

## Excess nitrogen accumulation in activated sludge in sequencing batch reactor with a single-stage oxic process

Xiao-ming Li, Dong-bo Wang, Qi Yang, Wei Zheng, Jian-bin Cao, Xiu Yue, Ting-ting Shen, Guang-ming Zeng and Jiu-hua Deng

### ABSTRACT

It was occasionally found that a significant nitrogen loss in solution under neutral pH value in a sequencing batch reactor with a single-stage oxic process using synthetic wastewater, and then further studies were to verify the phenomenon of nitrogen loss and to investigate the pathway of nitrogen removal. The result showed that good performance of nitrogen removal was obtained in system. 0–7.28 mg L<sup>-1</sup> ammonia, 0.08–0.38 mg L<sup>-1</sup> nitrite and 0.94–2.12 mg L<sup>-1</sup> nitrate were determined in effluent, respectively, when 29.85–35.65 mg L<sup>-1</sup> ammonia was feeding as the sole nitrogen source in influent. Furthermore, a substantial nitrogen loss in solution (95% of nitrogen influent) coupled with a little gaseous nitrogen increase in off-gas (7% of nitrogen influent) was determined during a typical aerobic phase. In addition, about 322 mg nitrogen accumulation (84% of nitrogen influent) was detected in activated sludge. Based on nitrogen mass balance calculation, the unaccounted nitrogen fraction and the ratio of nitrogen accumulation in sludge/nitrogen loss in solution were 14.6 mg (3.7% of nitrogen influent) and 0.89, respectively. The facts indicated that the essential pathway of nitrogen loss in solution in this study was excess nitrogen accumulation in activated sludge.

**Key words** | excess nitrogen accumulation, nitrogen loss, nitrogen mass balance, sequencing batch reactor, single-stage oxic process

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### INTRODUCTION

In recent years, it is increasingly evident that nitrogen (N) compounds cause the phenomenon of water pollution and water eutrophication. Currently, the most effective approaches to treat NH<sub>4</sub><sup>+</sup>-N in the wastewater are the biological processes, and nitrification, in most cases, is regarded as the pathway to remove NH<sub>4</sub><sup>+</sup>-N in the biological processes. In this pathway, ammonia is oxidized to nitrite by ammonia oxidizing bacteria and further oxidized to nitrate by nitrite oxidizing bacteria. Moreover, the classic biological N removal theory contains a classic denitrification process, which occurs under anoxic conditions with organic carbon as electron donor and nitrate as electron acceptor. In addition to the classic pathway, aerobic denitrification, heterotrophic nitrification, Anammox (anaerobic

ammonium oxidation), and methane oxidation are potentially important processes in N removal systems (van Loosdrecht & Jetten 1998; Strous *et al.* 1999; Raghoebarsing *et al.* 2006).

Theoretically, treatment of nitrogenous influent under aerobic conditions should result in very close influent and effluent concentrations of total nitrogen (TN), if classic nitrification predominates and assimilatory N uptake is negligible. However, significant N loss in solution has been detected in an aerobic environment by several researchers lately (Hippen *et al.* 1997; Kim *et al.* 2005; Sun & Austin 2007; Yu *et al.* 2007). Deficiently, the N loss pathways of those researches were only reasonable hypothesis and conclusions drawn from analysis of experimental data

were also inadequate. N mass balance provides one way of checking the reliability of the data. Surprisingly this is seldom done, most likely because gathering the data to conduct these balances may necessitate additional sampling and monitoring of the experimental system, beyond that regarded as necessary for addressing a particular research problem. Also, in certain cases it may not be feasible to gather the required data; for example, on a full-scale plant with dynamic influent loading.

There are few reports on N loss in sequencing batch reactors (SBR) with single-stage oxic processes, but it was found by our research group under neutral pH (about 7.0) condition in April 2006, and then detailed researches including N mass balance were conducted from April 2006 to September 2007. The aims of this paper were to verify the phenomenon of N loss in solution and to investigate the pathway of N removal in this study.

## MATERIALS AND METHODS

### Experimental device and operational methods

Experiments were tested in a tightly sealed 12 L-SBR (working volume) with two branches. One branch which was equipped with a septum on the mouth was used for instantaneous gaseous N analysis, and the other one was connected with a gaseous collected tank (50 L) for total gaseous N mass determination (schematics diagram shown in Figure 1). The 480 min cycles consisted of approximately 240 min aerobic period, followed by 28 min settling, 2 min decanting and 210 min idle periods. Synthetic wastewater (composition detailed below) was fed to reactor during the

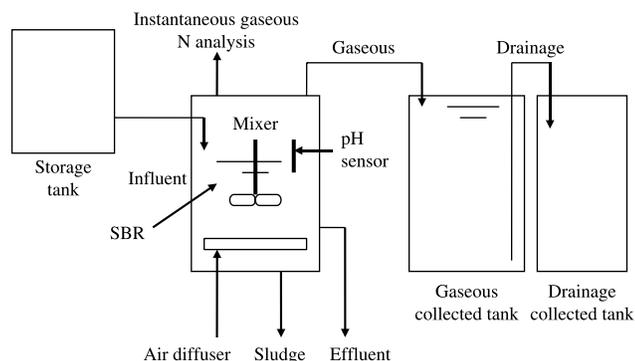


Figure 1 | Schematics diagram of the sequencing batch reactor.

