Environ. Eng. Res. Vol. 13, No. 3, pp. 125~130, 2008
Korean Society of Environmental Engineers

Analysis of Free Ammonia Inhibition of Nitrite Oxidizing Bacteria Using a Dissolved Oxygen Respirometer

Dong-Jin Kim†, Dong-Ig Lee, Gi-Cheol Cha[1], and Jürg Keller[2]

Department of Environmental Sciences and Biotechnology, Hallym University, Chunchon, Korea
[1]Division of Environmental Engineering, Yonsei University, Wonju, Korea
[2]Advanced Water Management Centre, The University of Queensland, Brisbane, Australia

Received January 2008, accepted June 2008

Abstract

Free ammonia (NH_3-N) inhibition of nitrite-oxidizing bacteria (NOB) has been widely studied for partial nitrification (or nitrite accumulation) and denitrification via nitrite (NO_2^--N) as a low-cost treatment of ammonium containing wastewater. The literature on NH_3-N inhibition of NOB, however, shows disagreement about the threshold NH_3-N concentration and its degree of inhibition. In order to clarify the confusion, a simple and cheap respirometric method was devised to investigate the effect of free ammonia inhibition of NOB. Sludge samples from an autotrophic nitrifying reactor were exposed to various NH_3-N concentrations to measure the maximum specific nitrite oxidation rate ($\hat{K}_{NO}$) using a respirometer. NOB biomass was estimated from the yield values in the literature. Free ammonia inhibition of nitrite oxidizing bacteria was reversible and the specific nitrite oxidation rate (K_{NO}) decreased from 0.141 to 0.116, 0.100, 0.097 and 0.081 mg NO_2^--N/mg NOB · h, respectively, as the NH_3-N concentration increased from 0.0 to 1.0, 4.1, 9.7 and 22.9 mg/L. A nonlinear regression based on the noncompetitive inhibition mode gave an estimate of the Inhibition concentration (K_I) of free ammonia to be 21.3 mg NH_3-N/L. Previous studies gave $\hat{K}_{NO}$ of *Nitrobacter* and *Nitrospira* as 0.120 and 0.032 mg/mg VSS · h. The free ammonia concentration which inhibits *Nitrobacter* was 30 ~ 50 mg NH_3-N/L and *Nitrospira* was inhibited at 0.04 ~ 0.08 mg NH_3-N/L. The results support the fact that *Nitrobacter* is the dominant NOB in the reactor. The variations in the reported values of free ammonia inhibition may be due to the different species of nitrite oxidizers present in the reactors. The respirometric method provides rapid and reliable analysis of the behavior and community of the nitrite oxidizing bacteria.

Keywords: Free ammonia inhibition, Nitrification, Nitrite-oxidizing bacteria, Respirometer

1. Introduction

Microbial nitrification, the sequential oxidation of ammonium (NH_4^+-N) to nitrate (NO_3^--N) via nitrite (NO_2^--N), is carried out by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), respectively. The reaction stoichiometry and metabolic flux analysis including the biomass syntheses are as follows:[1)]

$$55NH_4^+ + 76O_2 + 109HCO_3^- \rightarrow C_5H_7NO_2 + 54NO_2^- + 57H_2O + 104H_2CO_3 \quad (1)$$

$$400NO_2^- + NH_4^+ + 4H_2CO_3^- + HCO_3^- + 195O_2 \rightarrow C_5H_7NO_2 + 3H_2O + 400NO_3^- \quad (2)$$

When the nitrite generation rate is higher than its consumption or conversion rate, nitrite can be accumulated in the presence of free ammonia (NH_3) which depends on NH_4^+-N concentration, temperature and pH as follows:[2)]

$$NH_3 - N = \frac{NH_4 - N \times 10^{pH}}{K_a / K_w + 10^{pH}} \quad (3)$$

$$K_a/K_w = e^{(6334/273+T(°C))} \quad (4)$$

Novel nitrogen removal technologies via a nitrite pathway, such as the SHARON and Anammox processes, attract researchers' interest because of their potential savings in the costs of aeration and organic carbons.[3)] The above processes rely on the competition, elimination or selective inhibition of nitrite-oxidizing bacteria (NOB) so that the oxidation of nitrite to nitrate is blocked or inhibited. Nitrite concentration in the conventional

† Corresponding author
E-mail: dongjin@hallym.ac.kr
Tel: +82-33-248-2154, Fax: +82-33-256-3420

nitrogen removal process remains very low due to the simultaneous transformation of nitrite to nitrate. Several mechanisms are suggested for the inhibition of nitrite oxidation,[2,4-6] but they may not be equally effective. The nitrite oxidation rate and degree of inhibition may be different for each NOB species. Studies on NOB identification in wastewater treatment plants showed complicated and ambiguous results depending on the growth conditions and the wastewater used in the experiments as shown in Table 1.

