H}^+(\text{aq})$	-230.75	0.88	0.12
R-3	$\frac{1}{2}\text{NH}_2\text{OH}(\text{aq}) + \text{NO}(\text{g}) \rightarrow \frac{3}{4}\text{N}_2\text{O}(\text{g}) + \frac{3}{4}\text{H}_2\text{O}(\text{l})$	-320.75	0.84	0.16
R-4	$\frac{1}{2}\text{NH}_2\text{OH}(\text{aq}) + \frac{1}{2}\text{NO}_2^-(\text{aq}) + \frac{1}{2}\text{H}^+(\text{aq}) \rightarrow \frac{1}{2}\text{N}_2\text{O}(\text{g}) + \text{H}_2\text{O}(\text{l})$	-228.90	0.88	0.12
R-5	$\frac{1}{4}\text{NH}_3(\text{aq}) + \frac{1}{2}\text{NO}_2^-(\text{aq}) + \frac{1}{2}\text{H}^+(\text{aq}) \rightarrow \frac{3}{8}\text{N}_2\text{O}(\text{g}) + \frac{5}{8}\text{H}_2\text{O}(\text{l})$	-142.13	0.92	0.08
R-6	$\frac{1}{4}\text{NH}_4^+(\text{aq}) + \frac{1}{2}\text{NO}_2^-(\text{aq}) + \frac{1}{4}\text{H}^+(\text{aq}) \rightarrow \frac{3}{8}\text{N}_2\text{O}(\text{g}) + \frac{5}{8}\text{H}_2\text{O}(\text{l})$	-138.89	0.93	0.07

Table 4. The overall reaction for net synthesis and yield coefficients (Y).

Reactions	Stoichiometric reactions	Y (g cell/g N)
S-1	$0.024\text{CO}_2 + 0.006\text{NH}_4^+ + 0.006\text{HCO}_3^- + 0.880\text{NO} + 0.250\text{NH}_3$ $\rightarrow 0.006\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.565\text{N}_2\text{O} + 0.369\text{H}_2\text{O}$	0.194
S-2	$0.024\text{CO}_2 + 0.006\text{NH}_4^+ + 0.006\text{HCO}_3^- + 0.880\text{NO} + 0.250\text{NH}_4^+$ $\rightarrow 0.006\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.565\text{N}_2\text{O} + 0.369\text{H}_2\text{O} + 0.250\text{H}^+$	0.194
S-3	$0.032\text{CO}_2 + 0.008\text{NH}_4^+ + 0.008\text{HCO}_3^- + 0.840\text{NO} + 0.500\text{NH}_2\text{OH}$ $\rightarrow 0.008\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.670\text{N}_2\text{O} + 0.742\text{H}_2\text{O}$	0.129
S-4	$0.024\text{CO}_2 + 0.006\text{NH}_4^+ + 0.006\text{HCO}_3^- + 0.44\text{NO}_2^- + 0.500\text{NH}_2\text{OH} + 0.44\text{H}^+$ $\rightarrow 0.006\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.490\text{N}_2\text{O} + 0.964\text{H}_2\text{O}$	0.097
S-5	$0.016\text{CO}_2 + 0.004\text{NH}_4^+ + 0.004\text{HCO}_3^- + 0.460\text{NO}_2^- + 0.250\text{NH}_3 + 0.46\text{H}^+$ $\rightarrow 0.004\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.355\text{N}_2\text{O} + 0.601\text{H}_2\text{O}$	0.129
S-6	$0.014\text{CO}_2 + 0.0035\text{NH}_4^+ + 0.0035\text{HCO}_3^- + 0.465\text{NO}_2^- + 0.250\text{NH}_4^+ + 0.215\text{H}^+$ $\rightarrow 0.0035\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.3575\text{N}_2\text{O} + 0.604\text{H}_2\text{O}$	0.113

during nitrification, N_2O was a byproduct of oxidation of NH_2OH to NO [28], chemical decomposition of NH_2OH [29], and other chemical reactions [30]. As oxidation of NH_2OH to NO_2^- was an energy-generating step for AOB [31], the conversion of NH_3 to NO_2^- and NO_3^- were the dominant pathways. Nevertheless, from a thermodynamic standpoint, AOB can also gain energy from the reactions listed in Table 3. The reasonable explanation of why the reactions listed in Table 3 were not favorable for AOB was probably the relatively low energy-generating efficiency. When AOB carried out the reactions listed in Table 3, although the net energy of the reactions was positive, a great part of electrons were used to supply the energy for cell synthesis instead of cell synthesis itself. Consequently, the portions of the electrons into microbial cells were small.

