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Optimal operational factors for nitrite accumulation in batch reactors

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Abstract

The environmental factors that affected the accumulation of nitrite in nitrifying reactors were investigated using a mixed culture. A batch reactor with 50 mg-N/l of ammonia was used. The pH, temperature and dissolved oxygen concentration were varied. The concentration of unionized free ammonia also changed with the oxidation of ammonia and the variation of pH and temperature. The accumulation of nitrite was affected sensitively by pH and temperature. A higher nitrite concentration was observed at pH 8-9 or temperature around 30 °C. The dissolved oxygen also affected, giving the highest nitrite accumulation at around 1.5 mg/l. These were the favored conditions for nitrite production. The free ammonia concentration influenced the nitrite accumulation also, by inhibiting nitrite oxidation. The inhibition became apparent at a concentration of approximately 4 mg/l or above, but insignificant at below 1 mg/l. Thus, simultaneously high free ammonia concentration and maximum specific ammonia-oxidation rate (above 15×10^{-3} mg-N/mg-VSS·h) were needed for a significant nitrite accumulation. When the two conditions were met, then the highest accumulation was observed when the ratio of the maximum specific oxidation rate of ammonia to the maximum specific oxidation rate of nitrite (k_a/k_n) was highest. Under the optimal operating conditions of pH 8, 30 °C and 1.5 mg/l of dissolved oxygen, as much as 77% of the removed ammonia accumulated in nitrite.

Abbreviations: k = maximum specific rate of substrate utilization; k_a = maximum specific rate of ammonia oxidation (mg-N/mg-VSS·h); k_n = maximum specific rate of nitrite oxidation (mg-N/mg-VSS·h); K_b = ionization constant of the ammonia equilibrium equation; K_w = ionization constant of water

Introduction

Ammonia is oxidized to nitrate by two different groups of bacteria. The first group of bacteria, ammonia oxidizers, converts ammonia to nitrite (NO_2^-); then, the second group, nitrite oxidizers, further oxidizes the intermediate product to nitrate. In the environment, nitrite is seldom observed at a high concentration. This is probably due to a low $S_{\min}$ (minimum substrate concentration capable of supporting steady-state biomass) value (Rittmann & McCarty 2001) and a relatively high substrate-utilization rate of the nitrite oxidizers. However, a sizable amount of nitrite is accumulated in certain circumstances such as a transient phase (Anthonisen et al. 1976; Gee et al. 1990) or an inhibitory environment (Alleman 1985).

If the oxidation of nitrite could be controlled, a significant advantage can be taken in nitrogen removal, by using a shortcut biological nitrogen removal process (Bae & Chung 2002; Turk & Mavinc 1987; Beccari et al. 1983). The concept of the shortcut nitrogen removal is denitrifying nitrite to nitrogen gas before it oxidizes to nitrate. With this, up to 25% of oxygen and approximately 40% of carbon source can be saved in the biological nitrogen removal process (Bae & Chung 2002; Turk & Mavinc 1987; Turk & Mavinc 1989).

Anthonisen et al. (1976) reported that unionized ammonia (NH_3, free ammonia, FA) inhibited nitrification reaction. Inhibition of nitrite oxidation began at 0.1–1.0 mg/l of FA, while that of ammonia oxidation was observed at 10–150 mg/l. Thus, a selective inhibition of nitrite oxidation could be achieved at a range (1.0–10 mg/l) of FA concentration. Recent studies also suggested that free hydroxylamine (NH_2OH), an intermediate of ammonia oxidation, might be a key factor that caused inhibition to nitrite oxidation (Jetten et al. 1999; Yang & Alleman 1992). Other factors that could be used for control of nitrite oxidation were pH, temperature, dissolved oxygen (DO), solid retention time, and so on (Alleman 1985). Among them, the pH and temperature also affect the formation of FA by involving in the ionization of ammonia. Since FA concentration rises with the increase of total ammonia concentration, nitrogen-rich high-strength wastewaters, such as the supernatant of the anaerobic sludge digesters, livestock wastewaters, or landfill leachates, will be ideal for application of the shortcut biological nitrogen removal process.

