

Increased hydrazine during partial nitrification process in upflow air-lift reactor fed with supernatant of anaerobic digester effluent

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Abstract—The optimal balance of ammonium and nitrite is essential for successful operation of the subsequent anammox process. We conducted a partial nitrification experiment using an upflow air-lift reactor to provide operational parameters for achieving the optimal ratio of ammonium to nitrite, by feeding supernatant of anaerobic digester effluent, high-nitrogen containing rejection water. Semi-continuous operation results show that HRT should be set between 15 and 17 hours to achieve the optimum ration of 1.3 of $\text{NO}_2\text{-N}/\text{NH}_4\text{-N}$. In the UAR, nitrification was the dominant reaction due to high concentration of ammonia and low biodegradable organics. The influent contained low concentrations of hydroxylamine and hydrazine. However, hydrazine increased during partial nitrification by ~60-130% although there was no potential anammox activity in the reactor. The partial nitrification process successfully provided the ratio of nitrogen species for the anammox reaction, and relived the nitrite restraint on the anammox activity by increasing hydrazine concentration.

Key words: Hydrazine, Hydroxylamine, Nitrite, Partial Nitrification, Anammox

INTRODUCTION

The anaerobic ammonium oxidation, anammox, has been recognized as a promising alternative lithoautotrophic process for nitrogen removal from wastewaters [1]. It has been applied to wastewater of high nitrogen concentration and low biodegradable organics such as rejection water in wastewater treatment plants [2-4].

Nitrite accumulation by partial nitrification is an essential step toward the anammox process. An optimal balance of the electron donor (ammonium) and the electron acceptor (nitrite) should be provided for a successful anammox reaction. The golden molar ratio of ammonium to nitrite was assumed to be 1 : 1 [5], and this was a theoretical postulation before realizing anammox. 1.3 nitrite/ammonium ratio for anammox reaction was used due to the nitrate production during anammox [6]. To summarize all relevant studies, this ratio varies 1 : 0.5-4 depending on substrates, operating conditions, and reactor configuration [7]. Much research was conducted to accumulate nitrite in the presence of free ammonia and dissolved oxygen limitation [8-11]; however, high nitrite concentration was demonstrated to halt the anammox process [12].

For successful anammox process, partial nitrification was performed to provide optimum operational parameters for the optimal ratio of ammonium to nitrite of 1 : 1.3. An upflow air-lift reactor (UAR) was used to create a similar operating condition to complete stirred tank reactor. Supernatant of anaerobic digester effluent was injected into the UAR to enrich nitrifying community because it has high ammonium concentration and low concentration of biodegradable

carbon. We provided optimum operational parameters for partial nitrification in the UAR fed supernatant of anaerobic digester effluent. Intracellular anammox intermediates were reported to restart anammox activity, which had been paused due to nitrite accumulation in the previous anammox enrichment study [12]. However, intracellular anammox intermediates have not been found, either in anammox reactors or in partial nitrification reactors. No studies have reported the increased hydrazine in partial nitrification. Here, we report that hydrazine and hydroxylamine, anammox intermediates, were found without anammox activity and hydrazine increased during the partial nitrification process. The increased hydrazine in UAR will provide better performance for successful anammox operation and design.

MATERIALS AND METHODS

1. Reactor Construction and Operation

An upflow air-lift reactor (UAR, 6 cm of internal diameter and 1 L of total effective liquid volume) was made with Pyrex glass (Fig. 1). A ceramic porous air diffuser was installed in the bottom of the reactor (Kukje Hydraulic Co. Ltd., Hwasung, South Korea), and it equalized the air supply to the UAR throughout the experiment. An inlet for substrate feeding was installed at 1 cm above from the air diffuser. Nitrifying activated sludge (2.3 g VSS/L and 75% VSS/TSS) used for inoculum was collected from a municipal wastewater treatment plant (Gyungsan, South Korea). This plant was operated as an anaerobic/anoxic/oxic process for simultaneous nitrogen and phosphorus removal. Anaerobic digester effluent in the plant was settled for about 2 hours, and its supernatant was used as an influent. Characteristics of the influent were measured and monitored peri-