Kinetic parameters and inhibition characteristics of NOB can be estimated from nitrification kinetic studies. Generally, complete nitrification (ammonia oxidation + nitrite oxidation) was monitored and the nitrification kinetic parameters were calculated. In simple case, ammonia oxidation was the most important reaction to measure because it was regarded as the rate-limiting step.[11-13] It became apparent that nitrite oxidation could also be the rate-limiting step when the accumulation of substrate (ammonia) or product (nitrite) inhibit nitrite oxidation[2] and the rate of ammonia oxidation exceeds the rate of nitrite oxidation at higher temperature[4] and/or at lower dissolved oxygen concentration.[6] A respirometric method based on nitrogeneous oxygen uptake rate was developed to calculate the rates of ammonia oxidation and nitrite oxidation separately using combined measurements from dissolved oxygen(DO) meters, respirometers, and titration off-gas gas analyzers.[14-17]

Several reaction kinetics and inhibition parameters of individual NOB species have been calculated using enrichment culture and molecular identification techniques.[18-21] However, it is unclear whether these kinetic parameters are applicable to a natural nitrification system because of competition with AOB and other bacterial groups. NOB depends on AOB for the energy source (nitrite) while they compete with AOB for oxygen in nitrification systems. Therefore, it is meaningful to obtain NOB kinetic and inhibition parameters in mixed culture systems with AOB. It is also important to find out the causes of the different ranges of free ammonia inhibition concentration of NOB in the literature.

A respirometric method with off-gas analysis can be a very expensive choice to investigate the nitrification kinetics because mass spectroscopy is needed for off-gas analysis to measure oxygen uptake.[19,20] In this study, the liquid phase oxygen concentration was monitored using a dissolved oxygen (DO) electrode. This is a much simpler and cheaper method than using mass spectrometry.

The objective of this study is to analyze free ammonia inhibition of the activities of NOB and to find out the cause of different kinetic results from the literature. For rapid measurement and analysis, a simple and cheap respirometric method based on DO was used to measure the kinetic parameters of NOB in completely nitrifying sludge while the activity of AOB was selectively repressed.

2. Materials and Methods

2.1. Airlift Bioreactor for the Growth of Nitrification Bacteria

Nitrification bacteria were grown in a laboratory scale airlift bioreactor (total reactor volume: 5 L) equipped with a three-phase separator was used. Reactor height, riser and down comer diameter were 77, 4 and 7 cm, respectively. Reactor pH was kept at 7.5 by the addition of 1 M $NaHCO_3$. During the reactor operation the temperature and dissolved oxygen were maintained at 26 ~ 28°C and 3 ~ 5 mg/L, respectively, with air flow rate of 5.2 L/min.

For inoculation the sludge from a local nitrifying wastewater treatment plant was used. The reactor was operated at an ammonium load of 1.1 kg/m3·d and complete nitrification was achieved in two weeks. Other detailed experimental methods can be found elsewhere.[22]

2.2. Artificial Wastewater

The artificial wastewater composition was as follows (mg/L): $CaCl_2 \cdot 2H_2O$, 7; $FeCl_3 \cdot 6H_2O$, 1; KCl, 7; KH_2PO_4, 11; $MgSO_4 \cdot H_2O$, 5; $NaHPO_4 \cdot 12H_2O$, 29; $NaHCO_3$, 3.57 g/g NH_4-N, $(NH_4)_2SO_4$, 335 mg-N/L. NH_4-N was measured by the Nesslerization method by reading the absorbance at 425 nm by UV-Visible spectrophotometer (UV 1601, Shimadzu). Both NO_2^--N and NO_3^--N were measured by ion chromatograph (DX 500, Dionex). All the other analytical methods were based on the Standard Methods.[23]

