

The influence of trace NO₂ on the kinetics of ammonia oxidation and the characteristics of nitrogen removal from wastewater

Daijun Zhang, Qing Cai, Bo Zu, Cui Bai and Ping Zhang

ABSTRACT

Ammonia oxidizing bacteria-enriched sludge was obtained in a sequencing batch reactor with synthetic wastewater. Batch tests of ammonia oxidation were carried out with the sludge, in the presence of only trace NO₂ and in the presence of O₂ and trace NO₂, respectively. The Andrews model is used to describe NO₂-dependent ammonia oxidation. The maximum ammonia oxidation rate of 139.11 mg N·(g sludge COD h)⁻¹, occurred in the presence of 21% O₂ and 100 ppm NO₂, which was 3 times higher than the aerobic ammonia oxidation rate without NO₂. The kinetic model of ammonia oxidation in the presence of O₂ and trace NO₂ is developed, and the function of NO₂ apparently enhancing ammonia oxidation is suggested. The maximal nitrogen removal of 34.19% occurred at the 21% O₂ and 100 ppm NO₂ in the mixed gases. Nitrogen removal principally depended on the denitrification activity and NO₂-dependent ammonia oxidation activity of ammonia-oxidizing bacteria (AOB).

Key words | ammonia-oxidizing bacteria-enriched sludge, kinetics analysis, nitrogen removal from wastewater, NO₂-dependent ammonia oxidation, trace NO₂ enhancing ammonia oxidation

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INTRODUCTION

In biological nitrogen removal from wastewater, nitrogen is converted to the side-products N₂O and NO besides the main product N₂. Autotrophic nitrification and heterotrophic denitrification were considered as the main origins of N₂O and NO (Stuven & Bock 2001; Tallec *et al.* 2008), and, nitrifier denitrification (Kuai & Verstraete 1998; Kampschreur *et al.* 2008a; Tallec *et al.* 2008), anaerobic ammonia oxidation (Strous *et al.* 1999; Kampschreur *et al.* 2008b) could also produce those nitrogen oxides. NO can be easily oxidized to NO₂ by chemical oxidation with O₂ oxygen as oxidant. Schmidt & Bock (1997) found that NO₂ could serve as an electron acceptor for *Nitrosomonas eutropha* to anaerobically oxidize ammonia, which was known as NO₂-dependent ammonia oxidation. The maximum NO₂-dependent ammonia oxidation activity

of *N. eutropha* occurred anaerobically at 25 ppm NO₂. The rate of NO₂-dependent ammonia oxidation increased at 0 ~ 25 ppm NO₂, and then decreased gradually with further enhancing the concentration of NO₂ (Schmidt *et al.* 2001b). NO was released in NO₂-dependent ammonia oxidation, in which the stoichiometric relationship among the NH₄⁺, NO₂ and NO was 1:2:2 (Schmidt & Bock 1998).

The aerobic ammonia oxidation rate, the growth rate, the maximum cell density and the denitrifying ability of the *N. eutropha* pure culture increased significantly when adding trace NO₂. The maximum ammonia oxidation activity occurred at 100 ppm NO₂ in mixed gases of trace NO₂ and O₂ (Zart & Bock 1998; Schmidt *et al.* 2001b). NO₂ (or NO) was considered as a signal molecule in ammonia oxidation (Schmidt *et al.* 2001b,c). In the presence

of O₂, NO was chemically oxidized to NO₂, and then took part in NO₂-dependent ammonia oxidation. Those processes resulted in NO_x-cycle in the interior of the cell, only little NO_x could be observed in the outside of the cell. The NO_x-cycle increased the availability of the O₂, and enhanced the ammonia oxidation activity of AOB (Zart & Bock 1998; Schmidt *et al.* 2001c). It was suggested that the NO_x-cycle was a new mechanism model of ammonia oxidation (Schmidt *et al.* 2001a). In that model, O₂ participates in aerobic ammonia oxidation only through the NO_x-cycle, the oxygen in the intermediate product NH₂OH in ammonia oxidation comes from O₂ provided in the outside of cell, and the NO_x-cycle plays the role of transferring O₂ from the outside of cell (Schmidt *et al.* 2001a, 2002; Schmidt 2008). Zart & Bock (1998) observed the nitrogen removal was 51% in the ammonia oxidation process of *N. eutropha* when the DO was kept between 3 mg L⁻¹ ~ 4 mg L⁻¹ by controlling aeration intensity and stirring rate in the mixed gases containing 50 ppm NO₂. It was demonstrated that trace NO₂ addition could increase the CANON (completely autotrophic nitrogen removal over nitrite) activity of sludge with aerobic ammonium oxidation bacteria (AOB) and anaerobic ammonium oxidation bacteria (ANAOB) (Zhang *et al.* 2010).

