# Respirometer를 이용한 아질산 산화균에 대한 Free ammonia의 저해 특성 분석

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# Analysis of free ammonia inhibition characteristics of nitrite oxidizing bacteria by a respirometer

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## 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. Nitrite can be accumulated in the presence of free ammonia ($NH_3$) which depends on $NH_4^+$-N concentration, temperature and pH as follows:

$$NH_3 - N = \frac{NH_4 - N \times 10^{pH}}{K_a / K_w + 10^{pH}} \quad (1)$$

$$K_a/K_w = e^{(6334/273+T(℃))} \quad (2)$$

Several mechanisms are suggested for the inhibition of nitrite oxidation, but they are not always equally effective and it is believed that the nitrite oxidation rate and degree of inhibition are different for each NOB species. A respirometric method was developed to measure the rates of ammonia oxidation and nitrite oxidation separately and it enabled the investigation of individual ammonia and nitrite oxidation kinetics.

The objective of this study is to analyze the effect of free ammonia inhibition on the activities of NOB and to find out the cause of different kinetic results from the literature.

## MATERIALS AND METHODS

For the growth of nitrification bacteria a laboratory scale airlift bioreactor (total reactor volume: 5 L) equipped with a three-phase separator was used. The reactor was operated at an ammonium load of

1.1 kg/$m^3$·d and complete nitrification was achieved in two weeks. The artificial wastewater composition and other detailed experimental and analytical methods can be found elsewhere [1].

For batch experiment to study the effect of free ammonia on the NOB, washed nitrifying bacteria with buffered mineral solution were equally 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. 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. The flasks were incubated at 25℃ and 150 rpm for 1 hour and transferred to the respirometer to measure the activity of NOB.

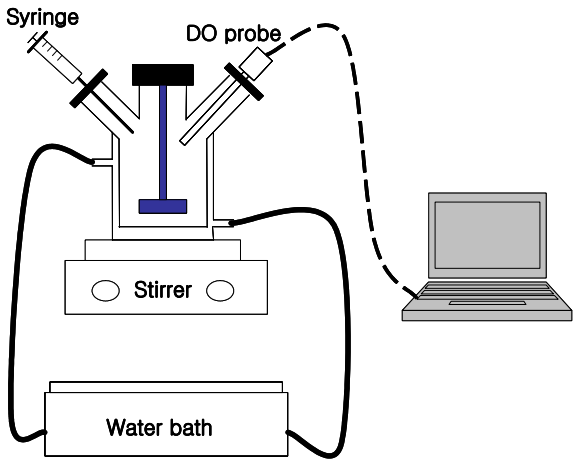


Fig. 1. Schematics of the respirometer.

The respirometric analyses were performed in a 730-mL water jacketed glass vessel with a magnetic stir-bar at 21±1℃ by water bath, which was completely filled with the sludge samples from the flasks and the aqueous medium aerated with pure oxygen. The respirometric vessel was sealed with DO meter and a syringe which provides substrate for nitrification (Fig. 1). A decrease in DO level in the vessel due to substrate oxidation was measured by the DO meter and continuously acquired by a personal computer for data analysis. The activity of NOB was estimated by measuring the profile of DO after injecting $NO_2$-N by a syringe. Total microbial concentration was converted from COD to volatile suspended solid (VSS) by the conversion factor of 1.4 mg COD/mg VSS.

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

$$\frac{dS}{dt} = -\frac{\mu_m X S}{Y(K_S + S)} \qquad (3)$$

$$\hat{K}_{NO} = \frac{\mu_m}{Y} \qquad (4)$$

where S is the substrate concentration, $\mu_m$ 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. 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 (3), (4) and the

substrate consumption curves, $\mu_{m,}$ $K_s$ and $\hat{K}_{NO}$ can be estimated. For the biomass yield coefficient of NOB, 0.042 g MLVSS/g $NO_2$-N was used.

