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Does denitrification via direct biological oxidation of ammonia to dinitrogen gas exist?

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ABSTRACT

To explore the possibility of directly biological oxidation of ammonium (NH_4^+) to dinitrogen gas (N_2) via oxygen (O_2), the stoichiometric equations of the reaction were established via a thermodynamic method. Moreover, the yields of the microbials responsible for the possible reactions under different energy-transfer efficiencies were evaluated. The results indicated that, the direct oxidation of NH_4^+ to N_2 by O_2 was an exergonic reaction, the microorganisms catalyzed this reaction were chemolithotrophs, and can obtain energy from this reaction for growth and synthesis. When energy-transfer efficiencies and sludge retention times were in the range of 20%–70% and 10–20 d, respectively, the microbial yields were in the range of 0.0225–0.2424 mg Cell/mg N. Compared with the known biological nitrogen removal processes, nitrogen removal via direct oxidation of NH_4^+ to N_2 has great advantages, for example, low sludge yield and low energy consumption. More efforts should be devoted to find the evidence of the existence of the direct oxidation of NH_4^+ to N_2 by O_2.

Keywords: Denitrification; Ammonium; Direct oxidation; Dinitrogen gas

1. Introduction

Nitrogen removal from wastewater can be achieved by biological and physico-chemical technologies. Physico-chemical technologies including ammonia air and steam stripping, ammonia vacuum distillation, ammonia precipitation as struvite, advanced oxidation processes can convert ammonia/ammonium (NH_3/NH_4^+) into dinitrogen gas (N_2) or nitrate (NO_3^-) [1]. However, compared with the physico-chemical removal methods, biological nitrogen removal processes are more economical and prevalent.

Wastewater can be treated under oxic, anoxic, and anaerobic conditions by different biological wastewater treatment processes, such as moving bed biofilm [2], stabilization pond [3,4], bio-filter and activated sludge combined process [5], constructed wetland [6], and so on. Conventional biological nitrogen removal process includes nitrification and denitrification. NH_4^+ is oxidized to NO_3^- via hydroxylamine (NH_2OH) and nitrite (NO_2^-), and then, NO_3^- is reduced to N_2 via NO_2^-, nitric oxide (NO) and nitrous oxide (N_2O). In 1977, based on the thermodynamic ground, Broda [7] predicts the existence of chemosynthetic bacteria which can oxidize NH_4^+ to N_2 by oxidants of oxygen (O_2), NO_2^-, or NO_3^-.

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Almost 20 y after the prediction, ANAMMOX (anaerobic ammonium oxidation) process is eventually discovered in a denitrifying fluidized bed reactor [8]. However, the major difference between the discovery and the prediction of Broda is that, NH_4^+ is oxidized to N_2 by NO_2^- instead of O_2 or NO_3^-, and NO_3^- is produced as the end-product of the ANAMMOX process. The discovery of ANAMMOX process leads to the development of new biological nitrogen removal processes, such as SHARON (single reactor for high ammonia removal over nitrite)-ANAMMOX process, OLAND (oxygen-limited autotrophic nitrification–denitrification) process, and CANON (completely autotrophic nitrogen removal over nitrite) process, and so on [9]. Fig. 1 summarizes the known and the possible pathways for biological denitrification.

From the perspective of thermodynamics, direct oxidation of NH_4^+ to N_2 by O_2 is an exergonic reaction. The standard Gibbs free energy change of the reaction (ΔG) is –105.42 kJ/e$^-$ eq.

$$\frac{1}{4}O_2 + \frac{1}{3}NH_4^+ = \frac{1}{2}H_2O + \frac{1}{6}N_2 + \frac{1}{3}H^+ \qquad \Delta G = -105.42\text{kJ/e}^-\text{eq} \tag{1}$$

If this reaction can be performed by microorganisms, microorganisms can gain energy from this reaction for synthesis and growth. Moreover, direct oxidation of NH_4^+ to N_2 by O_2 can eliminate the production of gaseous intermediates in conventional denitrification process, for example, NO and N_2O. As it is widely known, N_2O is a potent greenhouse gas and can be generated from biological wastewater treatment processes [10,11]. Reducing the N_2O emission is beneficial for the sustainability of municipal wastewater treatment plants [10]. However, more than 40 y after the prediction of Broda [7], experimental evidences of nitrogen removal by direct biological oxidation of NH_4^+ to N_2 by O_2 are still in the dark, and waiting to be discovered.