first 2 min of the aerobic period. For each SBR cycle, 7.8 L supernatant was discharged after the settling period, resulting in a hydraulic retention time (HRT) of 12 h approximately. 10.5–11 d of the sludge retention time (SRT) was maintained. In this process, anoxic phase did not exist but long-term idle zone (210 min) was operated during two cycles. Pure oxygen was fully pressurized into the systems at a rate of  $0.01 \text{ Nm}^3 \text{ h}^{-1}$  ( $\text{Nm}^3$  are based on  $0^\circ\text{C}$  and 1,013 mbar). The temperature in the reactor was controlled at  $20^\circ\text{C}$ . The pH was controlled at  $7.0 \pm 0.1$  during the aerobic period through addition of 0.5 M HCl and 0.5 M NaOH. Activated sludge, obtained from the first municipal wastewater treatment plant of Changsha, PR China, was seeded and cultured according to the way described above, and the initial concentration of sludge in reactor was set around  $4,000 \text{ mg L}^{-1}$ .

### Synthetic media

Synthetic wastewater was used in this research. Glucose was used as the organic carbon source, and N in the wastewater was simulated with chlorine ammonium. The chemical oxygen demand (COD) concentration in the feed was about  $300 \text{ mg COD L}^{-1}$ , while the N concentration was about  $35 \text{ mg NH}_4\text{-N L}^{-1}$ , which yielded a COD/N ratio of  $8.6 \text{ mg COD mg}^{-1} \text{ NH}_4\text{-N}$ . The concentration of the other nutrients in the synthetic feed are indicated below (per liter): 0.28 g  $\text{NaHCO}_3$ , 0.01 g  $\text{KH}_2\text{PO}_4$ , 0.02 g peptone, 0.01 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.005 g  $\text{CaCl}_2$ , and 0.5 mL of a trace metals solution. The trace metals solution has been described in Smolders *et al.* (1994) and consisted of (per litre): 1.5 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 0.15 g  $\text{H}_3\text{BO}_3$ , 0.03 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.18 g KI, 0.12 g  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.06 g  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 0.12 g  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.15 g  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  and 10 g EDTA.

### N mass balance calculation

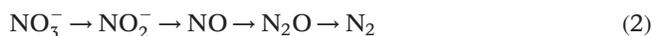
Since significant N loss was observed during the aerobic phase of SBR cycles, the N mass balance over system was just discussed during an aerobic period and was given by :

$$N_{\text{Inf}} = N_{\text{Eff}} + N_{\text{Slud}} + N_{\text{Gas}} + N_{\text{Unacc}} \quad (1)$$

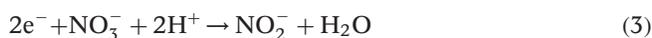
where,  $N_{\text{Inf}}$  and  $N_{\text{Eff}}$  the influent and effluent total nitrogen (TN) (mg),  $N_{\text{Slud}}$  N accumulation in the sludge (mg),  $N_{\text{Gas}}$

the gaseous N leaving the system (mg),  $N_{\text{Unacc}}$  the unaccounted fraction of N (mg).

Theoretically,  $N_{\text{Gas}}$  consists of the gaseous N leaving the system via ammonia volatilization and via denitrification. Ammonia volatilization will not occur until pH value in system is above 9.5 (pH values were just controlled at  $7.0 \pm 0.1$  in this study) or the wastewater temperature is high, e.g., above  $40^\circ\text{C}$  (the temperature was controlled at  $20^\circ\text{C}$  in this study) (Sun & Austin 2007). So, ammonia volatilization is negligible and  $\text{NH}_3$  of gaseous N was not detected in this study. Moreover, it has been proposed that denitrification is essentially a four step process (Barke & Dold 1995).



Each step may be represented by a half reaction where  $e^-$  denotes electron equivalents (COD) transferred from the organic substrate:



Equations (2)–(6) imply that, except for  $\text{N}_2$ , intermediates such as  $\text{N}_2\text{O}$ ,  $\text{NO}/\text{NO}_x$  may be produced during denitrification, and some of the intermediates did be observed under anoxic and anaerobic conditions or continuous biological aerobic condition by some researchers (Schulthess *et al.* 1994; Fuerhacker *et al.* 2000; Béline *et al.* 2001). Therefore,  $\text{N}_2$ ,  $\text{N}_2\text{O}$  and  $\text{NO}/\text{NO}_x$  were detected in off-gas samples and  $N_{\text{Gas}}$  was calculated by the sum of the three gaseous N in this study.

### Analytical methods

Sludge samples from the reactor were immediately filtered through a Whatmann GF/C glass microfiber filter ( $1.2 \mu\text{m}$ ). The filtrate was immediately analyzed for  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N, COD and total Kjeldahl Nitrogen (TKN), and

the filter was assayed for mix liquor volatile suspended solids (MLVSS), MLSS and TN in sludge.  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N, COD, TKN, MLVSS, MLSS and TN in sludge were measured according to Standard Methods (APHA 1995), and TN in influent and effluent were calculated by the sum of TKN,  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N.

