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Effects of Free Ammonia and Dissolved Oxygen on Nitrification and Nitrite Accumulation in a Biofilm Airlift Reactor

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Abstract–The purpose of this research is to find out the effects of free ammonia concentration and dissolved oxygen on nitrification and nitrite accumulation in a biofilm airlift reactor. Free ammonia seriously inhibited the activity of nitrite oxidizers at the concentration higher than 0.1 mg NH_3-N/L and it was very effective for nitrite accumulation. Dissolved oxygen limitation in the biofilm also caused nitrite accumulation. Long term inhibition decreased the growth rate for nitrite oxidizers, and ammonia oxidizers were the dominant nitrifiers in the wastewater nitrification. Selective accumulation of ammonia oxidizers in the biofilm could be another reason for nitrite accumulation. Free ammonia inhibited nitrite oxidizers immediately, and adaptation to free ammonia was not observed. Therefore, the optimum control of free ammonia and dissolved oxygen concentration is critical for nitrite accumulation and the strategy can be used for selective accumulation of ammonia oxidizers in a bioreactor system.

Key words: Biofilm, Free Ammonia, Dissolved Oxygen, Nitrification, Nitrite Accumulation

INTRODUCTION

Microbial nitrification, the sequential oxidation of ammonium (NH_4^+) *via* nitrite (NO_2^-) to nitrate (NO_3^-), followed by denitrification under anoxic condition is the key process in the removal of ammonium from wastewater. In an integral nitrogen removal system, it is beneficial if ammonium is only oxidized to nitrite rather than nitrate and thereafter denitrified for four reasons: 1) an approximately 25% lower consumption of oxygen in the nitrification stage, 2) an approximately 40% lower electron donor requirement in the denitrification stage, 3) higher denitrification rate, and 4) lower sludge generation [Abelling et al., 1992; Garrido et al., 1997; Kuai et al., 1998].

Nitrite accumulation can be obtained by disequilibrating the numbers or activities between the ammonia oxidizers and nitrite oxidizers. The disequilibrium in numbers occurs when ammonia oxidizers prevail nitrite oxidizers in mixed culture nitrification system. Differences of the oxidation activities can be mainly imposed, for example, by an inhibition of the activity of the nitrite oxidizers by the presence of free ammonia, dissolved oxygen limitation in the nitrifying bacteria [Goreau et al., 1980; Hellinga et al., 1998; Joo et al., 2000; Kim et al., 2003; Kuai et al., 1998; Nam et al., 2004; Turk and Mavinic, 1989a, b; Villaverde et al., 1997; Wolfe et al., 1990]. Selective inhibition of free ammonia (NH_3) on nitrite oxidizers usually occurs at 0.1 to 10 mg NH_3-N/L [Randall and Buth, 1984], while ammonia oxidizers are inhibited at 10-150 mg N/L [Anthonisen et al., 1976]. Nitrous acid (HNO_2) is also known to inhibit nitrite oxidizers at 0.22-2.8 mg N/L [Anthonisen et al., 1976].

Low dissolved oxygen also limits nitrite oxidation since the oxygen saturation coefficients (K_o) of ammonia oxidation and nitrite oxidation are known to be 0.3 and 1.1 mg/L, respectively. Nitrite was known to accumulate most highly at 1.5 mg/L of dissolved oxygen in biofilm reactor [Antoniou et al., 1990; Garrido et al., 1997]. The above studies observed those effects separately on nitrite accumulation.

The purpose of this study is to determine the combined effect of both the free ammonia and oxygen limitation on nitrite accumulation. Hydraulic retention time (HRT) of the wastewater and ammonia load was adjusted to obtain different free ammonia concentrations in the reactor, and the biofilm system was used to achieve dissolved oxygen limitation due to oxygen transfer resistance within the biofilm. Both freshly developing biofilm and fully saturated biofilm were compared for this purpose.

