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Optimization of free ammonia concentration for nitrite accumulation in shortcut biological nitrogen removal process

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Abstract A shortcut biological nitrogen removal (SBNR) utilizes the concept of a direct conversion of ammonium to nitrite and then to nitrogen gas. A successful SBNR requires accumulation of nitrite in the system and inhibition of the activity of nitrite oxidizers. A high concentration of free ammonia (FA) inhibits nitrite oxidizers, but unfortunately decreases the ammonium removal rate as well. Therefore, the optimal range of FA concentration is necessary not only to stabilize nitrite accumulation but also to achieve maximum ammonium removal. In order to derive such optimal FA concentrations, the specific substrate utilization rates of ammonium and nitrite oxidizers were measured. The optimal FA concentration range appeared to be 5– 10 mg/L for the adapted sludge. The simulated results from the modified inhibition model expressed by FA and ammonium/nitrite concentrations were shown very similar to the experimental results.

Keywords Free ammonia $(FA) \cdot$ Nitrite $accumulation \cdot Shortcut biological nitrogen removal$ $(SBNR)$ · Specific substrate utilization rate

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Introduction

The biological nitrogen removal process is one of the most common and relatively cost-effective processes used to remove nitrogen from wastewater. During this process, ammonium is first oxidized to nitrate by aerobic autotrophic nitrifiers and then nitrate is reduced to nitrogen gas by heterotrophic denitrifiers under the anoxic condition. This process, however, possesses such intrinsic shortcomings as; (1) nitrification reaction is slow and requires a relatively large amount of oxygen and (2) denitrification reaction requires lots of external carbon source, frequently not sufficient in wastewaters. The shortcut biological nitrogen removal (SBNR) scheme which nitrifies ammonium to nitrite and then reduces nitrite to nitrogen gas [\[1–4\]](#page-7-0) may overcome these drawbacks because it can theoretically save 25% of oxygen demand needed for nitrification and approximately 40% of carbon sources needed for denitrification and can reduce 63% of biomass production [\[5–9](#page-7-0)].

To achieve a successful SBNR system, nitrite should stably be accumulated in the reactor by inhibiting the oxidation of nitrite to nitrate, while still maintaining the rate of ammonium oxidation at its maximum. Most researchers indicated that high free ammonia (FA) levels [\[10–12](#page-7-0)] and low dissolved oxygen (DO) concentrations [\[8](#page-7-0), [9,](#page-7-0) [13–15](#page-7-0)] were the main factors for nitrite accumulation. The phenomenon of nitrite accumulation by retarding or inhibiting nitrite oxidation was such that the effects of temperature, alkalinity, DO concentration, and ammonium load are masked when the concentration of FA is above certain values $[3, 16, 17]$ $[3, 16, 17]$ $[3, 16, 17]$ $[3, 16, 17]$ $[3, 16, 17]$. Thus, the optimal operational factors for nitrite accumulation were difficult to find since most researchers reported the different and incoherent values for those factors including FA.

The threshold concentrations of FA at which ammonium and nitrite oxidizers are partially or totally inhibited, have been reported by Anthonisen et al. [[10\]](#page-7-0) for the activated sludge. They reported that the inhibition of nitrite oxidation began at 0.1–1.0 mg/L FA while the ammonium oxidation was inhibited at 10–150 mg/L. Thus, a selective inhibition of nitrite oxidation could be achieved at the FA concentration range of 1–10 mg/L. However, high FA concentrations not only inhibit nitrite oxidizers but also decrease the ammonium removal rate. Therefore, it is essential to maintain the optimal FA concentration range for both stable nitrite accumulation and maximum ammonium removal, and the threshold FA concentrations at which the nitrite oxidation was inhibited vary significantly among the previous studies, from 0.05 to 20 mg/L [\[1](#page-7-0), [2,](#page-7-0) [12](#page-7-0), [18–22](#page-7-0)].