In this study, based on the prediction of Broda [7], a further discussion of biological denitrification by direct oxidation of NH_4^+ to N_2 by O_2 is presented based on the thermodynamic method proposed by McCarty [12,13], Rittman and McCarty [14], and McCarty [15]. The thermodynamic method based on the standard Gibbs free energy change of half-reaction is used to obtain the results of the reactions. Meanwhile, the possible stoichiometric equations of the processes are constructed to ascertain the microbial yields of the microorganisms responsible for direct oxidation of NH_4^+ to N_2 by O_2. At last, the benefits of nitrogen removal via direct oxidation of NH_4^+ to N_2 are discussed and the possible ways for discovering this process is proposed.

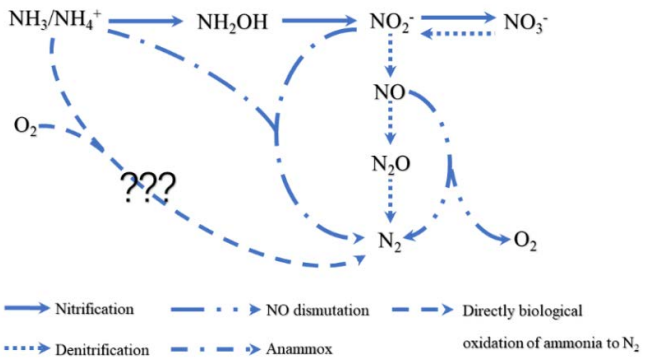

Fig. 1. Known and possible pathways for biological denitrification.

2. Methodology

2.1. 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 [12–14]. Recently, the previous method was modified by McCarty [15] and the effects of sludge retention time and organism decay rate on the portion of the electrons into microbial cells were taken into account. The details of the thermodynamic method are illustrated in Eqs. (2)–(10).

In a biological reaction, microorganisms must use an electron-donor substrate for synthesis. During the process, a portion of electrons (f_e^0) is initially transferred to the electron acceptor to provide energy for conversion of the other portion of electrons (f_s^0) into microbial cells. The sum of f_e^0 and f_s^0 is 1. The overall reaction for biological growth can be ascertained by an energy reaction (R_e) and a synthesis reaction (R_s) combined with f_e^0 and f_s^0.

The energy reaction (R_e) can be calculated by:

$$R_e = R_a + R_d \tag{2}$$

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

The synthesis reaction (R_s) can be calculated by:

$$R_s = R_c + R_d \tag{3}$$

where R_c is the half-reaction for synthesis. In the synthesis reaction, the nitrogen source is supposed to be the ammonia nitrogen.

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

$$R = f_e^0 \cdot R_e + f_s^0 \cdot R_s = f_e^0 \cdot R_a + f_s^0 \cdot R_c + R_d \tag{4}$$

f_s^0 and f_e^0 can be computed by:

$$f_s^0 = \frac{1}{(1+A)} \text{ and } f_e^0 = \frac{A}{(1+A)} \tag{5}$$

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

$$A \cdot \varepsilon \cdot \Delta G_r + \Delta G_s = 0 \tag{6}$$

A equals to:

$$A = -\frac{\Delta G_s}{(\varepsilon \cdot \Delta G_r)} \tag{7}$$

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

$$\Delta G_s = \left(\frac{\Delta G_p}{\nu}\right) + \left(\frac{\Delta G_{pc}}{\varepsilon}\right) \quad (8)$$