MATERIALS AND METHODS

For biofilm airlift reactors two (reactors A and B) laboratory scale concentric tubes equipped with a three-phase separator were used. Their height, riser diameter and down comer diameter are described in Table 1 and a schematic experimental set-up is shown in Fig. 1. The pH was controlled at 7.5 by the supply of 1 M $NaHCO_3$. The temperature of the reactors was maintained at 20-28 °C. Basalt with a mean diameter of 200 μm was the carrier material for biofilm for-

Table 1. Geometry of the applied biofilm airlift reactor

	Reactor A	Reactor B
Total reactor volume (L)	5	3.3
Total reactor height (cm)	77	102
Internal separator diameter (cm)	4	4
External separator diameter (cm)	18	10
Riser diameter (cm)	4	4
Downcomer diameter (cm)	7	7
Riser to bottom distance (cm)	1	1

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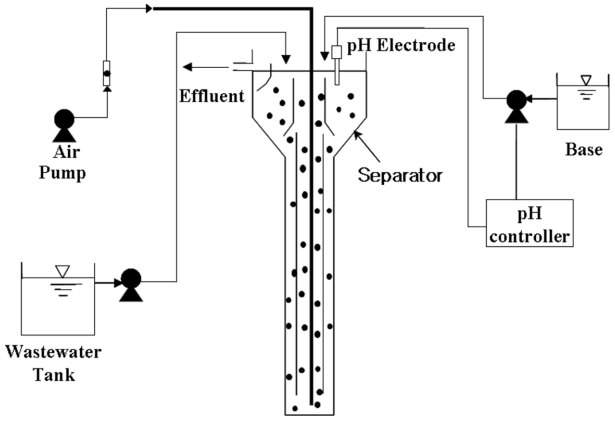

Fig. 1. Schematic of the nitrification experimental set-up of biofilm airlift reactor.

mation. The initial carrier concentrations in the reactors A and B were 16 and 23 g/L, respectively. The reactors were inoculated with the sludges from a nitrifying wastewater treatment plant. The reactor A was aerated at 4 L/min, and the reactor B was aerated at 1 and 2.5 L/min at low load (0.15 kg/m^3·d) and high load (1.9 kg/m^3·d), respectively. Other operating conditions of reactors A and B are summarized in Tables 2 and 3. The artificial wastewater composition is shown in Table 4. The analytical methods were based on the Standard Methods [APHA, 1992]. NH_4-N was measured by the Nesslerization method by reading absorbance at 425 μm by UV-Visible spectrophotometer (UV 1601, Shimadzu). Both NO_2-N and NO_3-N were measured by ion chromatograph (DX 500, Dionex). The

Table 2. Experimental conditions of biofilm airlift reactor A

Phase	Operation (days)	HRT (h)	NH_4-N (mg/L)	Load (kg/m^3·d)
(a)	1-31	10.2	450	1.6
(b)	32-74		280	
(c)	75-145	6.0	391	2.3
(d)	146-160		243	1.5
(e)	161-185	12.2	225	0.7

Table 3. Experimental conditions of biofilm airlift reactor B at low (0.15 kg/m^3·d) and high (1.9 kg/m^3·d) NH_4-N load

	Phase	Operation (days)	HRT (h)	NH_4-N (mg/L)	Nitrification (%)
Low load	(a)	1-56	16.5	103	92
	(b)	57-76	11	69	99
	(c)	77-119	8.2	52	97
	(d)	120-133	6.6	41	100
	(e)	134-148	4.6	29	100
High load	(a')	149-213	13.2	1,045	91
	(b')	214-276	8.3	653	92
	(c')	277-291	5.5	435	87
	(d')	292-312	4.1	326	81
	(e')	313-329	2.8	218	80

Table 4. Composition of the synthetic wastewater used for nitrification

Component	Concentration	Component	Concentration
$CaCl_2·2H_2O$	7 mg/L	$NaHPO_4·12H_2O$	29 mg/L
$FeCl_3·6H_2O$	1 mg/L	$NaHCO_3$ (as$CaCO_3$)	3.57 g/g NH_4-N
KCl	7 mg/L	$(NH_4)_2SO_4$	29-1,045 mg-N/L
KH_2PO_4	11 mg/L	pH controller	1 M $NaHCO_3$
$MgSO_4·H_2O$	5 mg/L	- buffer soln.	

degree of nitrite accumulation was expressed by the fraction of nitrite among nitrite and nitrate (NO_2-N/(NO_2-N+NO_3-N)). Biofilm thickness was examined by microscopic measurement. Saturated biofilm thickness was between 100 and 150 μm. Other detailed experimental methods can be found elsewhere [Kim et al., 2003].