## RESULTS AND DISCUSSION

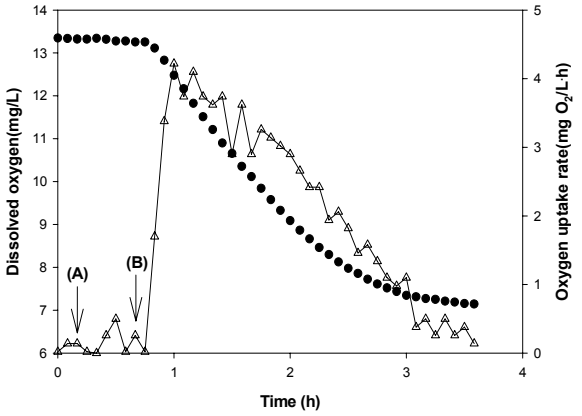


Fig. 2. Typical respirogram of acetate and nitrite oxidation of the nitrifying sludge.
(●: dissolved oxygen; △: oxygen uptake rate).
((A): Injection of 9.1 mg/L acetate, (B): Injection of 6.0 mg $NO_2$-N/L).

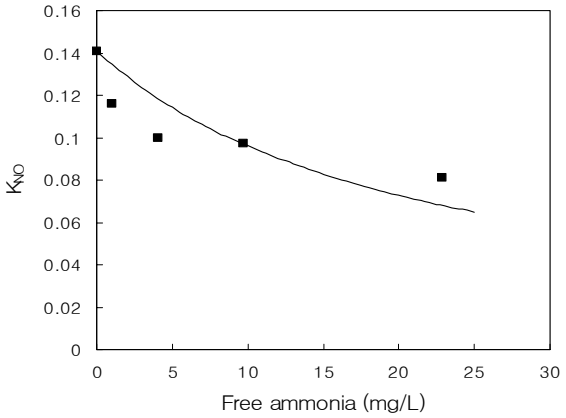


Fig. 3. Effect of free ammonia on the specific nitrite oxidation activities ($K_{NO}$) of the nitrite oxidation bacteria. (—) simulated $K_{NO}$ based on the noncompetitive inhibition kinetic model and the optimized $K_I$, ( ■ ) : respirometrically measured $K_{NO}$.

Fig. 2 shows a typical respirogram of a nitrifying sludge from the airlift reactor. As 9.1 mg/L sodium acetate (A) was injected to the vessel, oxygen uptake rate did not increase. It can be assumed that most of the bacteria in the sludge were nitrifying bacteria. As soon as 6.0 mg N/L of nitrite (B) was injected, dissolved oxygen concentration decreased continuously until nitrite is completely consumed. Mode of free ammonia inhibition of NOB was also examined by the respirometric measurements to find out whether the inhibition is reversible or not. 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) and allylthiourea (86 μM). After the incubation the sludge was washed 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.

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. The nitrifying sludge samples were transferred and exposed to the $NH_3$-N levels of 0.0 ~ 22.9 mg/L to measure the $K_{NO}$ values under the influence of free ammonia inhibition. 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. Vadivelu et al. [2] and Blackburne et al. [3] 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. From the $K_{NO}$ value of this study, 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). 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 concentration ($K_I$) of NOB reported in the literature are very diverse such as 1-3 mg/L, 1.06 mg/L, 8.9 mg/L, and 0.5 mg/L depending on the experimental conditions. Based on the $K_{NO}$ values under the presence of free ammonia, Blackburne et al. [3] observed free ammonia concentration which inhibits *Nitrobacter* and *Nitrospira*. *Nitrobacter* was inhibited at 30 ~ 50 mg $NH_3$-N/L, and 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 $K_{NO}$ and $K_I$. Hence, 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. This would seem to be critical in any future studies given the highly different inhibition impact of free ammonia on these two main nitrite-oxidizing bacterial groups.

$$K_{NO} = \frac{\hat{K}_{NO}}{1 + I / K_I} \qquad (5)$$

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