2.3. Batch Experiments for Free Ammonia Inhibition

Nitrifying bacteria grown in the airlift bioreactor were harvested and used for the analysis of free ammonia inhibition. The nitrifying bacteria removed from the reactor were further aerated for 2 hours to remove residual substrate in an aerated vessel and

Table 1. Various free ammonia inhibition of nitrite oxidizers from the literature

Free ammonia	Nitrite ratio[a]	Reactor type	Reference
8.1 mg NH_3-N/L	0.95	Activated sludge	Turk et al. (1989)[7]
22.1 mg NH_3-N/L	0.57		
1 mg NH_3-N/mg VAS[b]	0.8	Packed biofilm reactor	Fdz-Polanco et al. (1994)[8]
1.5 mg NH_3-N/g VAS	0.8 ~ 0.9	Packed biofilm reactor	Villaverade et. al. (1997)[9]
0.2 mg NH_3-N/L	1.0	Biofilm airlift reactor	Chang et al. (2000)[10]

[a] nitrite ratio = $\frac{NO_2 - N}{(NO_2 - N + NO_3 - N)}$

[b] VAS = volatile activated sludge

washed twice with buffered mineral solution. The washed nitrifying bacteria were transferred to five 500 mL flasks which have different NH_4^+-N (NH_3-N) solutions containing 0(0), 20(1.0), 80(4.1), 240(9.7), and 800(22.9) mg/L with minerals as used in the airlift reactor. NH_3-N concentrations were calculated from eq. (3). Allylthiourea (86 μM) was added to the flasks to keep the NH_4-N concentration constant during the incubation by selectively inhibiting AOB activity without affecting the activity of NOB.[24,25] The flasks were incubated at 25°C and 150 rpm for 1 hour and transferred to the respirometer to measure the activity of NOB. During these batch experiments, the growth of NOB was negligible due to the absence of the nitrite substrate.

2.4. Respirometric Measurements of NOB Activity

The respirometric analyses were performed in a 730-mL water jacketed glass vessel with a magnetic stir-bar at 21±1°C by water bath. This was completely filled with the sludge samples from the flasks and the aqueous medium saturated with pure oxygen. The respirometric vessel was sealed with the insertion of Clarke-type DO meter (Istek, Model 235D) and a syringe which provides substrate for nitrification (Fig. 1). DO levels were continuously monitored and stored on a personal computer for data analysis. The decrease in nitrite was estimated by measuring the profile of DO after injecting NO_2-N. All the respirometric experiments were carried out in triplicate and the average values were used for the analysis.

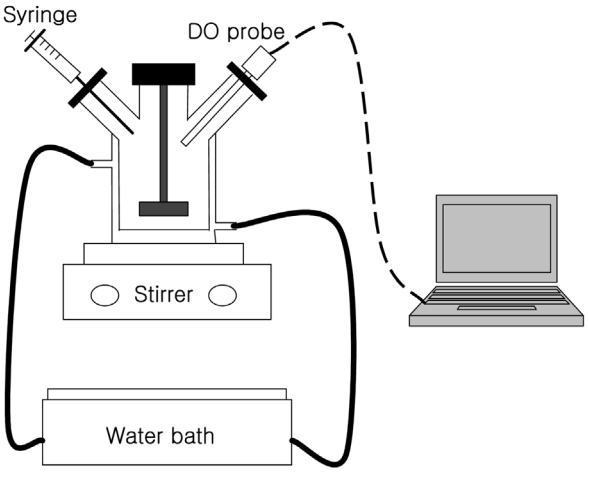

Fig. 1. Schematics of the respirometer for the measurement of nitrite oxidizing activity under free ammonia inhibition.

Biomass concentrations were measured after the kinetic analyses by means of total chemical oxygen demand (tCOD) using commercially available reagents (Hach Chemical Co., Loveland, CO). Total microbial concentration was converted from COD to volatile suspended solid (VSS) by the conversion factor of 1.4 mg COD/mg VSS.[26]

2.5. Estimation of the Maximum Specific Substrate Consumption Rate of NOB ($\hat{K}_{NO}$)

In order to estimate the maximum specific substrate consumption rate of NOB, the Michaelis-Menten equation was used.

$$\frac{dS}{dt} = -\frac{\mu_m X S}{Y(K_S + S)} \tag{5}$$

$$\hat{K}_{NO} = \frac{\mu_m}{Y} \tag{6}$$

where S is the substrate concentration, μ_m is maxmum specific growth rate, X is the biomass concentration, Y is the yield coefficient, K_s is the half-saturation constant, and $\hat{K}_{NO}$ is the maximum specific substrate consumption rate of NOB.