RESULTS AND DISCUSSION

1. Nitrification and Nitrite Accumulation in a Freshly Developing Nitrification Biofilm Reactor

Reactor A was operated at five different consecutive steps. From period (a) to (e), the ammonium loads to the reactor A were varied

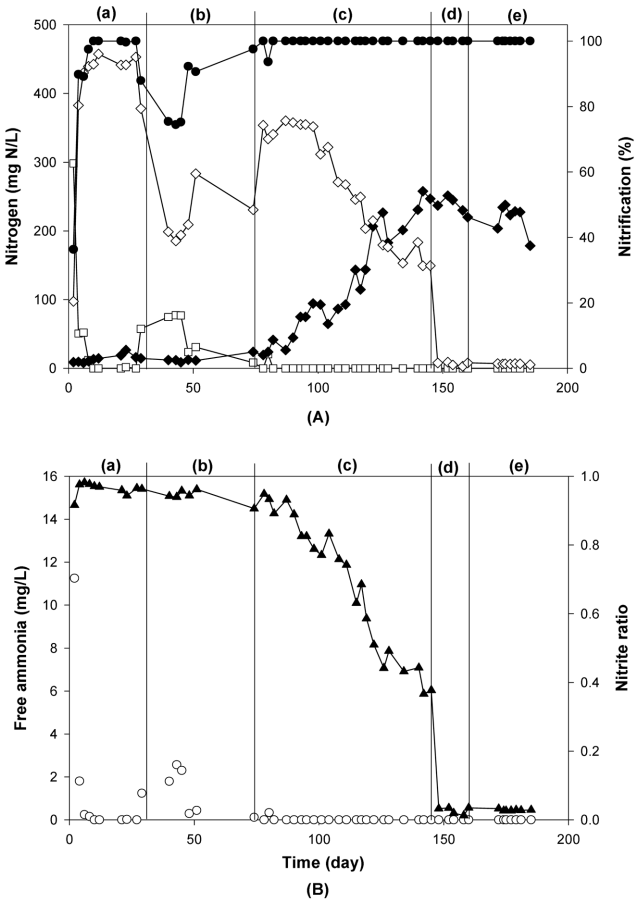

Fig. 2. Time dependence of nitrification and nitrite accumulation in the biofilm airlift reactor A (□: NH_4-N; ◇: NO_2-N; ◆: NO_3-N; ●: nitrification (%); ▲: nitrite ratio; ○: free ammonia).

1.6 (a and b), 2.3 (c), 1.5 (d) and 0.7 (e) kg/m^3·d. Fig. 2(A) shows the effluent concentrations of nitrogen compounds and nitrification efficiency of reactor A. Fig. 2(B) shows free ammonia concentration and nitrite ratio of the effluent.

In period (a), the reactor was operated at 1.6 kg NH_4-N/m^3·d and at an HRT of 10.2 hours for 31 days. Most of the ammonium was converted to nitrite after 10 days. As the HRT was reduced to 6 hours in period (b), nitrification efficiency decreased to 70% initially but it was recovered to higher than 95% in 2 weeks. Interestingly, only a very low nitrate concentration was found in the periods of (a) and (b). During the periods (a) and (b) free ammonia (NH_3-N) was maintained at less than 1.0 mg/L, and the average dissolved oxygen concentrations were 5.9 and 3.7 mg/L, respectively. In addition, the nitrite ratio was kept at 0.9-1.0. From the results, nitrite oxidation activity was supposed to be very low even though most of the ammonium was oxidized to nitrite.

In period (c) ammonium load was increased to 2.3 kg/m^3·d while HRT was kept constant at 6 hours as in (b). Despite the increased load, ammonium was completely oxidized so that free ammonia concentration decreased nearly 0 for 70 days at an average dissolved oxygen of 5.2 mg/L. On the other hand, the activity of nitrite oxidizers was slowly increased for 70 days from the fact of increased nitrate level in this period. In terms of nitrite ratio it decreased from 0.9 to 0.36.

In period (d) nitrite ratio decreased drastically to 0 as the ammonium load decreased from 2.3 to 1.5 kg/m^3·d. When comparing the nitrite oxidation rates at period (c) and (d), they have almost the same value of 1.47 and 1.46 kg NO_2-N/m^3·d, respectively. Therefore, the sudden decrease of nitrite ratio in (d) was due to the fact that just appropriate amount of nitrite was supplied by oxidation of ammonia which can be further oxidized to nitrate by nitrite oxidizers. Nitrite ratio in period (e) was 0 and it can also be explained by the insufficient supply of nitrite.