The Pathways for N_2O Production Simulation by Mathematical Models

There were three known pathways in the literature, i.e., the $\text{NH}_2\text{OH}/\text{HON}$ pathway, $\text{NH}_2\text{OH}/\text{NO}$, and AOB denitrification pathways, responsible for N_2O production in nitrification. The $\text{NH}_2\text{OH}/\text{HON}$ pathway was a chemical breakdown of the unstable NOH . Except for the $\text{NH}_2\text{OH}/\text{HON}$ pathway, there were six bio-chemical reactions (Table 4) relating to the other two N_2O production pathways in nitrification. The $\text{NH}_2\text{OH}-\text{NO}$, NH_3-NO , and $\text{NH}_2\text{OH}-\text{NO}_2^-$ reactions were successfully used in models of simulating N_2O production in nitrification. However, thermodynamically the $\text{NH}_3-\text{NO}_2^-$, NH_4^+-NO , and $\text{NH}_4^+-\text{NO}_2^-$ reactions also had the possibility to produce N_2O in nitrification as the negative Gibbs free energy changes.

Besides, the $\text{NH}_3\text{-NO}_2^-$, $\text{NH}_4^+\text{-NO}$, and $\text{NH}_4^+\text{-NO}_2^-$ reactions can theoretically be regarded as a simplification of the AOB denitrification pathway.

In biochemical reactions, electron donor and electron acceptor were always in pairs. NH_2OH and NO was the most popular combination in the model for simulating N_2O production in nitrification [32-33]. The combination of NH_2OH and NO_2^- [7], NH_3 , and NO [18, 34], and the chemical breakdown of NOH [35] were also used. However, every model had certain applicable conditions and weaknesses [16]. Wu et al. found that with the addition of NH_4^+ , N_2O emissions from the denitrification system increased compared to the experiments without NH_4^+ [36]. As NO_2^- and NO were intermediates of denitrification, Equations (1), (2), (5), and (6) can be carried out when NH_4^+ was added to the system, and N_2O emissions increased as a result. All reactions in Table 4 can be used for simulation of N_2O production, and which one should be used depends on the model proposed by the model-builder or model-user. In nitrification, there were several components relating to N_2O production. The processes or the components relating to N_2O production in different trials should be the core issues for model-builder or model-user in developing the mathematical model for N_2O simulation. Accordingly, the reactions relating to the process and the components model-builder or model-user intended to simulate by mathematical models should be selected as the pathway in mathematical models.

From the model itself, although the mechanism model was relatively accurate, there were many parameters needed to be calibrated and verified, such as the yields of cells on different substrates [14]. Consequently, development and application of the mechanism model was restricted to some extent. However, as a complex mechanism of N_2O production, the simulation effects of N_2O production with simplified model was not satisfactory. How to reconcile this contradiction required great skills and the art of simplification based on a solid theoretical foundation of the mechanism of N_2O production. Furthermore, as the mechanism of N_2O production was still not fully understood [15] and the present mathematical models cannot satisfy simulation of N_2O production in different conditions [16], whether the current mechanism for explaining the N_2O production in biological wastewater treatment process was complete or sufficient should be reconsidered. Probably, undiscovered pathways responsible for N_2O production in the biological wastewater treatment process may exist and need to be verified with more experiments.

Overall, the mechanism of N_2O production in the biological wastewater treatment process was not clear, in order to develop a unified model for better simulation of N_2O production and emissions in the biological wastewater treatment process, intensive studies should be carried out to reveal the undiscovered pathways relating to N_2O production. In simulation of N_2O production in nitrification, all the possible reactions should be considered and can be adopted, and eliminating any reaction might be a rash decision at present.

Conclusions

1. NH_3 , NH_4^+ , NH_2OH , NO , and NO_2^- are the formations relating to the N_2O production in nitrification. All the combination reactions comprised of the above formations had the possibilities of producing N_2O . In simulation of N_2O production in nitrification, all the possible reaction can be used.
2. Theoretically, the yield coefficient of autotrophs relating to N_2O production was in the range of 0.097~0.194 g cell/g N. AOB was responsible for the production of N_2O in nitrification.
3. All the possible reactions can be used as the pathways to describe N_2O production in nitrification in mathematical models, and which pathway was the best depended on the phenomenon or components the model described.
4. In order to develop a unified model for better simulation of N_2O production and emissions in the biological wastewater treatment process, intensive studies should be carried out to reveal the undiscovered pathways relating to N_2O production.

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