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Estimation of Nitrite Concentration in the Biological Nitritation Process Using Enzymatic Inhibition Kinetics

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Abstract Recently, interests to remove nitrogen in the nitritation process have increased because of its economical advantages, since it could be a short-cut process to save both oxygen for nitrification and carbon for denitrification compared to a typical nitrification. However, the kinetics related with the nitritation process has not yet been fully understood. Furthermore, many useful models which have been successfully used for wastewater treatment processes cannot be used to estimate effluent nitrite concentration for evaluating performance of the nitritation process, since the process rate equations and population of microorganisms for nitrogen removal in these models have been set up only for the condition of full nitrification. Therefore, the present study was conducted to estimate an effluent nitrite concentration in the nitritation process with a concept of enzymatic inhibition kinetics based on long-term laboratory experiments. Using a nonlinear least squares regression method, kinetic parameters were accurately determined. By setting up a process rate equation along with a mass balance equation of the nitrite-oxidizing step, an effluent nitrite concentration in the nitritation process was then successfully estimated.

Key words: Enzymatic inhibition kinetics, linearization of inhibition model, nitritation, nonlinear least squares regression, value of initial guess

A great deal of effort has been invested to biological wastewater treatment in order to achieve a stable quality of downstream water to avoid eutrophication [9, 11, 12, 15]. Recently, for the biological nitrogen removal from ammonium-rich wastewater, much more interests have been focused in the nitrification up to nitrite (nitritation) rather than a typical nitrification process [8, 14, 22]. Theoretically, the nitritation process could save up to 25% of the aeration power compared to the full nitrification processes [7, 20]. In addition, organic carbon could be required for the denitrification process [16, 17]. The denitrification of nitrite or denitritation could actually reduce the organic carbon requirement up to 40% compared to the nitrate denitrification [8]. Also, according to Picioreanu *et al.* [18], the denitrification rate of nitrite is 1.5–2.0 times faster than that from nitrate.

In spite of the recent highly increased interests in the nitritation process, the kinetic information on suppressing the nitrite-oxidizing step in this process has not been available. Many models, such as ASM3 [6], SSSP [1], and AQUASIM [19], have been successfully used for the design and optimization of the BNR (Biological Nutrient Removal) processes. However, these models cannot be used to estimate an effluent NO_2-N concentration for evaluating the nitritation process performance, since the process rate equations and microbial population for nitrogen removal have been set up only for the condition of full nitrification in these models.

Therefore, this study was undertaken for the purpose of estimating an effluent NO_2-N concentration in the nitritation process based on long-term laboratory experiments. For this objective, it was necessary to find out how the NO_2N-oxidizing step could be inhibited and to accurately determine the kinetic parameters. The method of NLSR (Nonlinear Least Squares Regression) was used for accurate determination of kinetic parameters and inhibition type. A theory of enzymatic inhibition kinetics was also applied to obtain initial kinetic parameters for NLSR analysis.

MATERIALS AND METHODS

Long-Term Operation of Laboratory Nitritation Reactor

A laboratory nitritation reactor was operated for 755 days. The recycle water taken from a large MWTP (Municipal Wastewater Treatment Plant) in Korea was used for the laboratory experiment. The strength of recycle water varied widely depending on the operational conditions of the plant, and all parameters of water quality were analyzed according

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to *Standard Methods* of American Public Health Association/ American Water Works Association/Water Environment Federation (Washington DC, U.S.A.) during the experiment. The operating conditions of the laboratory reactor have been explained by Gil and Choi in detail elsewhere [5]. They determined the inhibition type during the nitritation process. The FA (Free Ammonia) serving as an inhibitor of the nitrite-oxidizing step appeared to be the key factor of nitrite accumulation in the previous study [5].

Batch Experiments for the Enzymatic Inhibition Kinetics

Batch kinetic studies were performed by using a 1 liter-acrylic tube reactor at 35°C to determine FA inhibition type. Initial nitrate production rates (same as nitrite oxidation rates) were measured at different inhibition levels by varying the concentrations of FA and substrate (nitrite) during batch kinetic studies. The direct linear plot proposed by Eisenthal & Cornish-Bowden [4] was finally obtained using these initial reaction rates. According to the theory of direct linear plot, it was previously concluded that the type of FA inhibition as a key factor of nitrite accumulation was mixed inhibition. Also, the more detail procedures of the batch kinetic studies were explained by Gil and Choi [5]