where ΔG_p is the energy required to convert the carbon source and equals to 113.8 kJ/e⁻ eq under autotrophic condition; ε is the energy-transfer efficiency. Under the optimum conditions, transfer efficiencies of 55%–70% are typical; ΔG_{pc} is the energy required for converting pyruvate carbon to cellular carbon and is estimated to equal to 3.33 kJ per gram cells. As the empirical formula of bacterial cells is $C_5H_7O_2N$, ΔG_{pc} is 18.8 kJ/e⁻ eq; exponent *n* indicates energy produced or required in the conversion of external carbon source to pyruvate. When energy is produced, *n* is –1, and when energy is required, *n* is 1; ΔG_r is the energy released by oxidizing per equivalent of electron donor for energy generation, and is calculated by:

$$\Delta G_r = \Delta G_a^0 + \Delta G_d^0 \quad (9)$$

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

Eventually, *A*, f_e^0, and f_s^0 can be obtained. f_s is impacted by solid retention time (SRT) and decay rate as follows [15]:

$$f_s = f_s^0 \cdot (1 + 0.2 \cdot b \cdot \theta_x) / (1 + b \cdot \theta_x) \quad (10)$$

where *b* is the organism decay rate (d^{-1}), and θ_x is SRT (d^{-1}). Half reactions relating to the direct oxidation of NH_4^+ to N_2 are shown in Table 1, and different reaction conditions can be employed to explore the possibility of direct biological oxidation of NH_4^+ to N_2 theoretically.

2.2. Calculation scenarios

As the energy-transfer efficiency of the direct oxidation of NH_4^+ to N_2 was unknown, the values of ε were set at 20%, 30%, 40%, 50%, 60%, and 70%; *b* was equal to 0.05 [15]. As the microbials responsible for direct oxidation of NH_4^+ to N_2 were autotrophs, relatively long SRTs of 10, 15, and 20 d were used. The calculation scenarios are listed in Table 2. Table 3 exhibits the standard Gibbs free energies of different compounds under the standard condition used for calculating the standard Gibbs free energy changes of different reactions.

3. Results and discussion

3.1. Yield of chemolithotrophs capable of oxidizing NH_4^+ to N_2

Based on the thermodynamic method, the stoichiometric equations of direct biological oxidation of NH_4^+ to N_2 under different conditions are shown in Table 4. According to the stoichiometric equations, microbial yields can be ascertained (Table 4 and Fig. 2). When energy-transfer efficiencies were in the range of 20%–70%, the microbial yields were in the range of 0.0225–0.2424 mg cell/mg N. When energy-transfer efficiencies were lower than 0.4, the microbial yields were smaller than 0.1 g cell/mg N. Table 5 exhibits the yields of ammonia oxidizers and nitrite oxidizers in the published literatures.

When the energy-transfer efficiencies are higher than 40%, the yield of microbials responsible for the direct oxidation of NH_4^+ to N_2 is close to that of ammonia oxidizers (Table 5), and when the efficiency is lower than 40%, the microbial yield is close to that of nitrite oxidizers (Table 5). Nitrifiers, as autotrophs, conserve a small amount of energies in biomass, and the small f_s results in a low yield [14]. Similarly, microbials gained energy from the direct oxidation of NH_4^+ to N_2 is autotrophs, and the energy-transfer efficiency cannot be in a high level. Consequently, the yield of chemolithotrophs responsible for the direct oxidation of NH_4^+ to N_2 closing to that of nitrite oxidizers might be reasonable. In another words, the yield of chemolithotrophs responsible for the direct oxidation of NH_4^+ to N_2 is probably lower than 0.1 mg cell/mg N.

3.2. Comparisons of direct biological oxidation of NH_4^+ to N_2 with other biological nitrogen removal processes

Compared with the known biological nitrogen removal processes, biological nitrogen removal via direct oxidation of NH_4^+ to N_2 has a lot of benefits. Comparison of direct biological oxidation of NH_4^+ to N_2 with other biological nitrogen removal processes are shown in Table 6.