In this study, the environmental factors that affect the rates of ammonia oxidation and nitrite oxidation were investigated. For this, the pH, DO, temperature and FA concentration were chosen for variables. Since the Monod constant (the half-velocity coefficient) for nitrification reactions is usually not greater than 1.0 mg-N/l (Rittmann & McCarty 2001; U.S. EPA 1993), the maximum specific substrate-utilization rate, k, will govern the reaction rate in many wastewater treatment operations. Thus, we measured the k values for ammonia oxidation and nitrite oxidation using batch reactors, under various conditions. The k values for the two reactions were compared with the measured nitrite concentration, investigating the optimum conditions for nitrite accumulation.

Materials and methods

Cultivation of cells

A mixed culture that contained nitrifiers and heterotrophs was used. The inoculum was obtained from an activated sludge tank of a livestock wastewater treatment plant in Yongin City, Korea. The cells were grown and maintained in a continuous flow aerobic-anoxic reactor, by feeding a synthetic mineral medium that contained high concentrations of glucose (2,000 mg/l as chemical oxygen demand, COD) and ammonia (1,000 mg/l as N) (Table 1). The reactor was consisted with an anoxic tank (3.3L) followed by an aerobic tank (6.7L) and a settling tank. The aerobic tank was filled with polyvinyl alcohol sponge media (0.75-mm cubes) by 20% by volume. Thus, the reactor contained both attached cells (1500 mg VSS/l on average) and suspended cells (2500 mg VSS/l). The solids retention time for the suspended cells was maintained at 20–25 days. The temperature and the dissolved oxygen concentration in the reactor were kept at 30 °C and 2–3 mg/L, respectively. The pH was maintained around 8. During the period when the cells were being harvested, the removal rates of ammonia and COD were approximately 80% and 98%, respectively. The nitrite accumulation was just begun to be observed and approximate average concentrations of NO_2^-–N, NO_3^-–N and free ammonia in the aerobic tank were 250 mg/l, 30 mg/l, and 50 mg/l, respectively. More details on the reactor and its operation are described elsewhere (Bae & Chung 2002).

Batch experiments

The microorganisms for batch experiments were prepared by harvesting the suspended cells from the continuous reactor. A 300-ml cell suspension was concentrated in a centrifuge (3000 rpm × 15 min) in order to remove the remaining nitrogen species in the liquid. The concentrated cells were re-suspended with distilled water and concentrated again. The rinsed, concentrated cells were transferred to a 250-ml Erlenmeyer flask with 100 ml of medium that contained all necessary nutrients but nitrogen and glucose.

For determining the maximum specific substrate-utilization rate of ammonia oxidizers, 100 ml of 100-mg-N/l ammonia solution was added to the flask. The final volume and ammonia concentration, respectively, was 200 ml and 50 mg-N/l. For nitrite oxidizers, 100 ml of 100-mg-N/l nitrite solution was added to the flask. Thus, there was no FA inhibition in the nitrite oxidation tests. The constituents of the feed solution are given in Table 1. The concentration of cells in the flask was in the range of 2,000–2,500 mg/l as volatile suspended solids (VSS). The cells were consisted of ammonia oxidizers, nitrite oxidizers and heterotrophs.