The phenomena of nitrite accumulation in periods (a)-(b) and slow recovery of nitrite oxidizers activity for 70 days in period (c) were very interesting. The cause of these phenomena can be analogized in four possible ways.

First, free ammonia might inhibit nitrite oxidizers to cause lag time to their activity. Yoo et al. [1999] showed that nitrite oxidizers had lower activity than that of control when nitrite oxidizers were exposed to free ammonia. And it needed a lag time to recover nitrite oxidation activity after the removal of inhibition by free ammonia. In addition, the lag time effect of nitrite oxidizers was proportional to the free ammonia concentration and exposure time [Yoon, 2002]. However, it is speculated that the slow recovery of nitrite oxidizers activity in period (c) of Fig. 2(B) did not follow this mechanism because batch inhibition results showed that the lag time effect lasted less than several hours.

Secondly, nitrous acid (HNO_2) has been known to inhibit nitrite oxidizers from the concentration as low as 0.2 mg N/L [Anthonisen et al., 1976]. In period (c) the average HNO_2 concentration was calculated to be 0.03 mg N/L, which is one order of magnitude lower than the inhibition level. Therefore, it can be assumed that HNO_2 had little effect on nitrite accumulation.

Thirdly, low dissolved oxygen could be a cause for nitrite accumulation due to higher Ko values of nitrite oxidizers than that of ammonia oxidizers [Hanaki et al., 1990; Laudelout, 1974; Loveless and Painter, 1968; Peeters et al., 1969]. In periods (a) and (b), however, nitrite ratio was close to 1 even though the dissolved oxygen was maintained at least three times higher than the K_o (1.1 mg/L) of nitrite oxidizers. From the results we can assume that factors other than low dissolved oxygen were responsible for the nitrite accumulation. On the other hand, it could be thought that the dissolved oxygen concentration in the biofilm might be lower than the K_o value due to mass transfer resistance in the biofilm [Picioreanu et al., 1997]. However, recent results from Vogelsang et al. [2002] showed that biofilm thickness had increased more than 80 days during nitrification, and it meant increasing oxygen limitation in the biofilm as time goes. If low dissolved oxygen in the biofilm caused nitrite accumulation, nitrite ratio should have increased from low value to high value during the experiment but the result did not coincide with the hypothesis. In addition, Garrido et al. [1997] obtained a nitrite ratio of 0.5 at 1.5 mg/L of dissolved oxygen as the maximum nitrite accumulation in biofilm, but we could achieve nitrite ratio of about 1 at much higher dissolved oxygen concentration. Nogueira et al. [2002] also reported that no nitrite was observed at dissolved oxygen level between 2.0 and 5.0 mg/L under similar nitrification condition. Furthermore, slow recovery of nitrite oxidizers activity in period (c), which takes about 70 days, cannot be explained by low dissolved oxygen hypothesis because oxygen transfer rate in the biofilm is a very fast phenomenon which take only a few minutes. From the above results and examples from former studies we can rule out low dissolved oxygen from the main factors for nitrite accumulation in this study.

Fourth, the relatively lower number of nitrite oxidizers than that of ammonia oxidizers in the reactor could be a cause for nitrite accumulation. Long term selective inhibition on nitrite oxidizers could lower its specific growth rate and population number in the biofilm. In periods (a) and (b) the amount of produced nitrate was negligible when considering the oxidized ammonium. Considering that nitrite oxidizers are obligatory autotrophic bacteria which obtain all the necessary energy for biomass synthesis and maintenance from the oxidation of nitrite to nitrate, and considering the low yield of nitrite oxidizers [Beccari et al., 1979; Wiesmann, 1994] and little oxidation of nitrite, very few nitrite oxidizers are present at the nitrifying biofilm in periods (a) and (b). The relatively low number of nitrite oxidizers than ammonia oxidizers can accumulate nitrite in periods (a) and (b). In the absence of free ammonia inhibition on nitrite oxidizers, nitrite was further oxidized to nitrate to provide energy for the synthesis of nitrite oxidizers. The slow recovery of nitrite oxidizer activity in terms of nitrite ratio in period (c) could be explained by the increasing number of nitrite oxidizers under no inhibition condition by lowering ammonium load.

The above results showed the possibility of selective growth of ammonia oxidizers during wastewater nitrification under the presence of appropriate free ammonia inhibition. Nitrification sludge enriched with ammonia oxidizers selectively oxidizes ammonia to nitrite without further oxidation to nitrate, and it can make the nitrogen removal *via* nitrite pathway more feasible to achieve.