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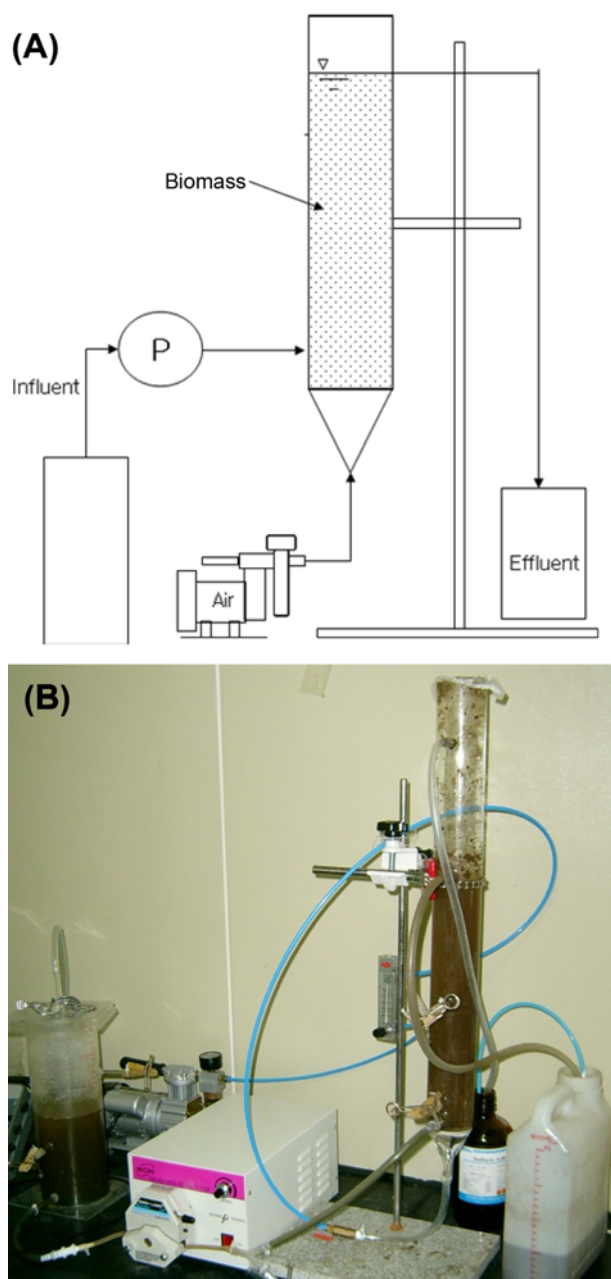


Fig. 1. Schematic (A) and picture (B) of the upflow air-lift reactor (UAR). The UAR has 6 cm of internal diameter and 1 L of total effective liquid volume.

odically to maintain a similar influent quality throughout the experiment.

The reactor was operated for about 250 days (Fig. 2) in batch mode at phase I and the last day of phase IV, and in semi-continuous mode in phases II, III and IV (Table 1). HRT was reduced as operation continued from 48 to 15 hours by controlling feeding frequency and amount (Table 1). Phases were divided by differences in feed flux and daily nitrogen loading. The reactor was operated at a temperature controlled room (30 °C).

2. Analysis

Water qualities of influent and effluent were analyzed by using protocols in Standard Methods for Examination of Water & Waste-