In this study, sludges from two different sources, a bench-scale SBNR hybrid process reactor and a pilotscale livestock wastewater treatment plant (WWTP) reactor, each with significantly different microbial distribution, were employed in order to investigate the optimal FA concentration range for the maximum ammonium removal as well as the high and stable nitrite accumulation, and the specific substrate utilization rates of ammonium oxidizers (q_{AO}) and nitrite oxidizers (q_{NO}) were estimated under various FA concentrations. Also, in order to ascertain whether the optimal FA concentration obtained from the batch experiments is accurate, the results obtained from the modified kinetics model considering the FA inhibition were compared with experimental results.

Materials and methods

Cell cultivation and batch tests

Cells for the nitrification experiments were obtained from two different sources. One was a bench-scale SBNR hybrid process reactor. A mixed culture containing nitrifiers and heterotrophs was obtained, as an inoculum, from the activated sludge tank of a pilot-scale livestock WWTP in Yongin, Korea. Cells were grown and maintained in a bench-scale continuous flow reactor (total working volume, 10 L), consisted of an anoxic tank for denitrification and two aerobic tanks for nitritification. The second aerobic tank was a fluidized-bed type reactor filled with porous polyvinyl alcohol (PVA) sponge-cubes (1.5 cm³ and 20% in v/v) to provide an attachable surface to ammonium oxidizers. This reactor was operated at solids retention time (SRT) of 20– 25 days and overall hydraulic retention time (HRT) of 1.5 days, and the internal and sludge recycle ratios were 2Q and 1Q, respectively. The composition of the medium was as follows (in mg/L): $NH₄HCO₃$ as N, 1,000; acetate as chemical oxygen demand (COD), 1,600–2,000; K_2HPO_4 , 944; $MgSO_4$ ⁻⁷H₂O, 500; FeSO₄⁻⁷H₂O, 20; $CaCl₂$, 40; MnSO₄·H₂O, 50; and KCl, 70. When the cells were being harvested, the removal rates of ammonium and COD were approximately 80 and 98%, respectively. The nitrite accumulation was stably observed and the average concentrations of $NO₂⁻N$, $NO₃⁻N$, and FA in the second aerobic tank were 250, 5, and 35 mg/L,

respectively. Details on the reactor and its operation are further described elsewhere [\[4](#page-7-0)].

The other source of cells was a field-scale WWTP (capacity, 3,800 m³/day) in Kwangju, Korea, fed with S Electorics (semiconductor manufacturing) wastewater containing 375 mg/L of COD and 180 mg-N/L of ammonium. This reactor consisted of an anoxic tank $(1,321 \text{ m}^3)$ followed by an aeration tank $(1,840 \text{ m}^3)$, a post-denitrification tank (294 m^3) , a deaeration tank (137 m^3) , and a clarifier tank (443 m^3) . The ammonium oxidation tank was filled with PVA sponge media (15 mm cubes), at 20% (v/v). The SRT for the suspended cells was maintained at 11.2 days and the internal and sludge recycle rates were 2Q and 1Q, respectively.

Microorganisms for the batch experiments were prepared by harvesting the suspended cells from the aerobic tank of two continuous reactors. A 300-mL cell suspension was first centrifuged (3,000 rpm for 15 min) in order to remove the remaining nitrogen species in the liquid. The concentrated cells were re-suspended in distilled water and then cells were transferred to a 250-mL Erlenmeyer flask with 100 mL of the medium which contained all the necessary nutrients but nitrogen and carbon sources.

Specific oxygen uptake rate (SOUR) test

The SOUR test was performed to investigate the distribution characteristics of the two kinds of microorganisms used, ammonium and nitrite oxidizers. The concentrated cells and the feed solution stated below (refer to Table [1\)](#page-2-0) were first separately maintained at 20°C and were continuously aerated to saturate with a sufficient DO concentration. About 30 min later, 100 mL of the concentrated cells and 200 mL of the substrate solution were mixed in the 300 mL biochemical oxygen demand (BOD) bottles, and the initial biomass concentration was maintained at $1,300 \pm 90$ mg volatile suspended solids (VSS) per liter. The SOUR test vessel equipped with a Clark-type oxygen electrode (YSI, Model 5100) was then carefully closed, leaving no air bubbles inside. Samples were mixed, during measurements, using a magnetic stirrer at 150 rpm, and temperature and pH in the vessel were maintained at 20°C and 8, respectively. The DO drop in each bottle filled with each medium was continuously monitored. The SOUR of each sample equaled the slope of DO depletion vs. time divided by the VSS concentration in the bottle.