2. Nitrification and Nitrite Accumulation in a Nitrification Biofilm Reactor with Balanced Ammonia Oxidizers and Nitrite Oxidizers

In the second part of this experiment the possibility of nitrite accumulation was investigated when nitrite oxidizers were fully grown

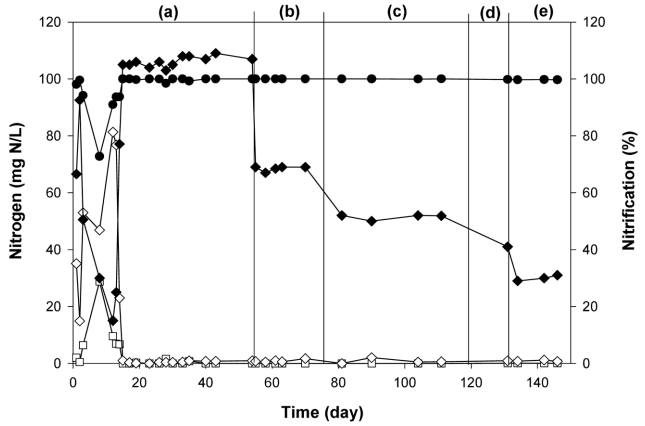

Fig. 3. Time dependence of nitrification in the biofilm airlift reactor B at low ammonia load (0.15 kg/m^3·d) (□: NH_4^+-N; ◇: NO_2^--N; ◆: NO_3^--N; ●: nitrification (%)).

in equilibrium with ammonia oxidizers. For this purpose the biofilm airlift reactor B was operated at low ammonium load (0.15 kg/m^3·d) to obtain complete nitrification from ammonium to nitrate for 5 months. After the balanced growth of ammonia oxidizers and nitrite oxidizers, ammonium load was increased to 1.9 kg/m^3·d to examine the effect of free ammonia on nitrite accumulation (Table 3).

Fig. 3 shows the nitrogen compounds in the effluent and nitrification efficiency under the low load operation. Nitrification efficiency reached almost 100% during the operation except for the initial 15 days as a start-up period. HRT reduction did not give any adverse effect on nitrification efficiency and nitrite accumulation during the period. The average dissolved oxygen concentration was about 7 mg/L and free ammonia concentration was maintained almost 0 except for the start-up period.

In many biofilm systems, oxygen (electron acceptor), other than nutrient substrates (electron donor), has been regarded as the limiting factor in the aerobic process because of its low solubility and mass transfer resistance in the biofilm. In this low load experiment, however, overall nitrification rate was governed by ammonium because ammonium concentration was maintained nearly 0 in the reactor. Therefore, both ammonia oxidizers and nitrite oxidizers could grow in balance without oxygen limitation and free ammonia inhibition to reach equilibrium in their activities and population numbers.

Fig. 4(A) and (B) show the effluent nitrogen concentrations, nitrification efficiency, free ammonia and nitrite ratio with decreasing HRT as ammonium load was increased to 1.9 kg/m^3·d in reactor B. In the periods (a′)-(e′) HRTs were 13.2, 8.3, 5.5, 4.1, 2.8 h, respectively, and the influent ammonium concentrations were 1045, 653, 435, 326, 218 mg/L, respectively, to keep the ammonium load constant as shown in Table 3. In contrast to low load condition, nitrification efficiency was decreased and nitrite was accumulated as HRT decreased. Detailed nitrification results of each periods are given as follows.

Nitrite ratio was maintained 1 throughout the period (a′) even though free ammonia concentration decreased to 0 after 200 days due to complete nitrification. In period (b′) as both the HRT and influent ammonium concentration decreased to 8 h and 653 mg/L, average nitrite ratio decreased to 0.82 with average nitrification effi-