Procedure of Determining Kinetic Parameters and Estimating Effluent NO_2-N in the Nitritation Process

The kinetic parameters were estimated by the linearization of inhibition model determined from a direct linear plot. Then, these values were used as initial guesses for NLSR analysis in order to determine accurate kinetic parameters and inhibition type. The advantages of NLSR are as follows: 1) it does not require linearization of the nonlinear equation; 2) it can be used for complicated multiparameter models; 3) the estimated parameter values are reliable; and 4) it provides a sound basis for calculating the errors such as the RMSE (Root Mean Squares Error). The initial reaction rates from previous batch experiments for the enzymatic inhibition kinetics were also used for the NLSR analysis. It is general consensus that the reverse reaction, inhibition by the product, progressive inactivation of the enzyme, and other complicating features can be avoided by using initial velocity [3]. For the NLSR analysis, MATLAB (Version 5.0) was used to fit the measured data to the inhibition model.

Using the kinetic parameters determined from NLSR, a process rate equation and mass balance equation of the nitritation process could be set up, and estimation of effluent NO_2-N concentration in the process could then be estimated. Finally, the estimated NO_2-N was compared with the measured values. Figure 1 shows the overall procedure to estimate effluent NO_2-N concentration in this study.

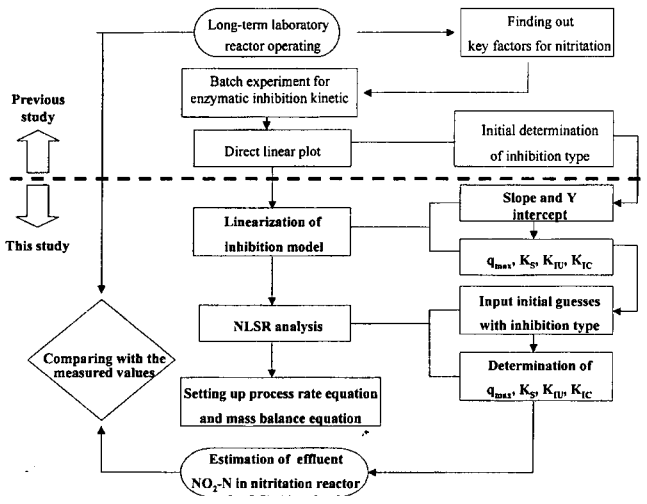

Fig. 1. Procedure of estimating effluent NO_2-N concentration in the nitritation process.

RESULTS AND DISCUSSION

Determination of $q_{max,NO_2\text{-}N}$, K_S, and K_I by the Linearization of Inhibition Model

Using apparent values of kinetic parameters, the Michaelis-Menten equation can be expressed as equation (1).

$$ {}^{app}q_{NO_2-N} = \frac{{}^{app}q_{max,NO_2-N} S_{NO_2-N}}{{}^{app}K_S + S_{NO_2-N}} \quad (1) $$

where,

${}^{app}q_{NO_2-N}$ =Apparent value of nitrite oxidation rate, mgN $mgVSS^{-1}$ d^{-1}

${}^{app}q_{max,NO_2-N}$ =Apparent value of maximum nitrite oxidation rate, mgN $mgVSS^{-1}$ d^{-1}

S_{NO_2-N} =Nitrite concentration, mg l^{-1}

${}^{app}K_S$ =Apparent value of half-saturation constant, mg l^{-1}

Descriptions of ${}^{app}q_{max,NO_2-N}$ and ${}^{app}K_S$ in equation (1) can be varied depending on the types of inhibition in the Michaelis-Menten model. According to biochemistry [21], inhibition types describing ${}^{app}q_{max}$ and ${}^{app}K_S$ are denoted as in Table 1.

Inhibition of the nitrite-oxidizing step with the mixed type inhibition can be described as in equations (2) and (3) from Table 1.

$$ {}^{app}q_{max,NO_2-N} = \frac{q_{max,NO_2-N}}{\left(1+\frac{I_{FA}}{K_{IU}}\right)} \quad (2) $$

$$ {}^{app}K_S = \frac{K_S\left(1+\frac{I_{FA}}{K_{IC}}\right)}{\left(1+\frac{I_{FA}}{K_{IU}}\right)} \quad (3) $$

where,

I_{FA}=FA concentration as inhibitor, mg l^{-1}

Table 1. The effects of inhibition at the parameters of the Michaelis-Menten equation.