The feed solution for the SOUR measurement for ammonium oxidizers was composed of 100 mg-N/L of ammonium and other minerals, but without organic compound and nitrite (Table [1\)](#page-2-0). Also, 20 mM of sodium chlorate (NaClO₃) was added to the mixed feed solution to prevent the nitrite oxidation [\[23–25\]](#page-7-0). The values of 20 mM sodium chlorate and 100 mg/L ammonium concentration were verified as the optimum for either the

Table 1 Composition of the feed solutions for the SOUR tests (unit: mg/L)

	Chemical	As	Ammonium oxidation	Nitrate oxidation	COD oxidation	Blank
Electron Donor	NH_4HCO3	N	100			
	NaNO ₂	N		100		
	Acetate	COD			100	
	NaNO ₃	N			15	
	K_2HPO_4	P	16.8	16.8	16.8	16.8
	$MgSO_4$ ⁻⁷ H_2O	Mg	5.0	5.0	5.0	5.0
Mineral	FeSO ₄ ·7H ₂ O	Fe	0.4	0.4	0.4	0.4
	CaCl ₂	Ca	1.6	1.6	1.6	1.6
	$MnSO_4 \cdot H_2$	Mn	1.6	1.6	1.6	1.6
	KC ₁	K	3.6	3.6	3.6	3.6
Inhibition reagent	NaClO ₃		20 mM			
	ATU			5		

selective inhibition of nitrite oxidation or the maximization of ammonium oxidition through preliminary experiments (data not shown). The feed solution for nitrite oxidizers was composed of 100 mg-N/L of nitrite and other minerals (Table 1). The allythiourea (ATU; at 5 mg/L), a selective inhibitor for the ammonium oxidizers [\[25,](#page-7-0) [26\]](#page-7-0), was added to the mixed feed solution to prevent the ammonium oxidation because there might be a small amount of ammonium left in the rinsed cells. Preliminary experiments showed that the ammonium oxidation was completely inhibited but there was no effect on nitrite oxidation when the ATU concentration was 5 mg/L (data not shown). On the other hand, the feed for heterotrophs was composed of 100 mg/L of acetate as COD, 15 mg/L of nitrate, and other minerals (Table 1). The endogenous respiration rate of the cells was measured using a blank containing only minerals. The net SOUR value for each electron donor was calculated by subtracting the endogenous respiration rate value from the measured SOUR value.

Specifc substrate utilization rates of ammonium and nitrite oxidizers

The concentrated cells and the feed solution were first separately maintained at 30°C. About 30 min later, 200 mL of the concentrated cells and 800 mL of the substrate solution were mixed in a 1-L Erlenmeyer flask, and the initial biomass concentration was $3,000 \pm 120$ mg-VSS/L. For the ammonium oxidation, maintaining constant FA concentrations was not possible because the FA concentration was changeable depending on the ammonium concentration. Therefore, the comparison of specific substrate utilization rates of ammonium oxidizers for two different sources of cells was based on the initial FA concentrations. To determine the maximum specific substrate utilization rate of ammonium oxidizers, 800 mL solution containing different ammonium concentrations was added to the flask, to have the final volume of 1,000 mL and the final FA concentrations of 1, 5, 10, 20, 30, and 50 mg/L. For nitrite oxidizers, 800 mL solution containing different

FA concentrations, same as those for ammonium oxidizers, was added to the flask containing nitrite at 50 mg-N/L, and ATU at 5 mg/L was added to the solution to prevent ammonium oxidation. Detailed constiuents of the feed solution for the specific substrate utilization rate expeiments are given in Table [2.](#page-3-0) For all the batch experiments, temperature, pH, and DO were maintained at 30 ± 0.5 °C, 8 ± 0.5 , and 5 mg/L, respectively.