The maximum specific substrate-utilization rates of the ammonia oxidizers and nitrite oxidizers were

Table 1. Composition of feeds for cell growth and batch tests

Chemicals	Concentration (mg/l)		
Chemicals	As of	For cell growth	For batch test
Glucose	COD	2,000	–
NH_4HCO_3	N	1,000	50 (For ammonia oxidation)
$NaNO_2$	N	–	50 (For nitrite oxidiation)
K_2HPO_4	P	168	8.4
$MgSO_4{\cdot}7H_2O$	Mg	50	2.5
$FeSO_4{\cdot}7H_2O$	Fe	4	0.2
$CaCl_2$	Ca	15	0.8
$MnSO_4{\cdot}7H_2O$	Mn	15	0.8
KCl	K	35	1.8

determined at various pH, DO and temperature. To test the pH effect, it was varied as 7, 8, 9 and 10, while the temperature and DO were fixed at 30 °C and 2–3 mg/l, respectively. The pH in the reactor was monitored closely and adjusted as it changed with the nitrification reaction. In order to see the effect of the DO concentration, it was varied like 0.5 ± 0.2 (nominally 0.5) mg/l, 1.5 ± 0.2 (nominally 1.5) mg/l and 2.5 ± 0.2 (nominally 2.5) mg/l, while the temperature and initial pH were fixed at 30 °C and 8.1 ± 0.1, respectively. A slight change of pH with reaction was unadjusted. Finally, to investigate the effect of temperature, it was varied from 10 °C to 20 °C, 30 °C and 40 °C, maintaining the DO and the initial pH at 2–3 mg/l and 8.1 ± 0.1, respectively.

Sampling and analysis

Samples were taken from the batch reactors at appropriate intervals and immediately filtered through a glass-fiber filter (Whatman, GF/C). All the samples were analyzed following the Standard Methods (APHA 1998). The concentration of ammonia was determined by the Phenate Method (4500-NH3 F in Standard Methods). The concentrations of nitrite and nitrate were determined by Methods 4500-NO_2^- B, and 4500-NO_3^- B, respectively. The COD and VSS were also measured following the Standard Methods. The DO concentration and pH were measured by using ordinary electrodes. The free ammonia concentration was estimated using the equation (Anthonisen et al. 1976),

$$\text{FA as NH}_3\ (\text{mg/l})$$

$$= \frac{17}{14} \times \frac{\text{Total ammonia as } N\ (\text{mg/l}) \times 10^{\text{pH}}}{K_b/K_w + 10^{\text{pH}}}$$

in which, $K_b/K_w = e^{\frac{6{,}344}{273+T(°\text{C})}}$.

Results and discussion

Effect of pH

The removal of ammonia under different pH was presented in Figure 1. The maximum specific ammonia utilization rate (note that the rate is specific to the mass of the mixed-culture cells), k_a, was determined from the initial slope of ammonia removal as illustrated in Figure 1. In estimating the slopes, initial three to five data points that had been obtained at relatively high substrate concentration were chosen for regression. Since the values of the coefficient of determination (R^2) in the regression were above 0.99, the reaction was virtually zero-order. The k_a value increased by 67% as the pH increased from 7 to 8, but it increased only slightly when the pH further increased to 9. At pH 10, the rate dropped and gave the lowest value among the tests. The oxidation of nitrite under different pH was shown in Figure 2 with the maximum specific nitrite utilization rate, k_n. The k_n increased gradually as pH rose from 7 to 9. But, it decreased drastically when the pH further rose to 10. The maximum specific oxidation rates of ammonia and nitrite under different pH conditions are shown together in Figure 3. The smooth lines were drawn to connect the data points using nonlinear curve fitting method of the MS-Excel program (Version: Microsoft Excel 2000). Figure 3 shows that the highest k values were obtained at pH 9 for both groups of bacteria. The k value of nitrite oxidizers was significantly higher than that of ammonia oxidizers at pH 9. It was reversed when the pH was 7 or 10 as the maximum rate of nitrite oxidation changed more sensitively with pH. At pH 8, the values of k_a and k_n were similar. Since the overall nitrification rate will be limited by the lower reaction rate in Figure 3, it will be controlled by the ammonia oxidation rate around pH 9, but by the nitrite oxidation rate around pH 7 or 10. It should be noted, however, that all the nitrite oxidation rates were estimated in an ammonia-free environment. Since free