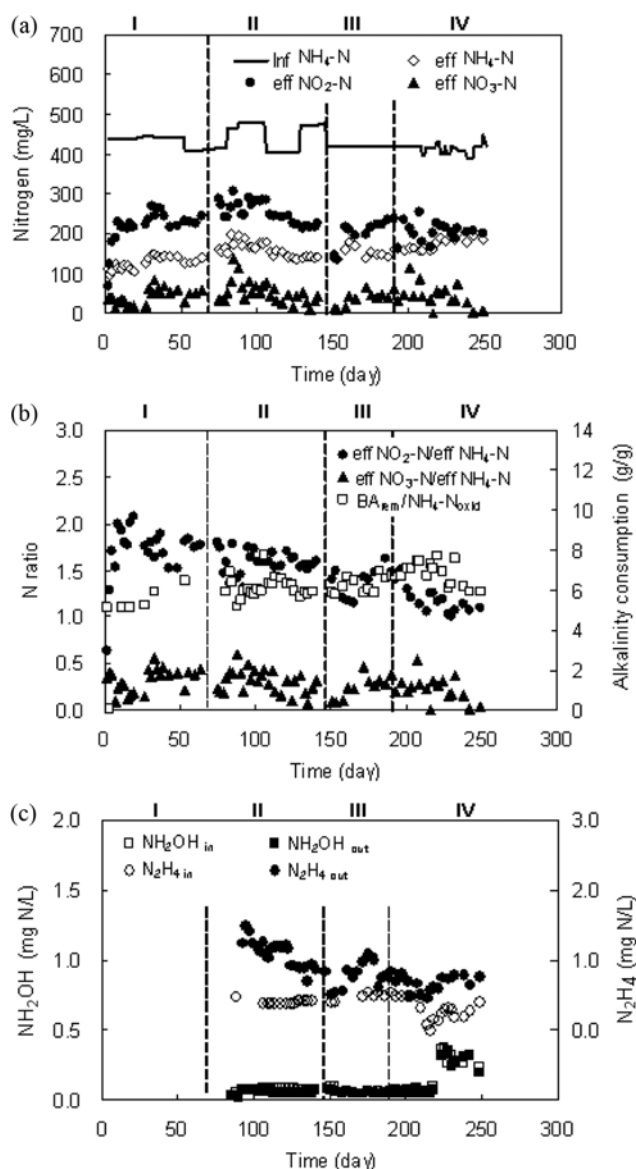


Fig. 2. Nitrogen species and bicarbonate consumption profiles in upflow air-lift reactor: (a) $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$, (b) $\text{NO}_2\text{-N}/\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}/\text{NH}_4\text{-N}$ and $\text{BA}/\text{NH}_4\text{-N}$ and (c) N_2H_4 and NH_2OH . Influent (Inf), effluent (Eff), bicarbonate alkalinity (BA), and removal (rem).

water [13]. pH was measured using pH/mV meter (Orion Research 720A), and bicarbonate and total alkalinity was measured by titration methods of Kapp [14]. COD, ammonia, nitrite, nitrate, hydrazine (N_2H_4), and hydroxylamine (NH_2OH) were quantified colorimetrically with a spectrophotometer (Spectronic 21D, Milton Roy or Hach Odyssey DR2500). Concentrations of hydrazine or hydroxylamine in cell-free suspension were measured using spectrophotometric methods as previously described [15–17]. At each phase, operation was considered to reach a steady state when variations of water quality parameters in the effluent were less than 10% (Fig. 2).

3. Calculation

Efficiency of nitrification and nitrification was calculated by using the following equations: nitrification efficiency (%) = $(\text{NO}_2\text{-N}_{\text{eff}})/(\text{NH}_4\text{-N}_{\text{in}}) \cdot 100$; Nitrification efficiency (%) = $(\text{NO}_3\text{-N}_{\text{eff}})/(\text{NH}_4\text{-N}_{\text{in}}) \cdot 100$. Max-

Table 1. Operating conditions of the upflow air-lift reactor for partial nitrification. Phases were divided by hydraulic retention time (HRT)

Phase	I ^a	II	III	IV
HRT (hour)	48	20	17	15
Q ^b (mL/d)	500	1200	1400	1600
Feeding amount at a time (mL)	500.0	75.0	87.5	100.0
Feeding frequency (1/d)	1	16	16	16
Feeding interval (hr)	24	1.5	1.5	1.5
NH ₄ -N (mg N/L)	423±16	432±34	420±13	432±25
Daily N load (mg N/L-d)	213±8	518±41	609±38	691±39
Aeration (mL/min)	100	200	200	200
DO (mg/L)	4-7	4-7	4-7	3-4

^aBatch operation^bFlow rate (Q) of influent and effluent

imum ammonia oxidation rate (AOR) was calculated on liquid-volumetric basis (AOR_L) or cell-mass basis (AOR_M) as follows: AOR_L (mgNH₄-N/L·hr)=(NH₄-N_{in}-NH₄-N_{eff})/(time), AOR_M (mgNH₄-N/gVSS·hr)=(NH₄-N_{in}-NH₄-N_{eff})/(time)/(VSS). Since microorganisms utilize only bicarbonate (HCO₃⁻) among all forms of alkalinity for

their cell synthesis, bicarbonate alkalinity (BA) is used as a numerator instead of total alkalinity to estimate alkalinity consumption for biological ammonia oxidation. Bicarbonate consumption per ammonia consumption was calculated as, BA/NH₄-N (mgCaCO₃/mgNH₄-N)=(BA_{in}-BA_{eff})/(NH₄-N_{in}-NH₄-N_{eff})·100.