Sampling and analysis

Samples were taken from the batch reactors with the following intervals: 10, 20, 40, 80, 120, 180, 240, and 300 min, and immediately filtered through a glass-fiber filter (Whatman, GF/C). All the samples were analyzed following the Standard Methods [\[27\]](#page-7-0). The concentration of ammonium was determined by the Phenate Method $(4500-NH₃ F)$ in Standard Methods). The concentrations of nitrite and nitrate were analyzed using an ion chromatograph (Dionex, DX500). The FA concentration was estimated using the following equation $[10]$ $[10]$,

FA as NH₃(mg/L) =
$$
\frac{17}{14}
$$

\n $\times \frac{\text{Total ammonia as } N(\text{mg/L}) \times 10^{\text{pH}}}{K_b/K_w + 10^{\text{pH}}}$ (1)
\nwhere $K_b/K_w = e^{6,344/273 + T(^{\circ}\text{C})}$

FA inhibition model for nitrification

The kinetic model used in this study predicts that the specific ammonium utilization rate (q_{AO}) decreases with the increase of the substrate concentration beyond certain value. This trend is in accordance with the observation by Anthonisen et al. [\[10](#page-7-0)] that the ammonium oxidation is inhibited by FA at a concentration above certain values (NH_X and FA are proportional to each other unless the pH in the reactor is changed). When the pH increases, the FA concentration also increases

Table 2 Composition of the feed solutions for the specific substrate utilization rates experiments under various FA concentrations (unit: $mg/L)$

Chemical	As	Concentration (mg/L)						
FA	NH ₃			10	20	30	50	
NH ₄ HCO ₃	N	10	50	100	200	300	500	
NaNO ₂ ^a	N			50				
K_2HPO_4		1.68	8.4	16.8	33.6	50.4	84	
$MgSO_4$.7H ₂ O	Mg	0.48	2.4	4.8	9.6	14.4	24	
$FeSO_4$.7H ₂ O	Fe	0.04	0.2	0.4	0.8	1.2		
CaCl ₂	Ca	0.1	0.5					
$MnSO_4 \cdot H_2$ O	Mn	0.16	0.8	1.6	3.2	4.8	8	
KCl	K	0.36	1.8	3.6	7.2	10.8	18	

^aAdded only when nitrite oxidation was tested

although the concentration of total ammonia (usually designated as $NH₄⁺$) unchanged. In order to incorporate FA rigorously in the model, Park [[28\]](#page-7-0) proposed a modified self-inhibition model using equation (2) which is equivalent to an uncompetitive inhibition by FA:

$$
q_{\rm AO} = \frac{\hat{q}_{\rm AO} S_{\rm NH_X}}{K_{\rm AO} + S_{\rm NH_X} + S_{\rm NH_X} S_{\rm FA}/K_{\rm I.FA.AO}}.\tag{2}
$$

where

$$
S_{NH_x}
$$
 total ammonium concentration as N (NH₄⁺–
N + NH₃-N),

- q_{AO} specific substrate utilization rate of ammonium oxidizers,
- \hat{q}_{AO} maximum specific substrate utilization rate of ammonium oxidizers,
- K_{AO} half-velocity constant of ammonium oxidizers (in mg/L),

$$
S_{FA}
$$
FA concentration = 17/14(S_{NH_X} × 10^{pt}/
(K_b/K_w) + 10^{pt} by Anthonisen et al. [10],

 K_b/K_w exp[6,334/(273+°C)]

 K_{LFAAO} inhibition constant of ammonium oxidizers by FA (in mg/L)