In this paper, the AOB-enriched sludge was obtained through the operation of a SBR reactor inoculated with the activated sludge and fed with the synthetic wastewater containing ammonium. The batch tests of ammonia oxidation were carried out with the AOB-enriched sludge, under the atmosphere with only trace NO₂ and with O₂ and trace NO₂ in mixed gases, respectively, in order to study the influence of trace NO₂ on the kinetics of ammonia oxidation and the characteristics of nitrogen removal from wastewater of the sludge.

MATERIALS AND METHODS

The experiment was carried out by two steps. The first step was to cultivate the AOB-enriched sludge in a sequencing batch reactor with 6 L working volume. The reactor was inoculated with 2 L activated sludge from a wastewater treatment plant in Chongqing, China. The operation duration was 24 h. The aeration time was 21.5 h, the settling time was

2 h and the emptying time was 0.5 h. The temperature of reactor was controlled between 28 and 32°C by a water bath. The DO concentration was kept at 0.8 ~ 1.2 mg L⁻¹ through controlling aeration, and the pH at 7.8 ~ 8.5 by adding NaHCO₃ (2 mol L⁻¹). The original sludge was washed with the distilled water and aerated for 6 h before inoculation. Initial NH₄⁺-N concentration of synthetic wastewater was controlled at 50 mg L⁻¹. After reactor was stable, NH₄⁺-N concentration of influent water increased to 250 mg L⁻¹ by gradually adding solution (50 mg L⁻¹ once), and lasting for 120 d. The main component of the synthetic wastewater was NH₄Cl, and the other components (mg L⁻¹) were as follows: KH₂PO₄ (30), KCl (110), MgSO₄ (50), and the trace nutrients liquid (0.4 mL L⁻¹), which consisted of (mg L⁻¹) ZnSO₄·5H₂O (80), FeSO₄·7H₂O (100), CuCl₂·2H₂O (20), MgCl₂·6H₂O (50), and H₃BO₃ (20). 4.6 × 10⁸ AOB cell (g MLSS)⁻¹ in the AOB-enriched sludge was determined by Most Probable Numbering method (Kuai & Verstraete 1998).

In the second step, the kinetic batch tests of ammonia oxidation with the AOB-enriched sludge were carried out in a reactor with 2.5 L working volume. The reactor was inoculated with 50 mL mixed culture sludge cultivated in the first step. The AOB-enriched sludge was washed 3 times with distilled water and settled for 40 min before inoculation. Then 450 mL synthetic wastewater was added in the reactor. The temperature of reactor was remained between 28 and 32°C, and the pH was controlled at 7.8 ~ 8.2. The mixed gases (Table 1) were directly vented over the base of reactor at the flow rate of 60 mL min⁻¹. The reactor was well stirred by the rotor (40 r min⁻¹) in the bottom of the reactor. Each experimental duration was about 2 ~ 3 h. The samples were taken out from the sample outlets every 20 min in the tests. The average concentrations of NH₄⁺-N, NO₂⁻-N, NO₃⁻-N were determined by measuring triple samples. The aerobic ammonia oxidizing kinetics coefficients of the AOB-enriched sludge were determined with a respirometer (Bioscience 2000, USA), in which there was 1 L working volume with 55 mL sludge.

A 721 type of Spectrophotometer (The Third Analysis Apparatus Company of Shanghai, China) was used for the measurements of NO₂⁻-N (GB/T13580. 7-1992, China), NO₃⁻-N (GB/T7480-1987, China) and NH₄⁺-N (GB/T7479-1987, China). A precision pH meter (PB10, Beijing