From the oxygen consumption curves of the respirometer, dissolved oxygen was converted to substrate (nitrite) concentration by subtracting endogeneous oxygen consumption. This was taken as the oxygen uptake rate when nitrite was absent. For the conversion coefficients of NH_4-N to NO_2-N and NO_2-N to NO_3-N, 3.43 and 1.14 mg O_2/mg N were used, respectively. Utilizing equation (5) and the substrate consumption curves, μ_m, and K_s can be estimated from the Lineweaver-Burk plot. Maximum specific substrate consumption rate of NOB ($\hat{K}_{NO}$) was calculated from equation (6). For the biomass yield coefficient of NOB, 0.042 g MLVSS/g NO_2-N was used.[1]

3. Results and Discussion

3.1. Respirogram of Nitrite Oxidation

Fig. 2 shows a typical respirogram of a nitrifying sludge from the airlift reactor. In Fig. 2(a), as 9.1 mg/L sodium acetate (A) was injected to the vessel, dissolved oxygen concentration remained constant and oxygen uptake rate did not increase. The absence of an increase in oxygen uptake with the addition of acetate indicates that the sludge has a very low heterotrophic activity or biomass. It can also be assumed that most of the bacteria in the sludge were nitrifying bacteria as NH_4-N was the only energy source provided. Therefore, the effects of heterotrophic bacteria on this respirometric measurement were ignored.

As soon as 6.0 mg N/L of nitrite (B) was injected to the vessel as shown in Fig. 2(a), dissolved oxygen concentration decreased continuously until the nitrite was completely consumed. The oxygen uptake rate was estimated from the slope of the dissolved oxygen profiles. The oxygen uptake rate increased sharply when nitrite was introduced and decreased slowly. The decrease of oxygen uptake rate in the later phase seems to be due to the limitation of substrate (nitrite) because the dissolved oxygen concentration was kept higher than 7 mg/L and the half saturation constant of NOB on oxygen is 0.3 mg/L.[6]

Fig. 2(b) ~ (f) shows respirograms of the nitrifying bacteria from the airlift reactor which were incubated in solutions with minerals and allylthiourea to maintain constant NH_4-N (NH_3-N). As nitrite was injected, the rate of oxygen uptake increased sharply in ten minutes then gradually decreased.