On the other hand, the inhibition of nitrite oxidation has been modeled as uncompetitive inhibition by NH_4^+ [\[29](#page-7-0)], mixed inhibition by $NH₃$ [[30\]](#page-7-0), or noncompetitive inhibition by $HNO₂$ [[31\]](#page-7-0). In addition, Park [[28\]](#page-7-0) recently proposed the kinetic model by FA inhibition. Since it has also been reported that the nitrite oxidation was suppressed by FA, the model of Park, uncompetitive inhibition by FA, was chosen. Thus, the kinetic equation is:

$$
q_{\rm NO} = \frac{\hat{q}_{\rm NO} S_{\rm NO_2}}{K_{\rm NO} + S_{\rm NO_2} - S_{\rm NO_2} S_{\rm FA}/K_{\rm I, FA, NO}}.\tag{3}
$$

Where

- \hat{q}_{NO} maximum specific substrate utilization rate of nitrite oxidizers,
- K_{NO} half-velocity constant of nitrite oxidizers (in mg/L),
- $K_{I.FA.AO}$ inhibition constant of nitrite oxidizers by FA $(in \ mg/L)$

Results and discussion

Specific oxygen uptake rates

The SOUR experiments were performed to confirm the different microbial distribution for the two sources of cells used to measure the specific substrate utilization rates. Figure 1 shows decreases of DO concentrations per unit biomass (VSS) and time for three kinds of electron donors (ammonium, nitrite, and carbon source) for the cells obtained from WWTP and SBNR reactors. Each line, as the average of triplicates, represents the SOUR value (\hat{q}_{O_2}) for different microorganisms, corrected by the blank experiment.

As shown in Fig. 1, the SOUR value for the ammonium oxidation by cells from the SBNR reactor was about nine times higher than that from the WWTP, implying that cells in the SBNR reactor contained about nine times more of the ammonium oxidizers, assuming the ammonium oxidizers in two

Fig. 1 Specific oxygen uptake rates (SOURs) for cells obtained from the WWTP and the SBNR reactor, for different electron donors

sources with similar activities. For the nitrite oxidation, on the other hand, the SOUR value for the cells from the WWTP was about 2.5 times higher than that from the SBNR reactor. For the COD oxidation, the SOUR values for the cells from the SBNR reactor were about 6.4 times higher than that from the WWTP. The distribution ratio of ammonium oxidizers to nitrite oxidizers for the cells from the WWTP was estimated 1.7 assuming similar microbial activities, suggesting much more of ammonium oxidizers compared to nitrite oxidizers. In comparison, however, the ratio for the cells from the SBNR reactor was calculated to be about 38, meaning the ammonium oxidizers dominant in the reactor. These results, therefore, indicate there was a significant difference in microbial distribution between two sources of cells.

Specific substrate utilization rates of ammonium oxidizers at different FA concentrations

In order to measure the specific substrate utilization rate (q_{AO}) of ammonium oxidizers per unit biomass (VSS), the concentration change of ammonium as a substrate was investigated in a batch reactor. Figure 2 shows the q_a values, determined from the initial slope for the ammonium removal, for the cells from the WWTP and the SBNR reactor, at different FA concentrations (1, 5, 10, 20, 30, and 50 mg/L). The reaction was shown zeroorder because of the R^2 values above 0.99. As shown, the specific ammonium utilization rates of ammonium oxidizers obtained from the WWTP were 4–6 times lower than those from the SBNR reactor, implying cells from the WWTP containing smaller portions of ammonium oxidizers.

Figure [3](#page-5-0) further demonstrates the relationship between the q_{AO} values for each source of cells (WWTP and SBNR reactor) and the FA concentrations. As shown, the highest q_{AO} value for both types of cells was at 10 mg/L of the initial FA concentration. When the initial FA concentration was above 10 mg/L, the specific ammonium utilization rates of both cells decreased with increasing FA concentrations. This result was also in agreement with the results of Anthonisen et al. [\[10\]](#page-7-0) that showed the inhibition of ammonium oxidation by FA at 10–150 mg/ L. The decrease in the q_{AO} values for the cells from the SBNR reactor, however, was observed relatively lower $(10\%$ decrease) compared to the WWTP cells $(20\%$ decrease) at the initial FA concentration of 50 mg/L. This implies that cells in the SBNR reactor were adapted to the high FA concentration because the reactor had been operated with 30 mg/L FA for a long period. On the other hand, at the initial FA concentration of below 10 mg/L, the q_{AO} values decreased for both sources of cells. This can be explained by the Monod equation (bacterial growth kinetics), which was developed in 1940s by the famous French microbiologist Jacques Monod, implying that the q_{AO} value might be limited by the concentration of ammonium used as a substrate.