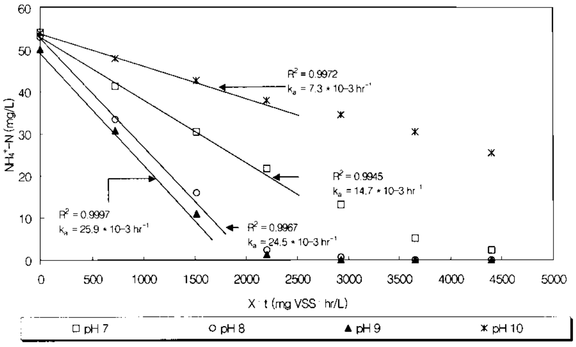

Figure 1. Maximum specific ammonia utilization rate (k_a) under different pH.

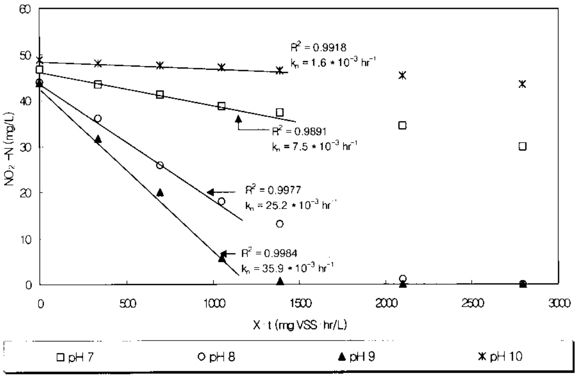

Figure 2. Maximum specific nitrite utilization rate (k_n) under different pH.

ammonia and/or its intermediate (hydroxylamine) affect the nitrite oxidation rate, the rate-limiting reaction at different pH may differ in usual situations in which ammonia is present in nitrite oxidation. The general pattern of the change of nitrification rate upon pH resembles those in the literature (U.S.EPA 1993; Barnes & Bliss 1983). Particularly the sharp drop of the rate near pH 10 might be due to the inhibition by free ammonia as indicated by Sauter and Alleman (1981).

The nitrite accumulation in batch reactors under various pH conditions is shown in Figure 4. The free ammonia (FA) concentrations were also shown. At pH 7, the accumulation of nitrite was insignificant, while about 90% of the removed ammonia (at time 40-min) converted to nitrate. Since the value of k_n was only a half of that of k_a (Figure 3), the rapid oxidation of nitrite was rather unexpected. At pH 8, the highest nitrite accumulation was observed, giving a maximum nitrite concentration, 24 mg-N/l. The nitrite-accumulation rate (the percentage of nitrite accumulation to the amount of ammonia removed) was as high as 60%. This means that only a 40% of the removed ammonia converted all the way to nitrate. The main reason for this nitrite accumulation when the k values for nitrite oxidation and ammonia oxidation were similar was most probably due to the high concentration of FA. The initial FA concentration (4.8 mg/l) was far above the threshold concentration of inhibition (0.1–1.0 mg/l) at which the inhibition of nitrite oxidation began (Anthonisen et al. 1976). After 40 minutes when the FA concentration was relatively low, the accumulated nitrite converted to nitrate quickly. At pH 9, an almost similar amount of nitrite accumulation to that in pH 8 was observed. Because the k value for nitrite oxidation was significantly higher than that for ammonia oxidation at pH 9, the FA inhibition probably had exerted more severely. At pH 10, the accumulation of nitrite was low, reflecting the low value of k_a at that pH. Again, a substantial oxidation of nitrite (approximately 60%) was observed in spite of the low k_n value at the pH. In summary, the optimum pH for nitrite accumulation appeared to be in the range of 8–9, under reaction conditions of 30 °C, $2 \simeq 3$ mg/l of DO, and 50 mg-N/l of initial ammonia concentration.