Table 1 | Mixed gas composition (with N₂ as equilibrium gas) and nitrogen removals from wastewater in the presence of O₂ and trace NO₂

| Gas composition (v/v) | | | | Nitrogen in the liquid | | | | | | NO _x in the mixed gas | | | | | |
|--------------------------|-----------------------|--------------------------|-----------------|---|---|---|---|---|---|----------------------------------|----------------------------|--------------|------------------------|----------------|-------------------------------|
| NO ₂ (ppm) | O ₂ (%) | CO ₂ (ppm) | Duration (h) | Initial state | | | Final state | | | Nitrogen removal (%) | In-gas | | Off-gas | | Total nitrogen removal (%) |
| | | | | NH ₄ ⁺ -N (mg) | NO ₂ ⁻ -N (mg) | NO ₃ ⁻ -N (mg) | NH ₄ ⁺ -N (mg) | NO ₂ ⁻ -N (mg) | NO ₃ ⁻ -N (mg) | | NO ₂ -N (mg) | NO-N (mg) | Nitrogen change (%) | | |
| 5 | / | 300 | 2.0 | 76.65 | 0.38 | 0.73 | 70.10 | 1.63 | 0.40 | 7.24 | 1.52 | 0.45 | 0.73 | -27.37 | 7.53 |
| 15 | / | 300 | 4.0 | 72.05 | 0.70 | 0.83 | 58.84 | 2.38 | 1.75 | 14.42 | 4.57 | 1.60 | 2.20 | -16.85 | 14.55 |
| 25 | / | 300 | 3.0 | 77.5 | 0.41 | 0.83 | 46.13 | 2.80 | 1.95 | 35.38 | 7.61 | 12.3 | 5.10 | +128.65 | 20.93 |
| 50 | / | 300 | 2.6 | 74.30 | 0.28 | 0.77 | 56.47 | 2.05 | 2.00 | 19.68 | 15.22 | 15.5 | 4.20 | +29.47 | 11.43 |
| 150 | / | 300 | 1.67 | 79.1 | 0.63 | 0.83 | 68.20 | 1.81 | 1.56 | 11.16 | 30.43 | 25.80 | 3.50 | -3.72 | 9.11 |
| 25 | 2 | 300 | 1.67 | 76.75 | 0.87 | 0.730 | 47.13 | 21.29 | 2.55 | 9.42 | 7.61 | 5.83 | 2.83 | +13.80 | 7.37 |
| 50 | 2 | 300 | 1.67 | 73.85 | 0.71 | 0.80 | 15.36 | 30.10 | 2.95 | 35.76 | 15.22 | 10.18 | 7.01 | +12.96 | 27.57 |
| 100 | 2 | 300 | 1.67 | 78.65 | 0.83 | 0.78 | 36.20 | 21.18 | 2.30 | 25.64 | 30.4 | 40.72 | 6.94 | +56.68 | 3.02 |
| 150 | 2 | 300 | 1.67 | 77.00 | 0.73 | 0.80 | 33.56 | 17.80 | 2.16 | 31.19 | 45.65 | 69.18 | 4.90 | +62.28 | -2.76 |
| 250 | 2 | 300 | 1.67 | 77.00 | 1.25 | 0.91 | 29.58 | 15.75 | 2.03 | 40.17 | 76.09 | 112.40 | 4.40 | +53.50 | -5.74 |
| 25 | 21 | 300 | 1.67 | 77.30 | 0.84 | 0.88 | 2.30 | 37.67 | 8.84 | 38.23 | 7.61 | 14.8 | 1.15 | +109.59 | 25.25 |
| 50 | 21 | 300 | 1.67 | 72.15 | 0.49 | 0.93 | 0.43 | 38.95 | 9.50 | 33.57 | 15.22 | 14.50 | 1.83 | +7.29 | 26.57 |
| 100 | 21 | 300 | 1.67 | 74.75 | 0.63 | 0.84 | 0.51 | 43.13 | 10.65 | 28.77 | 30.43 | 15.00 | 0.89 | -47.78 | 34.19 |
| 150 | 21 | 300 | 1.67 | 77.6 | 1.13 | 0.97 | 23.80 | 39.30 | 9.83 | 8.49 | 45.65 | 31.99 | 1.10 | -27.51 | 15.42 |
| 250 | 21 | 300 | 1.67 | 76.55 | 1.25 | 0.89 | 19.05 | 28.12 | 6.83 | 31.38 | 76.09 | 96.23 | 0.91 | +27.67 | 2.35 |

Note: Negative and positive value indicates the decreasing and increasing NO_x in the mixed gas, respectively. Total nitrogen removal contains N₂, organic nitrogen (microbial growth), and a little N₂O.

Sartorius Instrument System Company, China) was used for the measurement of pH. DO was measured by a precision DO meter (JYD-1A, Jiangsu Jiangfen Electro-analytical Instrument Company, China). NO₂ and NO were measured by a NO₂/NO gas analyzer (p-Gas200, Beijing Beidouxin Institute of Industrial Chemistry, China). The concentration of sludge was presented by COD, where 5 mL mixed solution in reactor was taken to measure sludge COD (GB/T11914-1989, China). Ammonia oxidation rate (q) was defined as NH₄⁺-N removal quantity per gram sludge COD in unit time.