Type of inhibition	$^{app}q_{max}$	$^{app}K_S$
Mixed	$\frac{q_{max}}{\left(1+\frac{I}{K_{IU}}\right)}$	$\frac{K_S\left(1+\frac{I}{K_{IC}}\right)}{\left(1+\frac{I}{K_{IU}}\right)}$
Noncompetitive	$\frac{q_{max}}{\left(1+\frac{I}{K_{IU}}\right)}$	K_S
Competitive	q_{max}	$K_S\left(1+\frac{I}{K_{IC}}\right)$
Uncompetitive	$\frac{q_{max}}{\left(1+\frac{I}{K_{IU}}\right)}$	$\frac{K_S}{\left(1+\frac{I}{K_{IU}}\right)}$

K_{IC}=Competitive inhibition constant, mg l^{-1}

K_{IU}=Uncompetitive inhibition constant, mg l^{-1}

Comparing equation (1) with equations (2) and (3), the following linearized inhibition model equation was derived.

$$\frac{1}{^{app}q_{max,NO_2-N}}=\frac{1}{q_{max,NO_2-N}}+\frac{1}{q_{max,NO_2-N}K_{IU}}I_{FA} \quad (4)$$

$$\frac{^{app}K_S}{^{app}q_{max,NO_2-N}}=\frac{K_S}{q_{max,NO_2-N}}+\frac{K_S}{q_{max,NO_2-N}K_{IC}}I_{FA} \quad (5)$$

The kinetic parameters such as q_{max,NO_2-N}, K_s, K_{IC}, and K_{IU} could be graphically estimated by using these linearized inhibition model of equations (4) and (5).

Equation (4) can be plotted by using known $^{app}q_{max,NO_2-N}$ and I_{FA}. Slope ($1/^{app}q_{max,NO_2-N}K_{IU}$) and y-intercept ($1/q_{max,NO_2-N}$) can be obtained from the linear plot of $1/^{app}q_{max,NO_2-N}$ versus I_{FA}. From the slope and y-intercept, q_{max,NO_2-N} and K_{IU} can be calculated. As shown in Fig. 2, a linear plot was achieved with an R^2 value of 0.98 by the equation (4). The linear squares regression yielded a q_{max,NO_2-N} value of 30.96 mgN mg VSS^{-1} d^{-1} and a K_{IU} value of 35.89 mg l^{-1}.

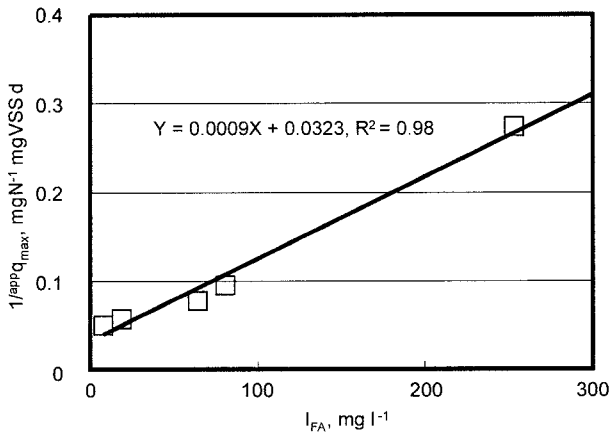

Fig. 2. Linearized equation of mixed inhibition model to determine q_{max,NO_2-N} and K_{IU}.

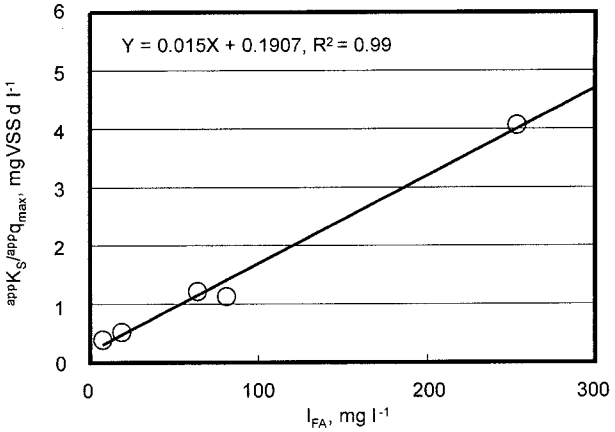

Fig. 3. Linearized equation of mixed inhibition model to determine K_S and K_{IC}.