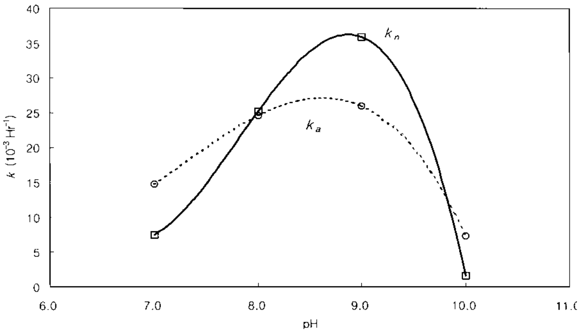

Figure 3. k vs. pH for ammonia oxidation (k_a) and nitrite oxidation (k_n).

Effect of DO

The maximum specific oxidation rates of ammonia and nitrite under various DO concentrations are presented in Figure 5. The coefficients of determination were above 0.99 in all sets of experiment (data not shown). The k values for both reactions were similar under DO = 0.5 mg/l, and increased as DO increased. The k_a increased 1.9 times as DO increased from 0.5 to 2.5 mg/l. The k_n increased even more, giving a 2.8-times higher value at DO=2.5 mg/l. Consequently, the nitrite oxidizers had a significantly higher maximum specific oxidation rate than the ammonia oxidizers had when DO=2.5 mg/l. Since dissolved oxygen is a co-

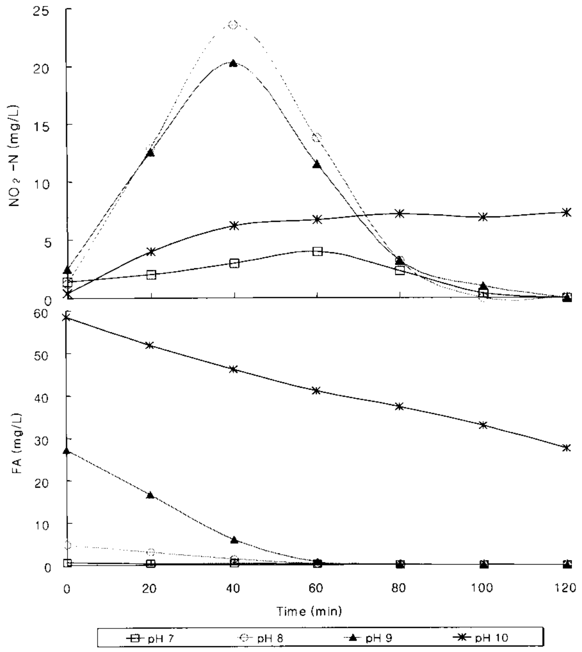

Figure 4. Effect of pH and FA concentration upon nitrite accumulation.

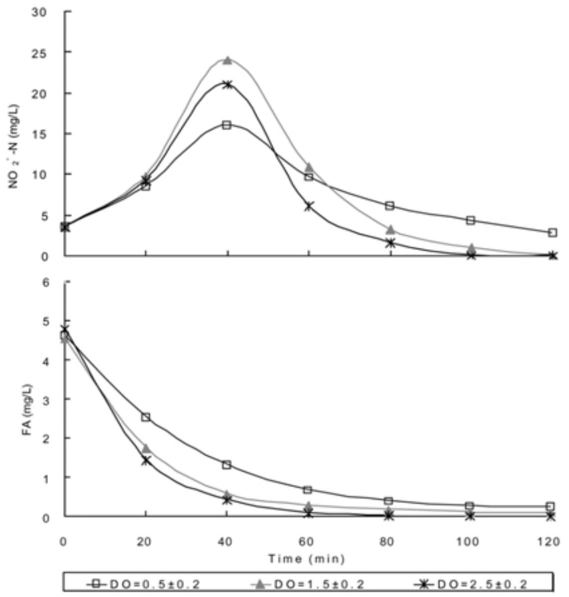

Figure 5. k vs. DO concentration for ammonia oxidation (k_a) and nitrite oxidation (k_n).