RESULTS AND DISCUSSION

The kinetics of NO₂-dependent ammonia oxidation

When there was no O₂ in the mixed gases, the relationship between ammonia oxidation rate and NO₂ concentration was shown in Figure 1 (left). The ammonia oxidation rate increased with NO₂ concentration, reaching maximum 18.37 mg N (g sludge COD h)⁻¹ at 25 ppm NO₂, but then decreased with further increasing NO₂ concentration. Schmidt & Bock (1997) got the maximum ammonia oxidation rate, 134.4 mol (g protein h)⁻¹, under the condition of 25 ppm NO₂ without O₂ in the mixed gases. And the influence of NO₂ concentration on the ammonia oxidation rate was basically accordant with our test results.

It was assumed that the quantity of AOB in the sludge do not change during the batch test, the kinetics process of NO₂-dependent ammonia oxidation should be controlled by NH₄⁺-N concentration and NO₂ concentration. Andrews inhibiting model (Andrews 1968) is applied to describe the influence of NO₂ on ammonia oxidation, so NO₂-dependent ammonia oxidation rate could be described as the following equation:

$$q_{\text{NO}_2} = q_{\text{NO}_2, \text{max}} \cdot \frac{S_{\text{nh}}}{K_S + S_{\text{nh}}} \cdot \frac{S_{\text{NO}_2}}{K_{\text{NO}_2} + S_{\text{NO}_2} + S_{\text{NO}_2}^2 / K_{\text{NO}_2, I}} \quad (1)$$

where, $q_{\text{NO}_2, \text{max}}$ is maximum NO₂-dependent ammonia oxidation rate (mg N (g sludge COD h)⁻¹), S_{nh} is NH₄⁺-N concentration (mg N L⁻¹), S_{NO_2} is NO₂ concentration (mmol NO₂ m⁻³), K_S is NH₄⁺-N half saturation constant (mg N L⁻¹), K_{NO_2} is NO₂ half saturation constant of NO₂-dependent ammonia oxidation (mmol NO₂ m⁻³), and $K_{\text{NO}_2, I}$ is NO₂ inhibition constant of NO₂-dependent ammonia oxidation (mmol NO₂ m⁻³).

The test for aerobic ammonia oxidation kinetics of mixed culture was carried out in a respirometer (Bioscience 2000, USA). The initial NH₄⁺-N concentration in the reactor was 150 mg NH₄⁺-N L⁻¹, and O₂ concentration measured under the condition of air aeration was 9.380 mg O₂ L⁻¹. The results were used to fit with the kinetic equation of aerobic ammonium oxidation, and the kinetic parameters were determined as follows: $q_{\text{max}} = 29.16$ mg N

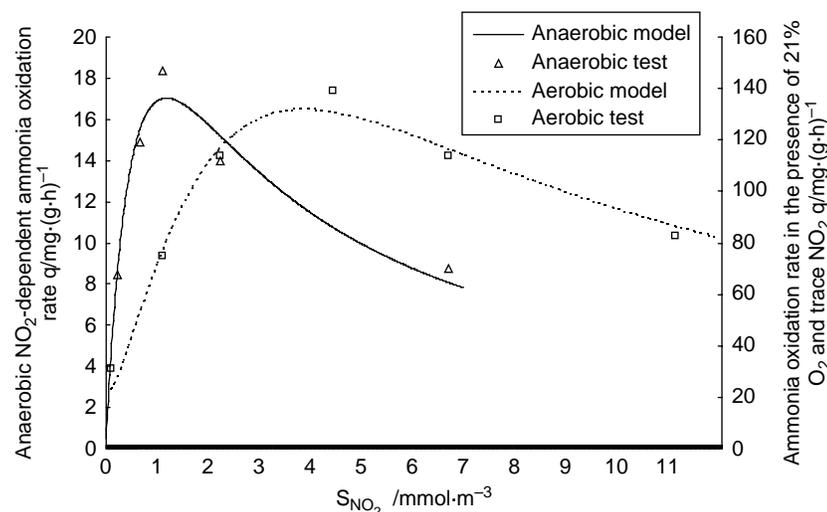


Figure 1 | Anaerobic NO₂-dependent ammonia oxidation rate (left) and ammonia oxidation rate in the presence of 21% O₂ and trace NO₂ (right).