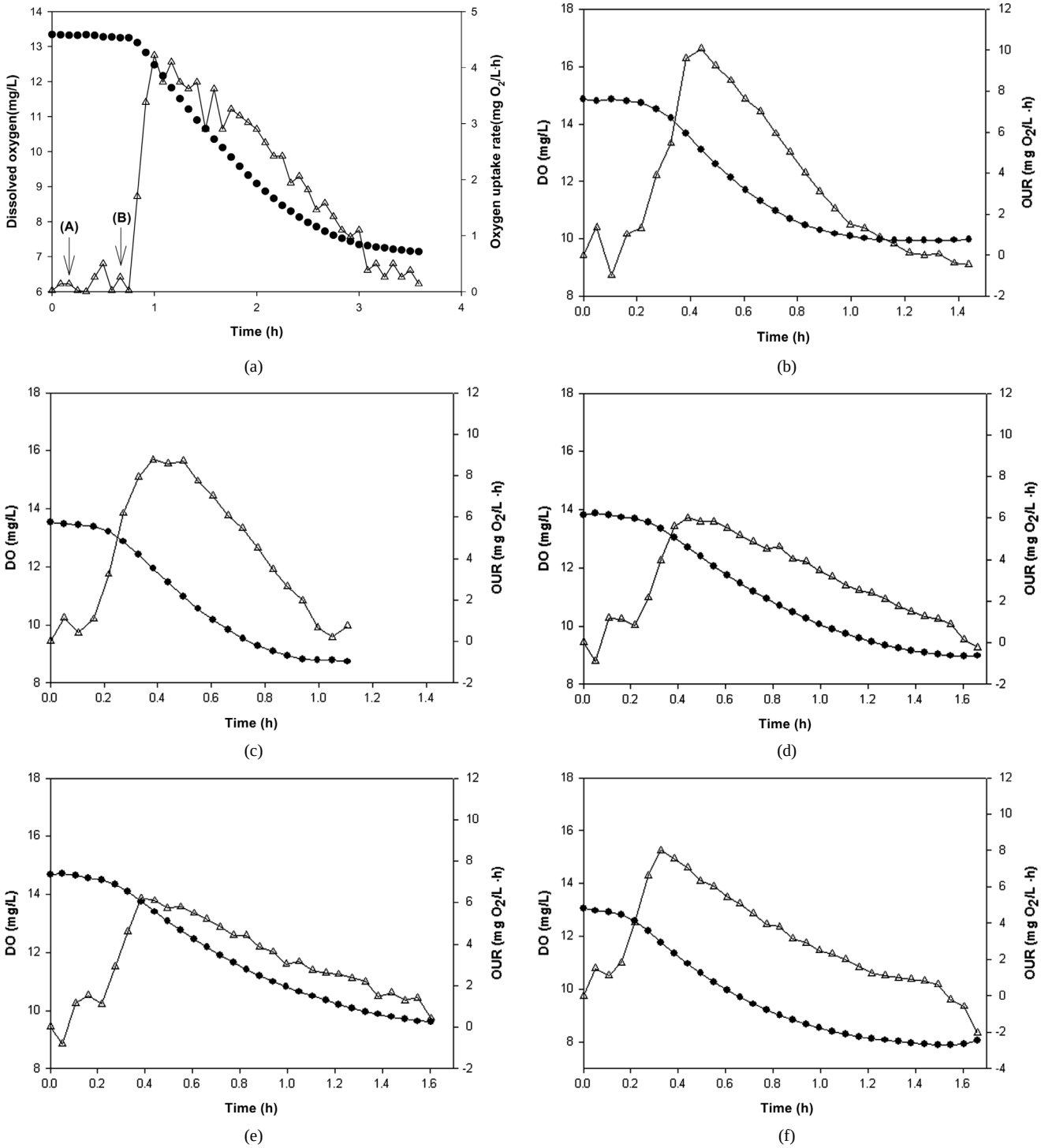

Fig. 2. (a): Typical respirogram of acetate and nitrite oxidation of the nitrifying sludge; (b~f): typical respirograms of nitrite oxidation under the presence of NH_3-N concentrations [0.0 mg/L (b), 1.0 mg/L (c), 4.1 mg/L (d), 9.7 mg/L (e), 22.9 mg/L (f)] with nitrifying sludge (●: dissolved oxygen; △: oxygen uptake rate). ((A): Injection of 9.1 mg/L acetate, (B): Injection of 6.0 mg NO_2-N/L).

Nitrifying sludge of the airlift bioreactor was incubated in the respirometer to compare the activities of NOB before and after free ammonia inhibition. For the free ammonia inhibition, the nitrifying sludge was incubated in a flask for 1 hour under the presence of free ammonia (3 mg N/L) for inhibition and allylthiourea (86 μM) to keep the free ammonia concentration constant. Allylthiourea of 86 μM did not inhibit NOB during the incubation while ammonia oxidation was selectively blocked. After the incubation the sludge was washed with mineral medium and transferred to the respirometer again for the activity measurement. The NOB activities before and after the free ammonia inhibition were 0.130 and 0.128 mg N/mg NOB · h, respectively, which were very close. Therefore, the inhibition of free ammonia on NOB can be regarded as reversible.

Smith et al. mentioned that the inhibition of NOB seems to be reversible as nitrite ceased to accumulate in river sediments when free ammonia concentrations declined below 50 μg N/L.[27] Philips et al. also indicated the reversible inhibition of NOB by free ammonia.[6] This present experiment agrees with these results and it also affirmed the accuracy of this respirometric method.