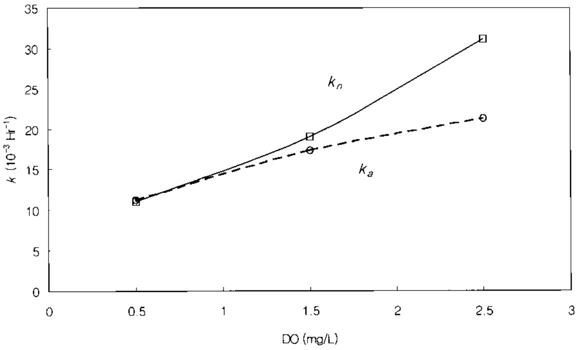

Figure 6. Effect of DO and FA concentration upon nitrite accumulation.

substrate in the nitrification reaction, its concentration will affect the reaction in a dual-limitation manner (Bae & Rittmann 1996). In this study, nitrite oxidation was affected more sensitively by the decrease of DO. The reported ranges of Monod constant for oxygen for *Nitrosomonas* and *Nitrobacter* were 0.3–1.3 and 0.25–1.3 mg/l, respectively (Barnes & Bliss 1983).

The nitrite accumulation in the ammonia oxidation reactions under various DO concentrations is shown in Figure 6 with the FA concentrations. In general, the nitrite accumulated for the first 40 minutes of the reaction time, during which the concentration of ammonia (and FA also) was relatively high. When the ammonia concentration was high, the ammonia oxidation was fast, while the nitrite oxidation was slow due to the free ammonia inhibition. The highest nitrite accumulation was observed when the DO concentration was 1.5 mg/l. At DO 2.5 mg/l, the accumulation was slightly lower than that at DO 1.5 mg/l, although the k_a value increased with DO increase. It was because the k_n value also increased, lowering the k_a/k_n ratio, with the increase of DO. In fact, the nitrite-accumulation rate (at time 40-min) decreased from 77% to 54%, as DO increased from 1.5 mg/l to 2.5 mg/l. The FA concentration in the earlier phase of reaction was within the optimum range for nitrite accumulation (1 to 10 mg/l). During the time the nitrite accumulated rapidly. After 40-min, the FA concentration was generally below 1 mg/l and a rapid consumption of nitrite was observed. The rate of nitrite consumption rose with the increase of k_n. Thus, the highest consumption rate was observed when DO=2.5 mg/l.

Effect of temperature

The maximum specific substrate-utilization rate of ammonia and nitrite oxidizers at different temperatures is shown in Figure 7. The coefficients of determination were above 0.98 in all sets of experiment. The k_a increased by 3.7 times as the temperature rose from 10°C to 30°C. This observation is in well accord with

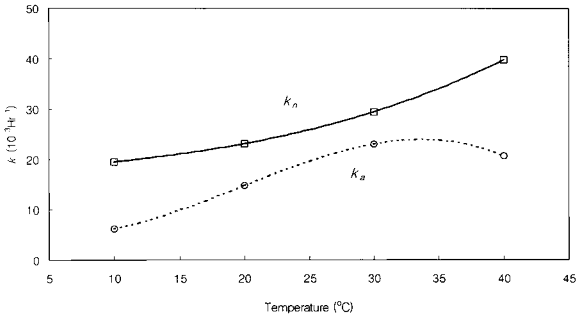

Figure 7. k vs. temperature for ammonia oxidation (k_a) and nitrite oxidation (k_n).

the classical temperature effect found in the literature (Downing & Hopwood 1964) in that the nitrification rate doubled as temperature rose by 10 °C over the range of 5–30 °C. At 40 °C, the rate slightly decreased. Thus, the optimum temperature most probably lied in the range of 30–40 °C. This coincides with the reported optimum temperature of 35 °C by Buswell (1954), but slightly differs from the results of Hellinga et al. (1998) in which the maximum respiration rate was observed near 40 °C. On the other hand, the k_n increased as the temperature rose from 10 °C to 40 °C. The increasing rate even accelerated as temperature rose. This is consistent with the reported optimum range of temperature, 35–42 °C (Deppe 1960; Laudelout 1960). The nitrite oxidizers had a significantly higher k value than the ammonia oxidizers over the whole range of temperature.