(mg sludge COD h)⁻¹, $K_{O_2} = 0.494 \text{ mg O}_2 \text{ L}^{-1}$, $K_S = 5.359 \text{ mg NH}_4^+ \text{-NL}^{-1}$. The typical values of K_{O_2} and K_S were $0.4 \text{ mg O}_2 \text{ L}^{-1}$ and $1.0 \text{ mg NH}_4^+ \text{-NL}^{-1}$ in ASM1, respectively (Henze *et al.* 2000). The experimental results of NO₂-dependent ammonia oxidation are used to fit with Equation (1). The fitted curve is shown in Figure 1 (left), and the parameters in equation are estimated with MATLAB 7.4.0 as follows: $q_{\text{NO}_2, \text{max}} = 42.01 \text{ mg N (g sludge COD h)}^{-1}$, $K_{\text{NO}_2} = 0.82 \text{ mmol NO}_2 \text{ m}^{-3}$, $K_{\text{NO}_2, I} = 1.72 \text{ mmol NO}_2 \text{ m}^{-3}$.

The kinetics of ammonia oxidation in the presence of O₂ and trace NO₂

The relationship between ammonia oxidation rate and NO₂ concentration in the presence of O₂ and trace NO₂ is shown in Table 2 and Figure 1. The ammonia oxidation rate increased obviously after adding 2% O₂ into the mixed gases. The maximum ammonia oxidation rate of about 57.74 mg N (g sludge COD h)⁻¹ occurred at 50 ppm NO₂. Compared with under the condition of 2% O₂ and trace NO₂ in mixed gases, the ammonia oxidation rate increased significantly under the condition of 21% O₂ and trace NO₂ in mixed gases. The maximum ammonia oxidation rate of about 139.11 mg N (g sludge COD h)⁻¹ occurred at 100 ppm NO₂ and 21% O₂, and then the ammonia oxidation rate decreased with further increasing NO₂ concentration.

Schmidt *et al.* (2001a) reported that acetylene (C₂H₂) could inhibit the activity of aerobic ammonia oxidation, rather than NO₂-dependent ammonia oxidation. The aerobic ammonia oxidation and the NO₂-dependent ammonia oxidation occurred at the different active positions of the ammonia monooxygenase (AMO). The cellular ultrastructure of *Nitrosomonas europaea* changed when trace gaseous

NO₂ was added in nitrification for some period (Schmidt *et al.* 2001c). These studies clearly demonstrate that it's necessary to distinguish between NO₂-dependent ammonia oxidation and general aerobic ammonia oxidation. In the presence of O₂ and trace NO₂, NO₂-dependent ammonia oxidation and general aerobic ammonia oxidation occur simultaneously, and the kinetics process of ammonia oxidation is controlled by NH₄⁺-N concentration, O₂ concentration and NO₂ concentration. The effect of trace NO₂ on ammonia oxidation could be described by the Andrews Equations (Andrews 1968). As the NO produced in NO₂-dependent ammonia oxidation is converted to NO₂ through NO_x-cycle in the interior of AOB cell, NO₂-dependent ammonia oxidation is also controlled by O₂ concentration. And, aerobic ammonia oxidation should be affected by O₂ and NO₂ concentrations because of the competition between the two types of ammonia oxidation. Therefore, the total ammonia oxidation rate is presented in the following equation:

$$q(\text{O}_2/\text{NO}_2) = q_{\text{max}} \cdot \frac{S_{\text{nh}}}{K_S + S_{\text{nh}}} \cdot \frac{S_{\text{O}_2}}{K_{\text{O}_2} + S_{\text{O}_2}} \cdot \frac{S_{\text{O}_2}}{S_{\text{O}_2} + K \cdot S_{\text{NO}_2}} + q_{\text{NO}_2, \text{max}}^{\text{O}_2} \cdot \frac{S_{\text{nh}}}{K_S + S_{\text{nh}}} \cdot \frac{S_{\text{O}_2}}{K_{\text{O}_2} + S_{\text{O}_2}} \cdot \frac{S_{\text{NO}_2}}{K_{\text{NO}_2}^{\text{O}_2} + S_{\text{NO}_2} + S_{\text{NO}_2}^2 / K_{\text{NO}_2, I}^{\text{O}_2}} \quad (2)$$

where, q_{max} , $q_{\text{NO}_2, \text{max}}^{\text{O}_2}$ are the maximal rate of aerobic ammonia oxidation and the maximal NO₂-dependent ammonia oxidation rate (mg N (g sludge COD-h)⁻¹) in the presence of O₂ and trace NO₂, respectively. S_{O_2} is DO concentration (mg O₂ L⁻¹) and K_{O_2} is DO half saturation constant (mg O₂ L⁻¹). K is the factor of O₂ competition with NO₂ (g O₂ (mmol NO₂)⁻¹), $K_{\text{NO}_2}^{\text{O}_2}$ is NO₂ half saturation constant of NO₂-dependent ammonia oxidation