3.2. Free Ammonia Inhibition of NOB from the Completely Nitrifying Airlift Bioreactor

Batch respirometric results of the nitrifying sludge from the completely nitrifying airlift bioreactor and mathematical calculations of the free ammonia inhibition model are shown in Fig. 3. As the NH_3-N increased from 0.0 to 1.0, 4.1, 9.7 and 22.9 mg/L, the K_{NO} decreased from 0.141 to 0.116, 0.100, 0.097 and 0.081 mg NO_2^--N/mg NOB · h, respectively. The K_{NO} values, expressed in percentage relative to the control value ($\hat{K}_{NO}$), were 100, 82, 71, 69 and 57%, respectively.

Vadivelu et al.[19] and Blackburne et al.[20] obtained maximum specific oxygen uptake rates of 0.120 mg/mg VSS · h and 0.032 mg/mg VSS · h from the enriched cultures of *Nitrobacter* and *Nitrospira*, respectively. They obtained the values from the enriched cultures of NOB by feeding nitrite as the only energy source, which is different from this study where ammonium was provided as the energy source for the nitrifiers. It is very rare in nature to have nitrite as the energy source and NOB as the dominant microorganism so that the specific oxygen uptake rate values of *Nitrobacter* and *Nitrospira* may not reflect the real situation. The $\hat{K}_{NO}$ value of this study was obtained during the normal nitrification (ammonium oxidation to nitrate via nitrite) and it has a meaning in this respect. The $\hat{K}_{NO}$ value of this study is close to that of *Nitrobacter*[19] and we can assume that the dominant NOB in the reactor is *Nitrobacter*.

A noncompetitive inhibition model was applied to the experimental data to estimate the inhibition concentration (K_I) of the noncompetitive inhibitor (free ammonia):

$$K_{NO} = \frac{\hat{K}_{NO}}{1 + I / K_I} \tag{7}$$

Polymath™ 5.1 (POLYMATH Software) was used to estimate the K_I from the experimental values by nonlinear regression and it gave 21.3 mg NH_3-N/L as the optimized K_I. At this free ammonia concentration, the K_{NO} is halved. The free ammonia inhibition concentrations (K_I) of NOB reported in the literature vary, eg. 1.06 mg/L,[28] 8.9 mg/L,[29] and 0.5 mg/L[30] depending on the experimental conditions. The K_{NO} also depends on dissolved oxygen, nitrous acid concentration, and biomass acclimation to free ammonia.[6] The above factors can be neglected in this respirometric experiment because the environment was well-controlled.

Based on the K_{NO} values in the presence of free ammonia, Blackburne et al.[20] observed free ammonia concentration inhibits *Nitrobacter* and *Nitrospira*. *Nitrobacter* was inhibited at 30 ~ 50 mg NH_3-N/L, which is quite similar to this study. On the other hand, *Nitrospira* had a much lower inhibition threshold for free ammonia (0.04 ~ 0.08 mg NH_3-N/L). Therefore, it can be assumed that *Nitrobacter* is the dominant NOB in the reactor from the estimated $\hat{K}_{NO}$ and K_I. The variations in the reported values of free ammonia inhibition may be due to the presence of different species of nitrite oxidizers in the various experiments reported in literature. The very different inhibition by free ammonia on these two main nitrite-oxidizing bacterial groups, and the suggestion that other groups may vary as well, indicates that the taxonomy of the nitifiers needs to be considered in future studies.

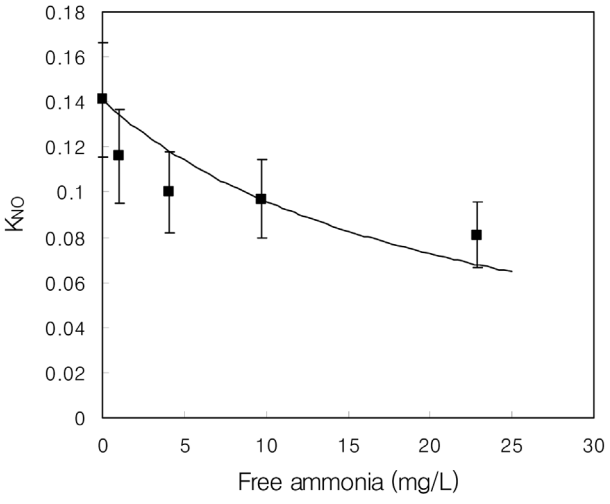

Fig. 3. Effect of free ammonia on the specific nitrite oxidation activities (K_{NO}) of the nitrite oxidation bacteria. (---) : simulated K_{NO} based on the noncompetitive inhibition kinetic model and the optimized K_I, (■) : respirometrically measured K_{NO}.