Concentrations of nitrogen species and bicarbonate during the operation of the UAR are profiled in Fig. 2, and all the average values during each steady state in phase II, III and IV are summarized in Table 2.

RESULTS AND DISCUSSION

1. Characteristics of Influent

The supernatant of anaerobic digester effluent has the following characteristics (n=10): pH 7.33±0.26, TCOD 1,762±624 mg/L, SCOD 131±59 mg/L, TS 1,657±335 mg/L, VS 783±100 mg/L, TSS 368±68 mg/L, VSS 290±57 mg/L, TKN 490±13 mg/L, NH₄-N 438±26 mg/L, alkalinity 1,694±80 mg/L, and alkalinity/NH₄-N 3.89±0.26 mg/L. Because biodegradable carbon was used for methanogenesis and negligible nitrogen conversion occurred during the anaerobic digestion, the influent had low VSS and SCOD, high TS and TCOD, and high TKN and NH₄-N. Because alkalinity also increases during anaerobic digestion, the influent had enough alkalinity

Table 2. Influent and effluent quality of the upflow air-lift reactor. Analysis was performed according to standard methods (13)

(n=3)

Phase	Steady state period (day)	HRT (hr)	Influent						
			TIN ^a	NH ₄ -N	HA ^b	HZ ^c	pH	BA ^d	
			(mg/L·D)	(mg/L)	(mg/L)	(mg/L)			
II	120-140	20	518±41	432±34	0.08±0.01	0.41±0.02	7.4±0.1	1653±22	
III	175-190	17	609±38	420±13	0.05±0.01	0.52±0.03	7.8±0.1	1742±117	
IV	216-250	15	663±17	415±10	0.26±0.10	0.43±0.10	7.8±0.2	1640±142	
Phase	HRT (hr)	Effluent							
		NO ₂ -N	NO ₃ -N	NH ₄ -N	HA ^b	HZ ^c	pH	BA ^d	
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)			
II	20	223±9	33±15	140±3	0.05±0.01	0.95±0.16	6.4±0.2	25±16	
III	17	227±8	47±9	152±6	0.05±0.02	0.86±0.29	6.4±0.1	15±24	
IV	15	208±10	24±25	185±11	0.23±0.11	0.70±0.09	6.5±0.1	110±59	
Phase	Effluent								
	DO	TSS	VSS	mg BA ^e	Nitritation	Nitrification	mgNO ₂ -N	AOR _L	AOR _M
	(mg/L)	(mg/L)	(mg/L)	/mgNH ₄ -N	(%) ^f	(%) ^g	/mgNH ₄ -N	(mgN/L-hr) ^h	(mgN/gVSS-hr) ⁱ
II	6.33±0.16	216±14	159±8	5.74±0.24	51.6±2.5	7.6±5	1.59±0.06	14.6±1.8	91.8±5.2
III	6.45±0.05	276±26	200±18	6.44±0.48	54.0±2.2	11.2±1.5	1.50±0.08	15.8±0.3	79.0±3.8
IV	6.53±0.17	260±32	227±21	6.65±0.74	50.1±3.8	5.8±5.1	1.13±0.09	15.3±1.3	71.0±7.5

^aTIN: total inorganic nitrogen^bHA: hydroxylamine^cHZ: hydrazine^dBA: bicarbonate alkalinity^eBA/NH₄-N (mgCaCO₃/mgNH₄-N)=(BA_{in}-BA_{eff})/(NH₄-N_{in}-NH₄-N_{eff})·100^fNitritation efficiency (%)=(NO₂-N_{eff})/(NH₄-N_{in})·100^gNitrification efficiency (%)=(NO₃-N_{eff})/(NH₄-N_{in})·100^hAOR_L (mgNH₄-N/L·hr)=(NH₄-N_{in}-NH₄-N_{eff})/(time)ⁱAOR_M (mgNH₄-N/gVSS·hr)=(NH₄-N_{in}-NH₄-N_{eff})/(time)/(VSS)