Table 2 | Ammonia oxidation rate in the presence of 2% O₂ and trace NO₂

| NO ₂ concentration S_{NO_2} (ppm) | 25 | 50 | 100 | 150 | 250 |
|--|--------|-------|--------|-------|-------|
| DO concentration S_{O_2} /mg L ⁻¹ | 0.375 | 0.375 | 0.374 | 0.378 | 0.379 |
| Calculated value of ammonium oxidation rate q /mg (gCOD h) ⁻¹ | 33.39 | 52.07 | 58.82 | 52.87 | 39.32 |
| Measured value of ammonium oxidation rate q /mg (g COD h) ⁻¹ | 28.78 | 57.74 | 49.87 | 48.12 | 45.20 |
| The proportion of general aerobic ammonia oxidation /% | 0.20 | 0.06 | 0.03 | 0.02 | 0.02 |
| The proportion of NO ₂ -dependent ammonia oxidation /% | 99.80 | 99.94 | 99.97 | 99.98 | 99.98 |
| Error /% | -16.23 | 9.82 | -17.95 | -9.88 | 13.02 |

(mmol NO₂ m⁻³) and $K_{\text{NO}_2, \text{I}}^{\text{O}_2}$ is NO₂ inhibition constant of NO₂-dependent ammonia oxidation in the presence of O₂ and trace NO₂ (mmol NO₂ m⁻³). Equation (2) is changed to

$$q(\text{O}_2/\text{NO}_2)' = q_{\text{max}} \cdot [\text{S}_{\text{nh}} / (K_{\text{S}} + \text{S}_{\text{nh}})] \cdot [\text{S}_{\text{O}_2} / (K_{\text{O}_2} + \text{S}_{\text{O}_2})] \times \left(\frac{\alpha_{\text{max}} \cdot \text{S}_{\text{NO}_2}}{K_{\text{NO}_2}^{\text{O}_2} + \text{S}_{\text{NO}_2} + \text{S}_{\text{NO}_2}^2 / K_{\text{NO}_2, \text{I}}^{\text{O}_2}} + \frac{\text{S}_{\text{O}_2}}{\text{S}_{\text{O}_2} + K \cdot \text{S}_{\text{NO}_2}} \right) \quad (3)$$

where, $\alpha_{\text{max}} = q_{\text{NO}_2, \text{max}}^{\text{O}_2} / q_{\text{max}}$. The function of NO₂ apparently enhancing ammonia oxidation is defined as $\alpha_{\text{O}_2/\text{NO}_2}$:

$$\alpha_{\text{O}_2/\text{NO}_2} = \frac{\alpha_{\text{max}} \cdot \text{S}_{\text{NO}_2}}{K_{\text{NO}_2}^{\text{O}_2} + \text{S}_{\text{NO}_2} + \text{S}_{\text{NO}_2}^2 / K_{\text{NO}_2, \text{I}}^{\text{O}_2}} + \frac{\text{S}_{\text{O}_2}}{\text{S}_{\text{O}_2} + K \cdot \text{S}_{\text{NO}_2}} \quad (4)$$

Therefore,

$$q(\text{O}_2/\text{NO}_2) = q_{\text{max}} \cdot [\text{S}_{\text{nh}} / (K_{\text{S}} + \text{S}_{\text{nh}})] \cdot [\text{S}_{\text{O}_2} / (K_{\text{O}_2} + \text{S}_{\text{O}_2})] \times \alpha_{\text{O}_2/\text{NO}_2} \quad (5)$$

The data from the batch tests in the presence of O₂ and trace NO₂ were used to fit with Equation (5). The fitted curve is shown in Figure 1 (right), and the parameters in equation are estimated with MATLAB 7.4.0 as follows: $\alpha_{\text{max}} = 36.27$, $K_{\text{NO}_2}^{\text{O}_2} = 12.60$ mmol NO₂ m⁻³, $K_{\text{NO}_2, \text{I}}^{\text{O}_2} = 1.22$ mmol NO₂ m⁻³, $K = 60.84$ g O₂ (mmol NO₂)⁻¹.