4. Conclusion

A batch respirometer was installed to measure the nitrite oxidizing kinetic characteristics of nitrite oxidizing bacteria by measuring the rate of oxygen uptake. Free ammonia inhibition of nitrite oxidizing bacteria was reversible and as the NH_3-N increased from 0.0 to 1.0, 4.1, 9.7 and 22.9 mg/L, the K_{NO} decreased from 0.141 to 0.116, 0.100, 0.097 and 0.081 mg NO_2^--N/mg NOB · h, respectively. Based on the values of $\hat{K}_{NO}$ and free ammonia inhibition threshold the dominant nitrite oxidizing bacteria in the reactor was characterized as *Nitrobacter*. The respirometric method provided a rapid and reliable way to analyze the inhibition characteristics of the nitrite oxidizing bacteria.

Acknowledgement

This research was supported by Hallym University Research Fund, 2006(HRF-2006-025).

References

1. Wiesmann, U., Choi, I. S., and Dombrowski, E. M., Funda-

mentals of biological wastewater treatment, Wiley-VCH, Weinheim, Germany, pp. 228-235 (2007).
2. Anthonisen, A. C., Loehr, R. C., Prakasma, T. B. S., and Srinath, E. G., "Inhibition of nitrification by ammonia and nitrous acid," *J. Wat. Pollut. Contr. Fed.*, **48**, 835-852 (1976).
3. Schmidt, I., Sliekers, D., Schmid, M., Bock, E., Fuerst, J., Kuenen, J. G., Jetten, M. S. M., and Strous, M., "New concepts of microbial treatment processes for the nitrogen removal in wastewater," *FEMS Microbiol. Rev.*, **27**, 481-492 (2003).
4. Hellinga, C., Schellen, A., Mulder, J. W., van Loosdrecht, M. C. M., and Heijnen, J. J., "The SHARON process: an innovative method for nitrogen removal from ammonium-rich waste water," *Wat. Sci. Technol.*, **37**, 135-142 (1998).
5. Kuai, L. P., and Verstraete, W., "Ammonium removal by the oxygen-limited autotrophic nitrification-denitrification system," *Appl. Environ. Microbiol.*, **64**, 4500-4506 (1998).
6. Philips, S., Laanbroek, H. J., and Verstraete, W., "Origin, causes and effects of increased nitrite concentrations in aquatic environments," *Re/Views Env. Sci. Bio/Technol.*, **1**, 115-141 (2002).
7. Turk, O., and Mavinic, D. S., "Stability of nitrite build-up in an activated sludge system," *J. WPCF.*, **61**, 1440-1448 (1989).
8. Fdz-Polanco, F., Villaverde S., and Garcia, P. A., "Temperature effect on nitrifying bacteria activity in biofilters: activation and free ammonia inhibition," *Wat. Sci. Tech.*, **30**, 121-130 (1994).
9. Villaverde, S., Garcia-Encina, P. A., and Fdz-Polanco, F., "Influence of pH over nitrifying biofilm activity in submerged biofilters," *Wat. Res.*, **31**, 1180-1186 (1997).
10. Chang, J. S., Cha, G. C., and Kim, D. J., "Nitrite accumulation characteristics in the nitrification of high strength ammonia wastewater with biofilm airlift suspension reactor," *HWAHAK KONGHAK*, **40**, 114-120 (2002).
11. Kong, Z., Vanrolleghem, P., Willems, P., and Verstraete, W., "Simultaneous determination of inhibition kinetics of carbon oxidation and nitrification with a respirometer," *Wat. Res.*, **30**, 825-836 (1996).
12. Surmacz-Gorska, J., Gernaey, K., Demuynck, C., Vanrolleghem, P., and Verstraete, W., "Nitrification monitoring in activated sludge by oxygen uptake rate (OUR) measurements," *Wat. Res.*, **30**, 1228-1236 (1996).
13. Gernaey, K., Vanderhasselt, A., Bogaert, H., Vanrolleghem, P., and Verstraete, W., "Sensors to monitor biological nitrogen removal and activated sludge settling," *J. Microbiol. Meth.*, **32**, 193-204 (1998).