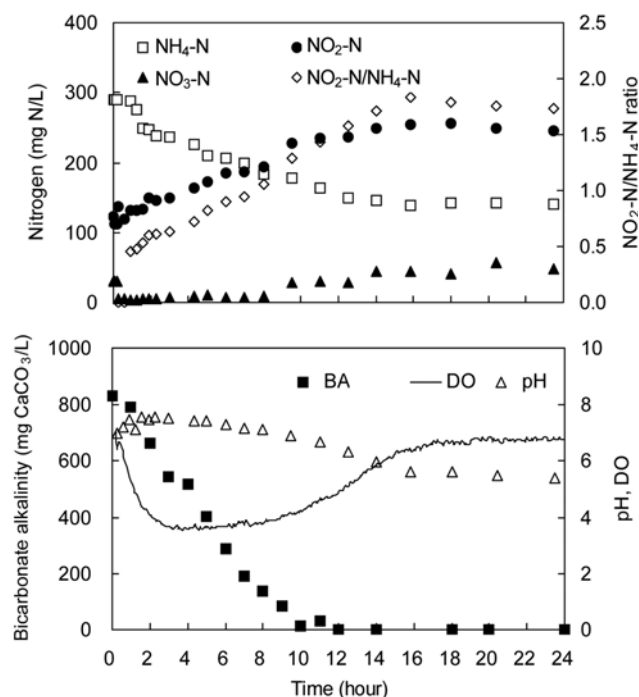


Fig. 3. Profiles of effluent water qualities during the batch test of Phase I.

ity for neutralizing acid production during nitrification. Its average alkalinity/ $\text{NH}_4\text{-N}$ (3.89 ± 0.26 mg/mg) was higher than 3.57 mg/mg for successful partial nitrification [7].

2. Batch Operation in Phase I

In the steady state period in phase I (day 43–67), $\text{NO}_2\text{-N}/\text{NH}_4\text{-N}$ was about 1.69 at 20 hours of HRT (Fig. 3), and this value was higher than the optimum stoichiometric ratio 1.3 for anammox reaction. Then, a batch test was performed during phase I to find optimum feeding condition for succeeded anammox process. The ratio of $\text{NO}_2\text{-N}$ to $\text{NH}_4\text{-N}$ reached 1 : 1.3 between 10–12 hours, and bicarbonate was consumed completely in 10 hours (Fig. 3). During the first 6 hours of the batch operation, maximum AOR_L was 14.0 mg $\text{NH}_4\text{-N}/\text{L}\cdot\text{hr}$, maximum AOR_M was 22.9 mg $\text{NH}_4\text{-N}/\text{gVSS}\cdot\text{hr}$ (VSS of mixed liquor 610 mg/L), and $\text{BA}/\text{NH}_4\text{-N}$ consumption was 6.47 mg $\text{CaCO}_3/\text{mgNH}_4\text{-N}$. Thereafter, $\text{NO}_2\text{-N}/\text{NH}_4\text{-N}$ increased and reached a plateau, and nitrate started to accumulate and reached ~50 mg $\text{NO}_3\text{-N}/\text{L}$ (Fig. 3).

3. Semicontinuous Operation

A batch test during phase I showed that bicarbonate was depleted and $\text{NO}_2\text{-N}/\text{NH}_4\text{-N}$ exceeded the optimum ratio 1 : 1.3 for anammox in 10 hours. Accordingly, the feeding interval was set at 1.5 hours and the feeding amount was reduced for semicontinuous operation. HRTs (20, 17 and 15 hour) were controlled by differing feeding amount (75, 87.5 and 100 mL) with the 1.5-hour feeding interval in phases II, III and IV, respectively (Table 2). At each phase, a steady state was achieved in days 120–140 for phase II, days 175–190 for phase III and days 216–250 for phase III (Table 2).

As hydraulic retention time decreased, concentration of nitrite, nitrate, hydroxylamine, and hydrazine also decreased along with declining NO_2/NH_4 and AOR_M (Table 2). Ammonia and BA/NH_4 consumption was higher in lower HRT due to less oxidation of am-

monia. These results show that ammonia conversion was dependent on the hydraulic or solid retention time. VSS and dissolved oxygen increased with decreasing HRT (Table 2), showing organic degradation and accompanied oxygen consumption increased as HRT became longer. $\text{NO}_2\text{-N}/\text{NH}_4\text{-N}$ decreased from 1.59 to 1.13 and $\text{BA}/\text{NH}_4\text{-N}$ consumption increased from 5.74 to 6.65 when HRT switched from 20 to 15 hours (Table 2). Small amount of bicarbonate alkalinity (15 mg CaCO_3/L for phase II and III, and 110 mg CaCO_3/L for phase IV) remained in the effluents. To achieve the golden ratio of 1.3 of $\text{NO}_2\text{-N}/\text{NH}_4\text{-N}$, HRT should be set between 15 and 17 hours in this reactor configuration fed the supernatant of digester effluent. Autotrophic nitrification was more active than heterotrophic carbon oxidation, possibly due to low concentration of biodegradable carbon source in the influent. Non-biodegradable or recalcitrant carbon fraction was high in the influent as indicated by high TCOD and TS and low SCOD and VSS. In phase IV, only ~22% of VSS was utilized, but ~60% of ammonia was oxidized.