a

b

Fig. 2 Specific ammonium utilization rates (q_{AO}) of the cells from the WWTP (a) and the SBNR reactor (b) at different FA concentrations

200 X*t (mg-VSSA*d)

0.5909 $q_{A0} = 0.59$
R² = 0.998

 300

400

Specific substrate utilization rates of nitrite oxidizers at different FA concentrations

The specific substrate utilization rate of the nitrite oxidizers, q_{NO} , from the WWTP and the SBNR reactor, was measured by nitrite concentration ($NO₂⁻N$) at different FA concentrations (similar to measuring q_{AO}), with 5 mg/L of ATU added into the batch reactor to prevent the ammonium oxidization. As shown in Fig. [4](#page-5-0), the q_{NO} values rapidly decreased until the FA concentration reached 5 mg/L, for both types of cells. However, those values showed a very little variation for the FA concentrations above 5 mg/L, implying that maintaining the FA concentration at above 5 mg/L would not be appropriate to suppress the nitrite oxidation. In addition, the q_{NO} values for the cells from the SBNR reactor were significant lower compared to the WWTP cells, suggesting that cells from the SBNR reactor operated at high FA concentrations for a long period, contained a relatively smaller number of nitrite oxidizers compared to ammonium oxidizers, due to the inhibition of nitrite oxidizers by the FA. In the case of WWTP cells, the inhibition for nitrite oxidizers was observed even at lower FA concentrations. This result indirectly implies the WWTP cells with relatively more nitrite oxidizers.

Fig. 3 Effect of FA concentrations on specific ammonium utilization rate $(q_{AO.})$

Application of modified inhibition model

Equations 2 and 3 can provide the specific information on FA inhibition. For example, it can explain how nitrifiers are inhibited by FA increase/decrease, whether different cultures give similar behavior against FA levels, and eventually how nitrite oxidizers gradually decreased in the SBNR reactor. Figures 3 and 4 show the simulated results from the modified inhibition model expressed by FA and ammonium/nitrite concentrations, compared with the experimental results. The fittings for maximum specific substrate utilization rate (\hat{q}) , a concentration giving one-half the maximum rate (K_S) , and an inhibition concentration of the inhibitor (K_I) values were performed mainly based on the experiment of Park [[28](#page-7-0)] and the obtained values are summarized in Table [3](#page-6-0). These results clearly demonstrate that the inhibition of ammonium and nitrite oxidizers was caused by the FA, and the kinetic models for the inhibition of nitrifiers

Fig. 4 Effect of FA concentrations on specific nitrite utilization rate $(q_{NO.})$

should be expressed with FA as inhibitor. Based on the results of modeling, the values of \hat{q} quantitatively and the change of K_S and K_I values caused by the inhibition can be obtained, which otherwise cannot be obtained by experiments. The change of K_S and K_I values in the mixed culture further implies the significant change of micobial community due to the acclimation to the high FA concentration. Figure [5](#page-6-0) shows the degree of inhibition depending on the change of K_S and K_I values. As shown in Fig. [5a](#page-6-0), the specific ammonium utilization rate gradually decreased as K_S increased when K_I was fixed at 10 mg/L. On the other hand, q_a increased as K_I values increased (Fig. [5](#page-6-0)b). Compared with the experimental results for ammonium oxidation (Fig. 3), K_S values of SBNR and WWTP cells were 15 and 5 mg-NH $_4^+$ -N/L and K_I values were 50 and 100 mg-FA/L, respectively, showing the K_S value of SBNR cells relatively higher than those of WWTP cells. It was probably because the cells taken from the SBNR reactor were acclimated to the higher FA concentration. The specific nitrite utilization rates gradually increased with the decrease of K_S and the increase of K_I (Fig. [5c](#page-6-0), d). The K_I values of SBNR and WWTP cells were predicted 10 and 2 mg- FA/L , respectively. This difference of K_I values might be caused by the fraction of nitrite oxidizers among total nitrifiers decreasing due to the long-term exposure to the relatively higher FA concentration.