Equation (5) can be plotted by using known $^{app}q_{max,NO_2-N}$, $^{app}K_S$, and I_{FA}. The slope ($K_S/q_{max,NO_2-N}K_{IC}$) and y-intercept ($K_S/q_{max,NO_2-N}$) can be obtained from the plot of $^{app}K_S/^{app}q_{max,NO_2-N}$ versus I_{FA}. Using the slopes, y-intercept, and a value of q_{max,NO_2-N} determined from Fig. 2, K_s and K_{IC} can be calculated. Figure 3 shows the linear plot drawn by equation (5). The R^2 value of the line in Fig. 3 was 0.99 and the regression yielded a K_s value of 5.90 mg l^{-1} and a K_{IC} value of 12.71 mg l^{-1}. Thus, all kinetic parameters, q_{max,NO_2-N}, K_S, K_{IC}, and K_{IU}, were estimated by the linearization of inhibition model.

Determination of q_{max,NO_2-N}, K_S, and K_I by NLSR Analysis

NLSR provides a tool for estimating the parameters of nonlinear models. NLSR has been successfully used to determine the kinetic parameters in many kinetic studies

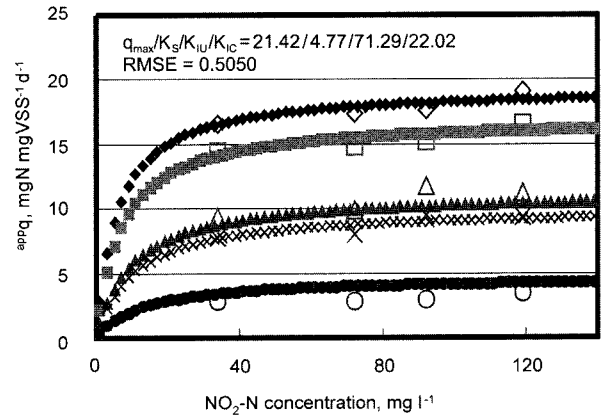

Fig. 4. NLSR best fit of the data to the mixed inhibition models. 7.6 mg FA l^{-1} (◇); 18.5 mg FA l^{-1} (□); 64.1 mg FA l^{-1} (△); 80.8 mg FA l^{-1} (×); and 253.2 mg FA l^{-1} (○).

Table 2. Kinetic parameters determined by NLSR fit in various inhibition models.

Type of inhibition	q_{max}, mgN mgVSS^{-1} d^{-1}	K_S, mg l^{-1}	K_{IU}, mg l^{-1}	K_{IC}, mg l^{-1}	RMSE
Mixed	21.42	4.77	71.29	22.02	0.5050
Noncompetitive	22.13	7.24	62.29	-	0.5224
Competitive	19.71	0.0004	-	0.0004	1.9559
Uncompetitive	22.62	8.94	55.15	-	0.5794

[2, 10, 13]. However, it seems that the NLSR has not been applied to determine kinetic parameters of FA inhibition on nitrite oxidation in the nitritation process, although FA inhibition of nitrite oxidation by FA has been known for decades. Kinetic parameters can be determined accurately by carrying out the NLSR analysis in various inhibition model equations. NLSR analysis requires an initial guess for each unknown parameter as input. If the model consists of complicated multiparameters, the guesses may be very useful for converging the regression. Therefore, the values of q_{max, NO_2-N}, K_S, K_{IC}, and K_{IU} determined from the previous linearization method were used as initial guesses for the NLSR fit of the experimental data. All kinetic parameters were determined by this NLSR fit with the mixed inhibition model, as shown in Fig. 4. Also, with the other type of inhibition models such as noncompetitive, competitive, and uncompetitive inhibition, the NLSR analyses were conducted to determine the kinetic parameters, as shown in Table 2.

Comparing the values of RMSE to each other, the RMSE in the mixed inhibition model in Table 2 was the smallest. Table 2 indicates that the type of FA inhibition on nitrite-oxidizing step was a mixed inhibition, which was in agreement with the result obtained in the previous study using a direct linear plot. NLSR in a mixed inhibition model yielded a q_{max, NO_2-N} value of 21.42 mgN mgVSS^{-1} d^{-1}, a K_S value of 4.77 mg l^{-1}, a K_{IU} value of 71.29 mg l^{-1}, and a K_{IC} value of 22.02 mg l^{-1}.