4. Batch Operation in Phase IV

After operation in semicontinuous mode for about 180 days, a batch test was performed at day 250 in phase IV to compare the performance of nitrifying communities. During the first 1.5 hour operation, maximum AOR_L was 10.0 mg $\text{NH}_4\text{-N}/\text{L}\cdot\text{hr}$, maximum AOR_M was 45.5 mg $\text{NH}_4\text{-N}/\text{gVSS}\cdot\text{hr}$ (VSS of mixed liquor 220 mg/L), and $\text{BA}/\text{NH}_4\text{-N}$ consumption was 6.9 mg $\text{CaCO}_3/\text{mgNH}_4\text{-N}$. Nitrate concentration was 10–20 mg $\text{NO}_3\text{-N}/\text{L}$, showing that nitrification activity decreased, but still continued in the reactor. Compared with initial batch operation of phase I, maximum AOR_L decreased by 29%, maximum AOR_M increased by 99%, VSS decreased by 64%, and $\text{BA}/\text{NH}_4\text{-N}$ consumption increased by 7%.

5. Detection of Anammox Intermediates

Pure hydrazine (N_2H_4) is a colorless flammable liquid with an ammonia-like odor, and pure hydroxylamine (NH_2OH), an unstable white crystalline compound, is commonly used as reducing agent in many industrial reactions. Hydrazine and hydroxylamine are the intermediates in the anammox process [18–20] (Fig. 5). It was found that supernatant of sludge digester effluent also contained hydrazine and hydroxylamine (Table 2). Hydrazine concentration increased during partial nitrification by about 60–130% (0.27–0.54 mg/L), but partial nitrification did not influence hydroxylamine concentration (Fig. 2(c), Table 2). When anammox process was completely inhibited by nitrite concentrations higher than 100 mg-N/L, addition of anammox intermediates (1.4 mg-N/L of hydrazine or 0.7 mg-N/L of hydroxylamine) restored anammox activity completely [21]. This partial nitrification process relieved potential nitrite stress by increasing hydrazine level, implying the UAR is an ideal forward process for anammox reaction.

While hydroxylamine can be produced in an oxic condition by catalysis of ammonia mono-oxygenase during nitrification [22], the anammox process is believed to be the only biological process of hydrazine production [23]. Our reactor could not perform any anammox reaction because the mass balance did not show any nitrogen removal via anammox and because HRT is too short for this reactor to harbor anammox bacteria. Anammox has 10.6 days of doubling time [19] and the UAR had 15–20 hours of HRT. It is believed that hydrazine is produced biologically only by anammox bacteria. And our results suggest that the previously known anammox bacteria might not be the only hydrazine-producing bacteria.

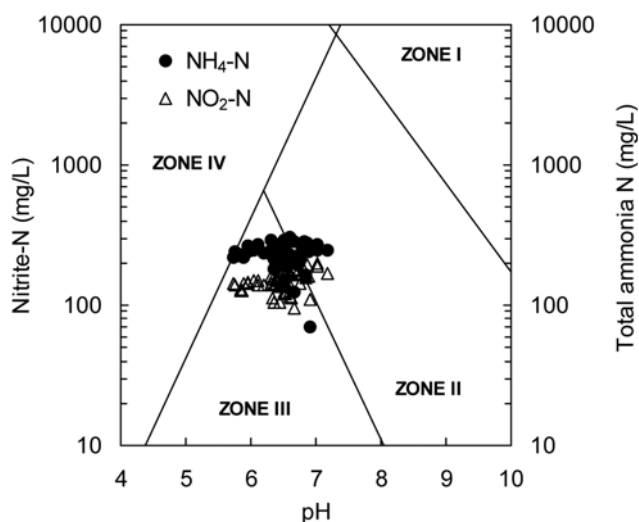


Fig. 4. Graphical representation of inhibition analysis at 30 °C. Zone I: FA inhibition to *Nitrobacter* and *Nitrosomonas*, Zone II: FA inhibition to *Nitrobacter*, Zone III: Complete nitrification, and Zone IV: FNA inhibition to *Nitrobacter*. FA: Free ammonia, FNA: Free nitrous acid.