The equation obtained in the presence of 21% O₂ and trace NO₂ was used to calculate the ammonia oxidation rate at 2% O₂ and trace NO₂. The results are shown in Table 2. It shows that the calculated ammonia oxidation rates by the equation are basically accordant with the measured ones.

The AOB-enriched sludge could use NO₂ as electron acceptor to oxidize ammonia when no molecule oxygen was present. After adding 2% O₂, the ammonia oxidation rate increased obviously, indicating that the activity of ammonia oxidation bacteria was improved. As O₂ concentration increased to 21%, the ammonia oxidation rate further increased. This indicated that the ammonia oxidation rate increased with increasing O₂ concentration in the presence of O₂ and trace NO₂. However, the ammonia oxidation rate decreased under the over-high NO₂ concentration because of the toxicity of NO_x.

The maximum rate of the NO₂-dependent ammonia oxidation was 18.37 mg N (g sludge COD·h)⁻¹, and was about 46% lower than the aerobic ammonia oxidation rate, 34.12 mg N (g sludge COD h)⁻¹. Schmidt and Bock reported the aerobic ammonia oxidation rate was 1377.6 mol (g protein h)⁻¹, and the anaerobic NO₂-dependent ammonia oxidation was only about 10% of the aerobic activity (Schmidt & Bock 1997). The rate of NO₂-dependent ammonia oxidation was high in this study, which indicated that NO₂-dependent ammonia oxidation was an important ammonia oxidation process and couldn't be ignored. By adding trace NO₂ to the mixed gases containing 21% O₂ or containing 2% O₂, the ammonia oxidation rates were 2.197 ~ 4.077 times and 0.844 ~ 1.692 times higher than aerobic ammonia oxidation rate, respectively. The maximum ammonia oxidation rate, 139.11 mg N (g sludge COD h)⁻¹, occurred at 21% O₂ and 100 ppm NO₂ in the mixed gases, and the value of the function of NO₂ apparently enhancing ammonia oxidation was 4.88. The ammonia oxidation rate was enhanced by adding trace NO₂ to the mixed gases containing O₂. The proportion of the aerobic ammonia oxidation and the NO₂-dependent ammonia oxidation in the total ammonia oxidation rate is shown in Table 2. It is obvious that NO₂-dependent ammonia oxidation was much more advantageous than aerobic ammonia oxidation when 2% O₂ and trace NO₂ were present. The case in the presence of 21% O₂ and trace NO₂ was the same (Data was not shown).

Although aerobic ammonia oxidation and NO₂-dependent ammonia oxidation possessed the different mechanisms of biochemical reactions, they could be coupled by NO_x-cycle, through which ammonia oxidation was enhanced. On the one hand, aerobic ammonia oxidation with O₂ as electron acceptor and the NO₂-dependent ammonia oxidation with NO₂ as electron acceptor occurred simultaneously (Schmidt *et al.* 2001a). On the other hand, the NO produced in the NO₂-dependent ammonia oxidation was chemically oxidized to NO₂ by O₂ in the cells, and the later again participated in NO₂-dependent ammonia oxidation. Therefore, as O₂ and trace NO₂ were present at the same time, O₂ enhanced, in the micro-mechanism, NO₂-dependent ammonia oxidation through NO_x-cycle. The NO_x-cycle resulted in O₂ consumption in NO₂-dependent ammonia oxidation, and finally resulted

in NO₂-dependent ammonia oxidation much more advantageous than aerobic ammonia oxidation. The coupling of two types of ammonia oxidation made the ammonia oxidation rate increase remarkably. Trace NO₂ apparently enhanced, in the macro-phenomena, ammonia oxidation when O₂ and trace NO₂ were present at the same time. The mechanism of trace NO₂ enhancing ammonia oxidation, especially the different enzymes related to NO₂-dependent ammonia oxidation and aerobic ammonia oxidation need to be further studied.

Nitrogen removal from wastewater in the presence of O₂ and trace NO₂

The nitrogen removals from wastewater in the tests are shown in Table 1. When only NO₂ was present, nitrogen removals increased with the increasing rates of ammonia oxidation and reached the maximum 20.93% at 25 ppm NO₂. When 2% O₂ and trace NO₂ were present in the mixed gases, the maximal nitrogen removal of 27.57% occurred at 50 ppm NO₂. As O₂ concentration increased to 21% in the mixed gases, the maximal nitrogen removal of 34.19% occurred at 100 ppm NO₂. Nitrogen removals in the presence of O₂ and trace NO₂ also increased with the increasing rates of ammonia oxidation. A significant amount of nitrogen removal from wastewater was converted to gaseous NO and NO₂, which resulted in the increasing amount of NO_x in the off-gas, especially in the limited oxygen conditions of 2% O₂ (Table 1).