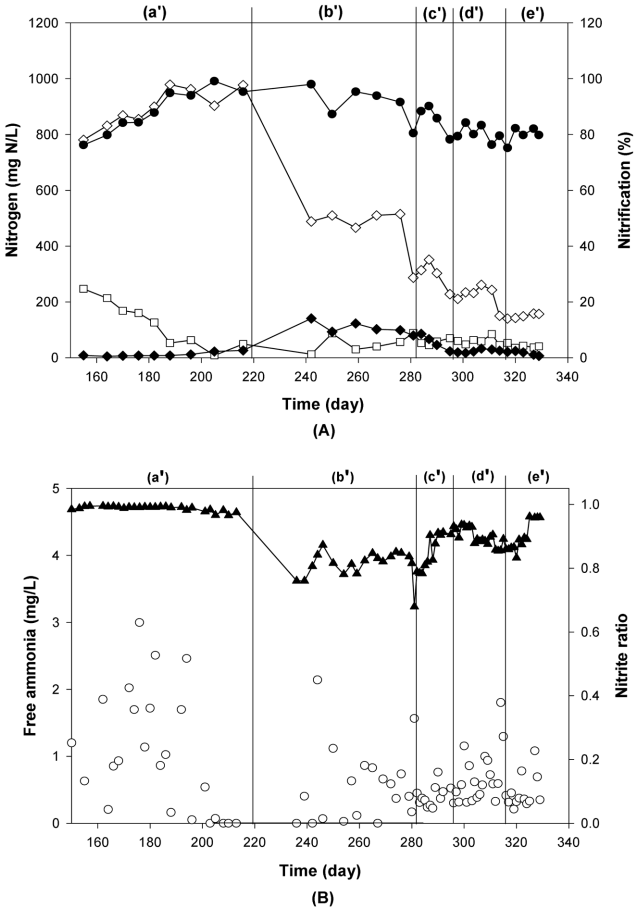

Fig. 4. Time dependence of nitrification in the biofilm airlift reactor B at high ammonia load (1.9 kg/m^3·d) (□: NH_4^+-N; ◇: NO_2^--N; ◆: NO_3^--N; ●: nitrification (%); ▲: nitrite ratio; ○: free ammonia).

ciency, average free ammonia and dissolved oxygen about 95%, 0.66 and 3 mg/L, respectively. In periods (c′), (d′) and (e′) average nitrite ratios were 0.81, 0.91, 0.89, respectively, with free ammonia concentrations of 0.47, 0.58, 0.59 mg/L, respectively. Free ammonia seems to be the main cause of nitrite accumulation in this high ammonium load condition, but the contribution by oxygen limitation in the biofilm might not be excluded. Relatively high concentration of ammonium than oxygen caused oxygen limitation and it can further deteriorate nitrite oxidizer activity. In consequence, nitrifying biofilm has an advantage over suspension system to accumulate nitrite by natural oxygen gradient formed in the biofilm in the presence of ammonium.

From the above results, nitrite can be highly accumulated by applying high ammonium load, and we can also conclude that nitrite accumulation was obtained by the following pathway. Long term inhibition of free ammonia and oxygen limitation decreased nitrite oxidizer activity and their growth rate, but not ammonia oxidizers to have unbalanced overwhelming growth of ammonia oxidizers in the nitrifying system. Nitrite oxidizers cannot oxidize enough nitrite which is provided by ammonia oxidizers even though their inhibition and limiting factors do not exist any more because of their limited population. Nitrite oxidizers grow slowly due to their slow nitrite

oxidation and low yield and that is why it took more than 70 days to have sufficient nitrite oxidizer population without nitrite accumulation as shown in Fig. 2. In addition, nitrite oxidizer adaptation to free ammonia could not be observed in this experiment during 330 days of operation.

CONCLUSION

In a biofilm airlift reactor wastewater nitrification under low ammonium load and high dissolved oxygen condition did not accumulate nitrite. At high ammonium load condition, residual free ammonia effectively inhibited nitrite oxidizers to have nitrite ratio higher than 0.8 whether the nitrite oxidizers were fully developed or not. Dissolved oxygen concentration (2-5 mg/L) did not show strong effect on nitrite accumulation. Therefore, free ammonia has more direct and strong inhibition effect on nitrite oxidizers than oxygen limitation.

Ammonia oxidizers became dominant nitrifiers by long term wastewater nitrification under the condition of selective inhibition on nitrite oxidizers. In this case nitrite could be accumulated even though there is no inhibition or limiting factors to nitrite oxidizers because of smaller number of nitrite oxidizers than that of ammonia oxidizers. Long term wastewater nitrification for 330 days showed that nitrite oxidizers did not show adaptation to free ammonia and nitrite could be accumulated by selective inhibition on nitrite oxidizers even though nitrite oxidizers were fully developed and in equilibrium with ammonia oxidizers.

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