Estimation of Effluent Nitrite Concentration in the Nitritation Process

The estimation of effluent NO_2-N concentration in a laboratory nitritation reactor was conducted by using equation (1) as a process rate equation, and also, mass balance equation. The following equation (6) could be used as a mass balance equation.

Effluent concentration of nitrite (S_{NO_2-N})
=Influent concentration of nitrite ($S_{NO_2-N_O}$)
-Oxidized concentration of nitrite (ΔS_{NO_2-N}) (6)

ΔS in equation (6) can be obtained by equation (7).

$$q_{NO_2-N}=\frac{S_O-S}{\theta_H X_{no}} \quad (7)$$

where,

θ_H=HRT, d

X_{no}=Concentration of nitrite oxidizer, mg l^{-1}

Equation (7) can be rearranged to equation (8).

$$(S_o-S)=q_{NO_2-N}\theta_H X_{no} \quad (8)$$

Therefore, equation (6) can be described as equation (9).

$$S_{NO-N}=S_{NO_2-N_O}-q_{NO_2-N}\theta_H X_{no} \quad (9)$$

For the estimation of effluent NO_2-N concentration, substituting equation (1) into equation (9) yields the following quadratic equation.

$$(S_{NO_2-N})^2+B(S_{NO_2-N})+C=0 \quad (10)$$

where,

$$B={}^{app}K_S-S_{NO_2-N_O}+{}^{app}q_{max, NO_2-N}\theta_H X_{no} \quad (11)$$

$$C=-{}^{app}K_S S_{NO_2-N_O} \quad (12)$$

Therefore, S_{NO_2-N} can be calculated as the following equation (13).

$$S_{NO_2-N}=\frac{-B+\sqrt{B^2-4C}}{2} \quad (13)$$

For the consideration of FA inhibition effect on the nitrite-oxidizing step, ${}^{app}q_{max, NO_2-N}$ and ${}^{app}K_S$ have to be obtained from the mixed inhibition model equation in Table 1. Figure 5 denotes the estimation of effluent NO_2-N concentration with RMSE of 131.51 and indicates that the estimated NO_2-N concentration was similar to the observed values in an overall operating period.

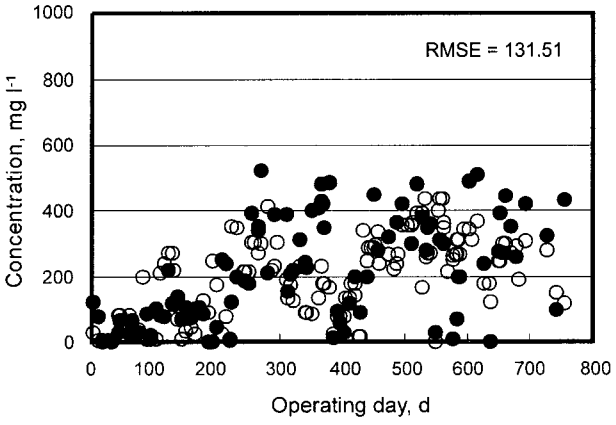

Fig. 5. Estimation of effluent NO_2-N concentration in the laboratory nitritation reactor.
Observed NO_2-N (●); Estimated NO_2-N (○).

In conclusion, a useful method to estimate effluent NO_2-N concentration in the biological nitritation process was proposed in this study. It is expected that this method can provide a new tool for design and operation of the biological nitritation process.

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NOMENCLATURE

I_A : Concentration of A inhibitor, mg l^{-1}
K_I : Inhibition constant, mg l^{-1}
K_{IC} : Competitive inhibition constant, mg l^{-1}
K_{IU} : Uncompetitive inhibition constant, mg l^{-1}
K_S : Half-saturation constant, mg l^{-1}
$^{app}K_S$: Apparent value of half-saturation constant, mg l^{-1}
q_A : Reaction rate for A substrate, mgA $mgVSS^{-1}$ d^{-1}
$q_{max,\ A}$: Maximum reaction rate for A substrate, mgA $mgVSS^{-1}$ d^{-1}
$^{app}q_{max,\ A}$: Apparent value of maximum reaction rate for A substrate, mgA $mgVSS^{-1}$ d^{-1}
S_A : Concentration of A substrate, mg l^{-1}
S_{Ao} : Influent concentration of A substrate, mg l^{-1}
X_n : Concentration of nitrite oxidizer, mg l^{-1}
θ_H : Hydraulic retention time, d

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