The nitrite accumulation at various reaction temperatures is presented in Figure 8. Even though the k_n value was significantly higher than the k_a value at each temperature a large amount of nitrite accumulated at temperatures 30 °C and 40 °C. This was probably due, in most part, to the high FA concentration at those temperatures as shown in Figure 8. However, the FA concentration was not the sole reason because the largest accumulation of nitrite was observed at 30 °C, at which the FA concentration was not highest.

Parameter analysis

Table 2 summarizes the key results of our experiment. The k values estimated in this experiment were significantly lower than the reported values in the literature. For instance, Gee et al. (1990) obtained 78 × 10^{-3} and 210 × 10^{-3} mg-N/mg-VSS·h for ammonia and nitrite oxidizers, respectively, under 23 °C and pH 8. These values are about five times higher then those obtained in our experiment under similar experimental conditions (e.g., Temp 20 °C in Table 2). The main reason was that the major part of the cell mass was heterotrophic in our experiment. The fraction of nitrifiers was only approximately 8% when estimated form the COD and ammonia removals (data not shown) and the typical cell-yield, 0.45 g-VSS/g-COD for heterotrophs, or 0.1 g-VSS/g-N for nirifiers (U.S.EPA 1993). Since nitrite was seldom observed in significant amount in the environment, it has been thought that k_a is considerably lower than k_n (U.S.EPA 1993). Our results revealed, however, that the ratio k_a/k_n was close to or greater than 1.0 in some experimental conditions, such as pH=8 (in that Temp.=30 °C and DO = 2-3 mg/l) or DO=1.5–2.5 mg/l (in that pH=8 and Temp.=30 °C).

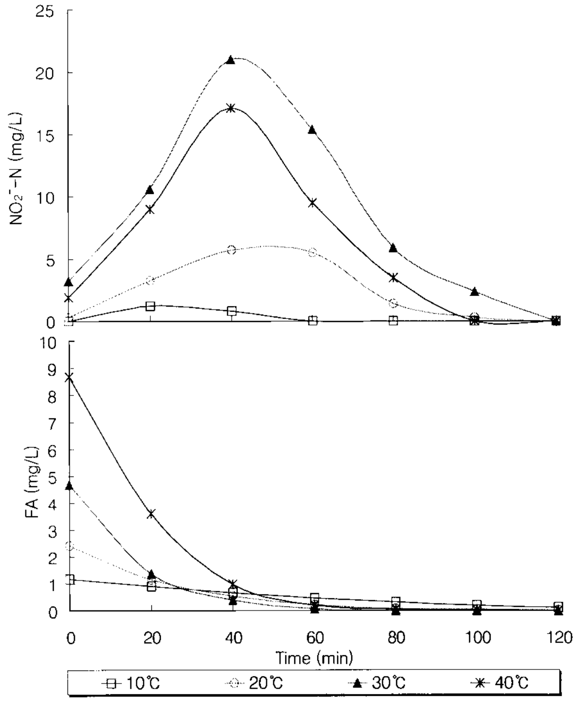

Figure 8. Effect of temperature and FA concentration upon nitrite accumulation.