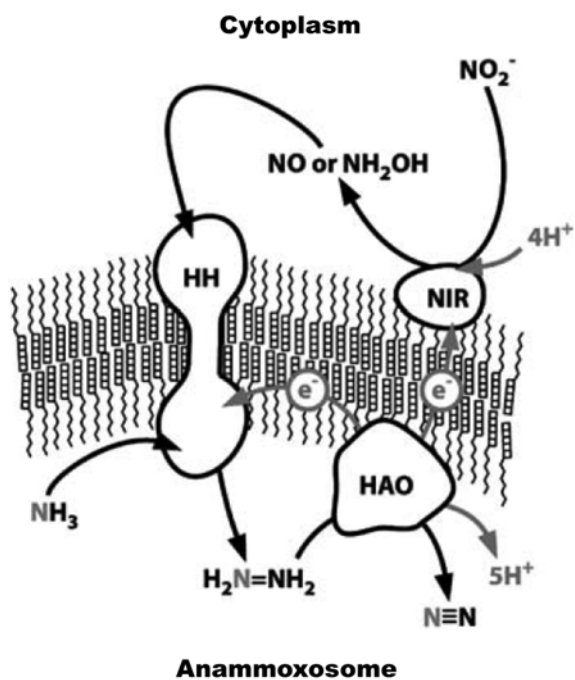


Fig. 5. Suggested pathway of anammox. Black and gray N-atoms highlight the production of $^{29}\text{N}_2$ from one of the ^{15}N -labeled substrates, ammonium or nitrite [20]. NIR is nitrite reductase, HH is hydrazine hydrolase, and HAO is hydroxylamine oxidoreductase.

6. Inhibition Analysis

Partial nitrification process is aimed for increasing nitrite concentration without minimizing nitrate production, and the reactor had a high concentration of nitrite (208–227 $\text{mgNO}_3\text{-N/L}$) of in effluent with some nitrate (24–47 $\text{mgNO}_3\text{-N/L}$) (Table 2). Potential inhibition of nitrification by free ammonia or free nitrous acid was diagnosed in our experiment using a graphical representation previously de-

scribed (Fig. 4) [24]. It showed that the reactor was in the complete nitrification domain, indicating that there was neither ammonia nor nitrite inhibition in the reactor. *Nitrosomonas* has 8–36 hours of doubling time and *Nitrobacter* has minimum 8–12 hours [25]. In real systems, they tend to have longer doubling time than the optimal values. Because *Nitrobacter* has a longer doubling time, it is highly possible that reactor condition offered a better ecological niche for *Nitrosomonas*, and *Nitrobacter* might be washed out more than *Nitrosomonas*. Accordingly, this might lead to nitrite concentration ~5–9 times higher than nitrate (Table 2). Another way to accumulate nitrite is to maintain low dissolved oxygen (DO) concentration. In the competition for DO, it appears that NOB is out-dominated by AOB [26]. Nitrite oxidation was strongly inhibited at 0.5 mg-DO/L , and nitrite concentration accumulated up to about 60 $\text{mgNO}_2\text{-N/L}$ in a suspended growth system. In addition to controlling HRT, low DO strategy would benefit realization of partial nitrification system.

CONCLUSIONS

In partial nitrification experiment using supernatant of anaerobic digester effluent, the UAR reactor successfully provided the stoichiometric ratio of 1.3 of $\text{NO}_2\text{-N/NH}_4\text{-N}$ for following anammox reaction. Alkalinity deficiency was not observed during overall test periods, and the optimal HRT was between 15 and 17 hours. AOR_L was 15 $\text{mgNH}_4\text{-N/L}\cdot\text{hr}$ and AOR_M was 75 $\text{mgNH}_4\text{-N/gMLVSS}\cdot\text{hr}$ (MLVSS 200–220 mg/L). Autotrophic nitrification was more dominant than heterotrophic carbon oxidation due to low concentration of biodegradable carbon source and high concentration of ammonia in the influent. Sludge digester effluent also contained some hydrazine and hydroxylamine, and hydrazine increased during partial nitrification by ~60–130% (0.27–0.54 mg/L), but hydroxylamine concentration was not changed though there was no potential anammox activity.