14. Ciudad, G., Werner, A., Bornhardt, C., Muñoz, C., and Antileo, C., "Differential kinetics of ammonia- and nitrite-oxidizing bacteria: A simple kinetic study based on oxygen affinity and proton release during nitrification," *Proc. Biochem.*, **41**, 1764-1772 (2006).
15. Chandran, K., and Smets, B. F., "Optimizing experimental design to estimate ammonia and nitrite oxidation biokinetic parameters from batch respirograms," *Wat. Res.*, **39**, 4969-4978 (2005).
16. Jubany, I., Baeza, J. A., Carrera, J., and Lafuente, J., "Respirometric calibration and validation of a biological nitrite oxidation model including biomass growth and substrate inhibition," *Wat. Res.*, **39**, 4574-4584 (2005).
17. Carrera, J., Jubany, I., Carvallo, L., Chamy, R., and Lafuente, J., "Kinetic models for nitrification inhibition by ammonium and nitrite in a suspended and an immobilised biomass systems," *Pro. Biochem.*, **39**, 1159-1165 (2004).
18. Kim, D. J., and Kim, S. H., "Effect of nitrite concentration on the distribution and competition of nitrite-oxidising bacteria in nitritation reactor systems and their kinetic characteristics," *Wat. Res.*, **40**, 887-894 (2006).
19. Vadivelu, V. M., Keller, J., and Yuan, Z., "Effect of free ammonia on the respiration and growth processes of an enriched Nitrobacter culture," *Wat. Res.*, **41**, 826-834 (2007).
20. Blackburne, R., Vadivelu, V. M., Yuan, Z., and Keller, J., "Kinetic characterisation of an enriched Nitrospira culture with comparison to Nitrobacter," *Wat. Res.*, **41**, 3033-3042 (2007).
21. Kim, S. J., "Estimation of active Nitrosomonas and Nitrobacter concentrations in activated sludge using nitrogeneous oxygen uptake rate," *Environ. Eng. Res.*, **9**, 130-142 (2004).
22. Kim, D. J., Ahn, D. H., and Lee, D. I., "Effect of free ammonia and dissolved oxygen on nitrification and nitrite accumulation in a biofilm airlift reactor," *Kor. J. Chem. Eng.*, **22**, 85-90 (2005).
23. American Public Health Association, Standard methods for the examination of water and wastewater, 18th ed. Washington, DC., U.S.A. (1992).
24. Ginestet, P., Audic, J. M., Urbain, V., and Block, J. C., "Estimation of nitrifying bacterial activities by measuring oxygen uptake in the metabolic inhibitors allylthiourea and azide," *Appl. Environ. Microbiol.*, **64**, 2266-2268 (1998).
25. Ficara, E., Musumeci, A., and Rozzi, A., "Comparison and combination of titrimetric and respirometric techniques to estimate nitrification kinetics parameters," *Water SA.*, **26**, 217-224 (2000).
26. Chandran, K., and Smets, B. F., "Applicability of two-step models in estimating nitrification kinetics from batch respirograms under different relative dynamics of ammonia and nitrite oxidation," *Biotechnol. Bioeng.*, **70**, 54-65 (2000).
27. Smith, R. V., Burns, L. C., Doyle, R. M., Lennox, S. D., Kelso, B. H. L., Foy, R. H., and Stevens, R. J., "Free ammonia inhibition of nitrification in river sediments leading to nitrite accumulation," *J. Environ. Qual.*, **26**, 1049-1055 (1997).
28. Balmelle, B., Nguyen, K. M. Capdeville, B., Cornier, J. C., and Deguin, A., "Study of factors controlling nitrite build-up in biological processes for water nitrification," *Wat. Sci. Tech.*, **26**, 1017-1025 (1992).
29. Mauret, M., Paul, E., Puech-Costes, E., Maurette, M. T., and Baptiste, P., "Application of experimental research methodology to the study of nitrification in mixed culture," *Wat. Sci. Tech.*, **34**, 245-252 (1996).
30. Fdz-Polanco, F., Villaverde, S., and Garcia, P. A., "Effects of causing nitrite build-up in biofilters," *Wat. Sci. Tech.*, **34**, 371-378 (1996).