Comparison of specific substrate utilization between ammonium and nitrite oxidizers

Figure [6](#page-7-0)a shows the effects of FA concentrations on q_{AO} and q_{NO} values for the cells from the two different sources, WWTP and SBNR reactor (also refer to Figs. 3, 4). The difference between q_{AO} and q_{NO} values shows the possibility of potential nitrite accumulation. As shown, this difference was the highest at approximately 10 mg/ L of FA concentration, and after that, little changed even though FA concentrations increased. This result implies that nitrifiers including both ammonia and nitrite oxidizers have very similar characteristics even though the distribution of biomass population was significantly different, indicating that nitrite accumulation can be easily achieved by manipulating FA. For the cells from the WWTP, the nitrite accumulation might occur stably at the FA concentration above 5 mg/L. The FA concentration of 5 mg/L could also have an advantage of the lower effluent ammonium concentration over 10 mg/L, for the WWTP. These results were in good agreement with the report of Bae et al. [\[32](#page-7-0)] which suggested 4 mg/L of FA concentration and a high q_{AO}/q_{NO} ratio were favorable to nitrite accumulation. Therefore, 10 mg/L of FA concentration was shown the optimal condition for maintaining the high ammonium removal efficiency, whereas 5 mg/L was more appropriate for the low ammonium concentration levels in the effluent.

In order to confirm the optimal FA range, the relationship between FA concentration and nitrite

Fig. 5 Change of inhibition degree with various K_S and K_I

accumulation rate is shown in Fig. [6](#page-7-0)b [[4,](#page-7-0) [32](#page-7-0)]. The ratio of NO₂–N to NO_x–N was shown the highest at around 10 mg-FA/L and gradually decreased with increasing FA concentrations, implying the optimal FA range was $5 < F A < 10$ mg/L.

Conclusions

In order to derive the optimal FA concentrations, the specific substrate utilization rates of ammonium and

Table 3 Comparison between fitting values using kinetic models for the inhibition on nitrifiers

	WWTP		SBNR		
	Ammonium oxidizers	Nitrate oxidizers	Ammonium oxidizers	Nitrate oxidizers	
\hat{q} , mg-N/mg- VSS-day	0.19	0.5	0.9	0.019	
K_S , mg-N/L K_I , mg-FA/L	10 100	3	15 150	10	

nitrite oxidizers were investigated at various FA concentrations through batch experiments. The highest specific ammonium oxidation rate was observed when the FA concentration was 10 mg/L, and the rate decreased slightly with increasing FA concentration. In case of nitrite oxidation, the specific nitrite utilization rate decreased significantly with increasing FA concentration up to 5 mg/L. Therefore, the optimal FA concentration range for both stable nitrite accumulation and maximum ammonium removal appeared to be 5– 10 mg/L for the adapted sludge. In order to ascertain the experimental results, the simulated results were obtained using the modified inhibition model expressed by FA and ammonium/nitrite concentrations and these simulated results were shown dramatically similar to the experimental results. These model results further support that the inhibition of ammonium and nitrite oxidizers was caused by FA and the fraction of nitrite oxidizers among total nitrifiers decreased due to the long-term exposure to the relatively higher FA level.

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Fig. 6 Effects of FA concentration on a specific ammonium and nitrite utilization rates (q_{AO} and q_{NO}) and **b** nitrite accumulation of cells from SBNR reactor

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