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REFERENCES

1. S. Bagchi, R. Biswas and T. Nandy, *Crit. Rev. Environ. Sci. Technol.*, **42**, 1353 (2012).
2. A. Joss, D. Salzgeber, J. Eugster, R. Konig, K. Rottermann, S. Burger, P. Fabijan, S. Leumann, J. Mohn and H. Siegrist, *Environ. Sci. Technol.*, **43**, 5301 (2009).
3. S. V. Kalyuzhnyi, M. A. Gladchenko, H. Kang, A. Mulder and A. Versprille, *Water Sci. Technol.*, **57**, 323 (2008).
4. U. van Dongen, M. S. Jetten and M. C. van Loosdrecht, *Water Sci. Technol.*, **44**, 153 (2001).
5. M. S. Jetten, M. Wagner, J. Fuerst, M. van Loosdrecht, G. Kuenen and M. Strous, *Curr. Opin. Biotechnol.*, **12**, 283 (2001).
6. C. Fux, M. Boehler, P. Huber, I. Brunner and H. Siegrist, *J. Biotechnol.*, **99**, 295 (2002).
7. Y.-H. Ahn, *Process Biochem.*, **41**, 1709 (2006).
8. D. J. Kim, D. H. Ahn and D. I. Lee, *Korean J. Chem. Eng.*, **22**, 85 (2005).

9. H. Nam, T. Lee, S. Park and T. Park, *Korean J. Chem. Eng.*, **21**, 635 (2004).
10. J. M. Yoon, D. J. Kim and I. K. Yoo, *Korean Chem. Eng. Res.*, **42**, 200 (2006).
11. J. S. Chang, G. C. Cha and D. J. Kim, *Korean Chem. Eng. Res.*, **40**, 114 (2002).
12. E. Bettazzi, S. Caffaz, C. Vannini and C. Lubello, *Process Biochem.*, **45**, 573 (2010).
13. APHA/AWWA/WEF (American Public Health Association, American Water Works Association, Environment Federation), *Standards for examination of water and waste water*, 21st Ed. United Book Press, Baltimore, MD (2005).
14. K. Buchauer, *Water S.A.*, **24**, 49 (1998).
15. N. Chamchoi, S. Nitisoravut and J. E. Schmidt, *Bioresour. Technol.*, **99**(9), 3331 (2008).
16. J. van de Vossenberg, J. E. Rattray, W. Geerts, B. Kartal, L. van Niftrik, E. G. van Donselaar, J. S. Sinninghe Damsté, M. Strous and M. S. M. Jetten, *Environ. Microbiol.*, **10**, 3120 (2008).
17. B. Kartal, M. Koleva, R. Arsov, W. van der Star, M. S. M. Jetten and M. Strous, *J. Biotechnol.*, **126**, 546 (2006).
18. Y.-H. Ahn and H.-C. Choi, *Process Biochem.*, **41**, 1945 (2006).
19. M. S. M. Jetten, M. Wagner, J. Fuerst, M. van Loosdrecht, G. Kuenen and M. Strous, *Curr. Opin. Biotechnol.*, **12**, 283 (2001).
20. M. Strous and M. S. Jetten, *Annu. Rev. Microbiol.*, **58**, 99 (2004).
21. E. Bettazzi, S. Caffaz, C. Vannini and C. Lubello, *Process Biochem.*, **45**, 573 (2010).
22. S. Otte, J. Schalk, J. G. Kuenen and M. S. Jetten, *Appl. Microbiol. Biotechnol.*, **51**, 255 (1999).
23. H. R. Harhangi, M. Le Roy, T. van Alen, B. L. Hu, J. Groen, B. Kartal, S. G. Tringe, Z. X. Quan, M. S. Jetten and H. J. Op den Camp, *Appl. Environ. Microbiol.*, **78**, 752 (2011).
24. S. M. Lee, J. Y. Jung and Y. C. Chung, *Biotechnol. Lett.*, **22**, 991 (2000).
25. S. A. Hawkins, K. G. Robinson, A. C. Layton and G. S. Sayler, *Environ. Eng. Sci.*, **23**, 521 (2006).
26. J. Guo, Y. Peng, S. Wang, Y. Zheng, H. Huang and Z. Wang, *Bioresour. Technol.*, **100**, 2796 (2009).