In all experiments, nitrite was accumulated more or less. The maximum net accumulation of NO_2^--N in each test, which was measured by subtracting the initial NO_2^- concentration from the peak concentration, ranged from 1.2 mg/l to 22.4 mg/l. The k_a/k_n was not the necessary condition of nitrite accumulation, although the degree of accumulation was insignificant when the ratio k_a/k_n was 0.32. In most cases, nitrite accumulated during the first 40 min. Two conditions were favorable for nitrite accumulation in that

Table 2. Summary of the experimental results

		Initial FA (mg/l)	k_a (10^{-3} mg-N/mg-VSS·h)	k_a/k_n *	Maximum net nitrite accumulation (mg-N/l)	Nitrite accumulatino rate (%)**
pH	7	0.5	14.7	1.96	2.6	6.7
	8	*4.8*	*24.5*	*0.96*	*22.4*	*60.2*
	9	27.2	25.9	0.76	17.8	45.4
	10	58.5	7.3	4.56	6.9	50.9
DO (mg/L)	0.5	4.6	11.2	1.02	12.5	76.7
	1.5	*4.5*	*17.3*	*0.91*	*20.5*	*77.4*
	2.5	4.8	21.3	0.68	17.5	54.2
Temp. (°C)	10	1.2	6.2	0.32	1.2	9.1
	20	2.4	14.7	0.64	5.4	25.4
	30	*4.7*	*23.0*	*0.78*	*17.7*	*53.0*
	40	8.7	20.6	0.52	15.2	49.7

*k_n was measured without FA inhibition.

**Percentage of nitrite accumulated to ammonia removed at reaction time 40 min.

period. The concentration of ammonia was higher than that of nitrite, and the FA concentration was relatively high. A nitrite accumulation over 15 mg-N/l (approximately 30% of the nitrogen in the feed) was observed when the initial FA concentration was above approximately 4 mg/l, and the maximum specific oxidation rate of ammonia, k_a, was above 15 × 10^{-3} mg-N/mg-VSS·h. (The k_a would have been around 190 × 10^{-3} mg-N/mg-VSS·h, or 4.5 mg-N/mg-VSSd if the cells were solely nitrifiers). Those runs that satisfied the two conditions were highlighted with shade in Table 2. It should be noted that the critical concentration of FA of 4 mg/l was found from batch experiments in which cells had not been allowed sufficient time to acclimatize to FA. Since microorganisms can adapt to FA in certain degree, the criteria value of FA can be altered with cells' acclimatization, particularly in a continuous-flow reactor. The nitrite accumulation at pH 7 or at 20 °C was small because the initial FA concentration was far below 5 mg/l, although the k_a value was near 15 × 10^{-3} h^{-1}. Also, the nitrite accumulation was small at pH 10 due to the low k_a value, in spite of its high initial FA concentration. When the above two conditions were met, then, the higher in the ratio of k_a/k_n, the more in the nitrite accumulation. The optimum operation condition in each set of experiment was underlined in the table, which were pH = 8, DO = 1.5 mg/l, and T = 30 °C. When all of the optimum conditions were met (The run of DO = 1.5 mg/l was the case), the nitrite accumulation rate reached 77%. It will be recognized that the data sets for DO = 32.5 mg/l and T=30 °C had identical experimental conditions and gave very similar results each other, demonstrating that our experiments had a reproduciblility.

Conclusions

The accumulation of nitrite was controlled by the production rate of nitrite and the inhibition of nitrite oxidation. The pH, temperature and DO concentration affected the production rate of nitrite or k_a. The FA, of which concentration was affected by the total ammonia concentration, pH and temperature, inhibited the nitrite oxidation at a relatively high concentration. Simultaneously high FA concentration and k_a value were essential for a significant nitrite accumulation in a batch reactor. The critical value for initial FA was approximately 4 mg/l. The minimum required k_a value was about 15 × 10^{-3} mg-N/mg-VSS·h. When the two conditions were met, then the highest accumulation was observed at the highest k_a/k_n ratio. The optimum operational criteria that could meet the above conditions were pH = 8, DO = 1.5 mg/l and T = 30 °C when the initial ammonia concentration was 50 mg-N/l, at which as much as 77% of the removed ammonia accumulated in nitrite.

Acknowledgments

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