Compared with conventional biological nitrogen removal processes and short-cut nitrification–denitrification process,

Table 1
Half reactions relevant to the direct oxidation of NH_4^+ to N_2

Equation	Description	Half reaction
R_d	Direct oxidation of NH_4^+ to N_2	$\frac{1}{3}NH_4^+ = \frac{1}{6}N_2 + \frac{4}{3}H^+ + e^-$
R_a	Aerobic reaction	$\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$
R_c	Cell synthesis (NH_4^+ as N source)	$\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$

Table 2
Calculation scenarios for direct oxidation of NH_4^+ to N_2 by O_2

Energy-transfer efficiency (ε)	Organism decay rate (d^{-1})	SRT (d)
0.2	0.05	10
		15
		20
0.3	0.05	10
		15
		20
0.4	0.05	10
		15
		20
0.5	0.05	10
		15
		20
0.6	0.05	10
		15
		20
0.7	0.05	10
		15
		20

Table 3
Standard Gibbs free energy of different compounds

No.	Substance	State	ΔG (1 atm, 25°C) (kJ/mol)	References
1	H^+ (10^{-7})	aq	–39.870	[16]
2	NH_4^+	aq	–79.370	
3	H_2O	l	–237.178	

the sludge yields of the processes are higher than those of direct oxidation of NH_4^+ to N_2 as heterotrophs participate in the denitrification process. Besides, as NH_4^+ is converted to NO_2^- or NO_3^-, more O_2 are required and energy requirements of the above processes are higher than those of direct oxidation of NH_4^+ to N_2.

Essentially, short-cut nitrification process combined with ANAMMOX process are the nitrogen removal pathways of SHARON-ANAMMOX process, OLAND process, and CANON process [20–22]. In the SHARON-ANAMMOX process, OLAND process, and CANON process, N removal is carried out completely by autotrophs, and sludge yields of the three processes are smaller than those of the conventional biological nitrogen process. Similar to the three processes, direct biological oxidation of NH_4^+ to N_2 is also an autotrophic N removal process, and the sludge yield of the direct biological oxidation of NH_4^+ to N_2 is also lower than conventional nitrogen removal process which can be confirmed by the theoretical analysis (Table 5). Except for the benefit of lower sludge yield, from the perspective of electron transfer, three electrons are transformed in the direct oxidation of NH_4^+ to N_2, while five electrons are transformed during the conversion of NH_4^+ to NO_2^- in SHARON-ANAMMOX process, OLAND process, as well as CANON process. Consequently, the oxygen supply of the direct oxidation of NH_4^+ to N_2 are lower than those of the three processes as the end-product was N_2 instead of NO_2^-, and energy requirements can be reduced.

3.3. Possible methods for discovering chemolithotrophs capable of oxidizing NH_4^+ to N_2

Generally, N_2 is the main end-product in biological nitrogen removal processes, for example, conventional denitrification process [14], aerobic denitrification process [23–25], simultaneous nitrification and denitrification process [26]. Furthermore, NO dismutation recently discovered also can produce N_2 [27]. As a result, it's hard to distinguish the direct oxidation of NH_4^+ to N_2 from other denitrification processes via the end-product.

How can we find the experimental evidence for the existence of the chemolithotrophs capable of direct oxidation of NH_4^+ to N_2? The ideal system for cultivating chemolithotrophs capable of oxidizing NH_4^+ to N_2 should possess several characteristics.

Firstly, as the specific growth rate of chemolithotrophs was small, the system should be operated in a relatively long SRT, and the chemolithotrophs population can be enriched; secondly, owning to the low yield of the chemolithotrophs, smaller than 0.1 mg cell/mg N, the system should be operated in a long period with enough substrates such as oxygen and NH_4^+; finally, chemolithotrophs catalyzing the reaction would compete with nitrifiers for substrate [7], and the nitrification process should be suppressed by C_2H_2, nitrapyrin or allylthiorea (ATU) [28,29] to avoid the conversion of NH_4^+ to NO_2^- or NO_3^-. The prerequisite of the known biological denitrification processes is nitrification process, no matter heterotrophic denitrification, autotrophic denitrification, or aerobic denitrification. If the nitrification process is stopped, all known denitrification processes cannot happen, and if N_2 can be detected in the exhaust of