The nitrogen removals from wastewater in this paper were basically accordant with literatures (Schmidt & Bock 1997; Zart & Bock 1998). The nitrogen removals in the presence of O₂ and trace NO₂ were related with O₂ concentration and NO₂ concentration, and the maximum nitrogen removal of 34.19% occurred at 21% O₂ and 100 ppm NO₂. There, nitrifier denitrification should take most responsibility for the nitrogen removals. The nitrifier denitrification of AOB is its inherent potential, and the nitrite reductases (Nir) gene and nitric oxide reductases (Nor) gene have been found in genome of *N. europaea* (Chain *et al.* 2003). The nitrifier denitrification, which occurred more easily under the oxygen-limited conditions, could accelerate energy metabolism and increase the growth rate of *N. europaea* remarkably (Zart & Bock 1998;

Schmidt *et al.* 2004b). Nitrite reductase (NirK) supports growth of *N. europaea* by supporting the oxidation of NH₃ to NO₂⁻ via NH₂OH, and NirK-deficient *N. europaea* grew more slowly, consumed less NH₃, had a lower rate of nitrite (NO₂⁻) production (Cantera & Stein 2007). It was also found that the putative *nirK*, which may reduce NO₂⁻ to NO, in *Nitrobacter winogradskyi* Nb-255 to be maximally transcribed under low O₂ (between zero and 4% O₂) in the presence of NO₂⁻ (Starkenburger *et al.* 2008).

NO could enhance nitrifier denitrification (Schmidt & Bock 1997; Schmidt *et al.* 2004a), and inhibit NO₂⁻-dependent respiration (Starkenburger *et al.* 2008). The addition of 200 ppm NO induced higher denitrification activity of *N. europaea* and the increase in N removal from 7% to about 50%, but the ammonia oxidation activity kept basically unchanged (Schmidt *et al.* 2004a). NO and little N₂O could be produced from both NO₂-dependent ammonia oxidation and nitrifier denitrification of AOB. Although net production of NO could not be detected from either aerobically grown or anaerobically incubated cells, exogenous NO was consumed by viable cells and concomitantly inhibited NO₂⁻-dependent O₂ uptake (Starkenburger *et al.* 2008). Therefore, trace NO₂ addition would increase the nitrogen removal due to the increased nitrifier denitrification activity by NO produced from the NO₂-dependent ammonium oxidation of AOB (Zhang *et al.* 2010). Since NO could be easily oxidized to NO₂ in the presence of oxygen, the actual NO amount produced in tests might be higher than those measured.

CONCLUSIONS

AOB used NO₂ as electron acceptor to oxidize ammonia when no O₂ was present. The maximum ammonia oxidation rate was 18.32 mg N (mg sludge COD h)⁻¹ at 25 ppm NO₂. Andrews model could be used to describe the kinetics of NO₂-dependent ammonia oxidation, in which the kinetic parameters have been estimated by the experimental data.

After adding 2% O₂ in the mixed gases, the ammonia oxidation rate was significantly increased, and the maximum ammonia oxidation rate was 57.74 mg N (mg sludge COD h)⁻¹ at 2% O₂ and 50 ppm NO₂. The ammonia oxidation rate increased greatly in the presence of

21% O₂ and trace NO₂, and reached 139.11 mgN (mg sludge COD h)⁻¹ at 100 ppm NO₂, which was 3 times higher than the aerobic ammonia oxidation rate without NO₂. The kinetics model of ammonia oxidation in the presence of O₂ and trace NO₂ is developed, in which the function of NO₂ apparently enhancing ammonia oxidation is given to describe the characteristic of trace NO₂ enhancing ammonia oxidation and the kinetic parameters have been estimated by the experimental data.

The nitrogen removals in the process of ammonia oxidation in the presence of O₂ and trace NO₂ were positively related with ammonia oxidation rate, the maximum nitrogen removal of 34.19% occurred at 21% O₂ and 100 ppm NO₂. The nitrogen removals resulted principally from AOB denitrification and NO₂-dependent ammonia oxidation.

ACKNOWLEDGEMENTS

This study was financially supported by the Natural Science Foundation of China (NSFC 50378094).

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