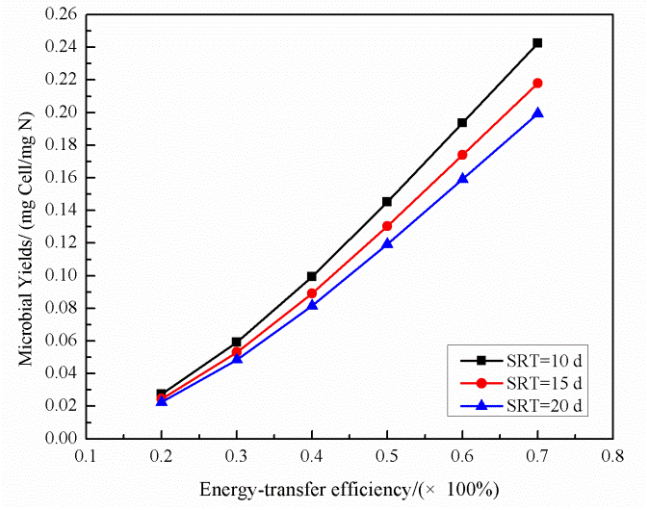

Fig. 2. Microbial yields under different SRTs and energy-transfer efficiencies.

Table 4
Stoichiometric equation of direct biological oxidation of NH^{4+} to N_2 and the microbial yield

ε	SRT (d)	f_s	f_e	Stoichiometric equation of directly biological oxidation of NH_4^+ to N_2	Microbial Yield (mg cell/ mg N)
	10	0.0227	0.9773	$0.0045CO_2 + 0.0011HCO_3^- + 0.3345NH_4^+ + 0.2443O_2 = 0.0011C_5H_7O_2N + 0.4989H_2O + 0.1667N_2 + 0.3333H^+$	0.0274
0.2	15	0.0204	0.9796	$0.0041CO_2 + 0.0010HCO_3^- + 0.3344NH_4^+ + 0.2449O_2 = 0.0010C_5H_7O_2N + 0.4990H_2O + 0.1667N_2 + 0.3333H^+$	0.0246
	20	0.0186	0.9814	$0.0037CO_2 + 0.0009HCO_3^- + 0.3343NH_4^+ + 0.2454O_2 = 0.0009C_5H_7O_2N + 0.4991H_2O + 0.1667N_2 + 0.3333H^+$	0.0225
	10	0.0492	0.9508	$0.0098CO_2 + 0.0025HCO_3^- + 0.3358NH_4^+ + 0.2377O_2 = 0.0025C_5H_7O_2N + 0.4975H_2O + 0.1667N_2 + 0.3333H^+$	0.0592
0.3	15	0.0441	0.9559	$0.0088CO_2 + 0.0022HCO_3^- + 0.3355NH_4^+ + 0.2390O_2 = 0.0022C_5H_7O_2N + 0.4978H_2O + 0.1667N_2 + 0.3333H^+$	0.0531
	20	0.0403	0.9597	$0.0081CO_2 + 0.0020HCO_3^- + 0.3353NH_4^+ + 0.2399O_2 = 0.0020C_5H_7O_2N + 0.4980H_2O + 0.1667N_2 + 0.3333H^+$	0.0485
	10	0.0832	0.9168	$0.0166CO_2 + 0.0042HCO_3^- + 0.3375NH_4^+ + 0.2292O_2 = 0.0042C_5H_7O_2N + 0.4958H_2O + 0.1667N_2 + 0.3333H^+$	0.0994
0.4	15	0.0745	0.9255	$0.0149CO_2 + 0.0037HCO_3^- + 0.3371NH_4^+ + 0.2314O_2 = 0.0037C_5H_7O_2N + 0.4963H_2O + 0.1667N_2 + 0.3333H^+$	0.0892
	20	0.0680	0.9320	$0.0136CO_2 + 0.0034HCO_3^- + 0.3367NH_4^+ + 0.2330O_2 = 0.0034C_5H_7O_2N + 0.4966H_2O + 0.1667N_2 + 0.3333H^+$	0.0815
	10	0.1221	0.8779	$0.0244CO_2 + 0.0061HCO_3^- + 0.3394NH_4^+ + 0.2195O_2 = 0.0061C_5H_7O_2N + 0.4939H_2O + 0.1667N_2 + 0.3333H^+$	0.1452
0.5	15	0.1095	0.8905	$0.0219CO_2 + 0.0055HCO_3^- + 0.3388NH_4^+ + 0.2226O_2 = 0.0055C_5H_7O_2N + 0.4945H_2O + 0.1667N_2 + 0.3333H^+$	0.1304
	20	0.0999	0.9001	$0.0200CO_2 + 0.0050HCO_3^- + 0.3383NH_4^+ + 0.2250O_2 = 0.0050C_5H_7O_2N + 0.4950H_2O + 0.1667N_2 + 0.3333H^+$	0.1192
	10	0.1639	0.8361	$0.0328CO_2 + 0.0082HCO_3^- + 0.3415NH_4^+ + 0.2090O_2 = 0.0082C_5H_7O_2N + 0.4918H_2O + 0.1667N_2 + 0.3333H^+$	0.1936
0.6	15	0.1469	0.8531	$0.0294CO_2 + 0.0073HCO_3^- + 0.3407NH_4^+ + 0.2133O_2 = 0.0073C_5H_7O_2N + 0.4927H_2O + 0.1667N_2 + 0.3333H^+$	0.1740
	20	0.1341	0.8659	$0.0268CO_2 + 0.0067HCO_3^- + 0.3400NH_4^+ + 0.2165O_2 = 0.0067C_5H_7O_2N + 0.4933H_2O + 0.1667N_2 + 0.3333H^+$	0.1591
	10	0.2064	0.7936	$0.0413CO_2 + 0.0103HCO_3^- + 0.3437NH_4^+ + 0.1984O_2 = 0.0103C_5H_7O_2N + 0.4897H_2O + 0.1667N_2 + 0.3333H^+$	0.2424
0.7	15	0.1850	0.8160	$0.0370CO_2 + 0.0092HCO_3^- + 0.3426NH_4^+ + 0.2038O_2 = 0.0092C_5H_7O_2N + 0.4908H_2O + 0.1667N_2 + 0.3333H^+$	0.2179
	20	0.1689	0.8311	$0.0338CO_2 + 0.0084HCO_3^- + 0.3418NH_4^+ + 0.2078O_2 = 0.0084C_5H_7O_2N + 0.4916H_2O + 0.1667N_2 + 0.3333H^+$	0.1994

Table 5
Yields of ammonia oxidizers and nitrite oxidizers

No.	Yields of ammonia oxidizers	Yields of nitrite oxidizers	References
1	0.33 mg VSS/mg N	0.083 VSS/mg N	[14]
2	0.18 g cell/mg N	0.06 mg cell/mg N	[17]
3	0.15 g cell/mg N	0.04 mg cell/mg N	[18]
4	0.21 g cell/mg N	0.05 g cell/mg N	[19]

Table 6
Comparisons of direct oxidation of NH_4^+ to N_2 with other biological nitrogen removal processes

No.	Process	Microbials	Sludge yield	Energy-transfer efficiencies	Energy requirement
1	Conventional biological nitrogen removal process	Heterotrophs and autotrophs	High	High	High
2	Short-cut nitrification and denitrification process	Heterotrophs and autotrophs	Medium	High/lows	Medium
3	SHARON + ANAMMOX process	Autotrophs	Low	Low	Medium
4	OLAND process	Autotrophs	Low	Low	Medium
5	CANON process	Autotrophs	Low	Low	Medium
6	Direct biological oxidation of NH_4^+ to N_2	Autotrophs	Low	Low	Low

the aforementioned process, there is a great opportunity to discover a completely new denitrification pathway.

4. Conclusions

There are different pathways for biological nitrogen removal, however, direct biological oxidation of NH_4^+ to N_2 is still undiscovered. From the perspective of thermodynamics, microorganisms can gain energy from the direct oxidation of NH_4^+ to N_2. Based on the thermodynamic method, the yield of the microbials responsible for this process was relatively small and was probably lower than 0.1 mg cell/mg N owing to the low energy-transfer efficiency. Besides, the end-product of directly biological oxidation of NH_4^+ to N_2 was as same as that of the known denitrification processes. More efforts should be made to discover the evidence for the existence of the chemolithotrophs capable of the direct oxidation of NH_4^+ to N_2.

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