Extractable  $\text{NH}_4^+$ -N: Samples were taken from the process tanks and immediately separated from the sludge by centrifugation or extracted in KCl. Samples for dissolved ions were centrifuged at 2,000 rpm. In order to get the same background concentration of KCl as the extracted samples, the supernatant was filtered (GF/F) and 2.0 ml was mixed with 2.0 ml 2 N KCl and frozen until analysis. Samples for extractable ions taken from the process tanks were immediately poured into test tubes with a 2 N KCl solution on a 1:1 volumetric basis. The samples were mixed and extracted for at least 15 min. After centrifugation and filtration the supernatant was frozen until analysis (Nielsen 1996).

The  $\text{N}_2\text{O}$  and  $\text{N}_2$  concentrations in the gas samples were determined with gas chromatography (Varian 5300, thermal conductivity detector), under the following conditions: column at  $35^\circ\text{C}$ , detector at  $110^\circ\text{C}$ , injector at  $100^\circ\text{C}$ , filament at  $135^\circ\text{C}$  and volume samples  $50 \mu\text{l}$ . Helium was employed as carrier gas (Cervantes *et al.* 2001).  $\text{NO}/\text{NO}_x$  was examined with continuous automatic facility (Nitrogen Oxides Analyzer Model 8840, Monitor Labs). Total mass of gaseous N in a typical cycle was calculated as the sum of measured gaseous N in gaseous collected tank and N accumulation in 50 L solution.

## RESULTS

### N loss in solution during long-term operation

Experiments were conducted in a SBR system and lasted for about 400 d. The performance of N removal and the significant N losses during steady-state operation were partly shown in Table 1 and 2.

As shown in Table 1,  $0\text{--}7.28 \text{ mg L}^{-1}$   $\text{NH}_4^+$ -N was obtained in effluent when  $29.85\text{--}35.65 \text{ mg L}^{-1}$  was fed to reactor, which indicated the efficiencies of  $\text{NH}_4^+$ -N were in the range of 79.05–89.63%. Surprisingly, low  $\text{NO}_2^-$ -N

**Table 1** | System performances during long-term operation

Parameters		Influent (mg L <sup>-1</sup> )	Effluent (mg L <sup>-1</sup> )	Removal	
				(mg L <sup>-1</sup> )	(%)
COD	Max	326	48	292	91.25
	Min	284	16	248	83.78
	Avg	305	28	277	90.82
NH <sub>4</sub> <sup>+</sup> -N	Max	35.65	7.28	31.80	89.63
	Min	29.85	0	26.94	79.05
	Avg	35.16	3.98	31.18	88.68
NO <sub>2</sub> <sup>-</sup> -N	Max	0.27	0.38		
	Min	0.06	0.08		
	Avg	0.21	0.24		
NO <sub>3</sub> <sup>-</sup> -N	Max	1.67	2.12		
	Min	0.84	0.94		
	Avg	1.36	1.51		

Data were obtained from about 300 days steady-state operation. Influent data were calculated at 2 minutes of aeration.

(0.08–0.38 mg L<sup>-1</sup>) coupled with low NO<sub>3</sub><sup>-</sup>-N (0.94–2.12 mg L<sup>-1</sup>) was detected in effluent after 4-h aerobic period. Moreover, Table 2 showed an average N loss in solution was achieved at 31.85 mg L<sup>-1</sup> (86.13% of TN influent).

Equations (7)–(9) in 3.2 show that the total amount of NH<sub>4</sub><sup>+</sup>-, NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N should be nearly constant during nitrification. From Table 1 and 2, it may be concluded that if nitrification is regarded as the only pathway to remove NH<sub>4</sub><sup>+</sup>-N, 79.05%–89.63% of the removed NH<sub>4</sub><sup>+</sup>-N was lost, which strongly indicated other pathways for removal besides nitrification.

**Table 2** | Nitrogen loss during long-term operation

	TN (mg L <sup>-1</sup> )		N loss in solution	
	Influent	Effluent	(mg L <sup>-1</sup> )	(%)
Max	37.86	9.54	33.36	88.58
Min	31.54	2.58	27.72	78.21
Avg	36.98	4.75	31.85	86.13

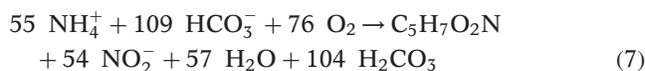
Data were obtained from about 300 days steady-state operation. Influent data were calculated at 2 minutes of aeration.

## N loss in solution and gaseous N increase paradox

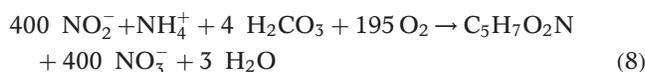
Equations (2)–(6) in 2.3 indicate that N<sub>2</sub>O, NO/NO<sub>x</sub> and N<sub>2</sub> may be formed during denitrification, and can result in N losses in solution. Recently, “aerobic deammonification” (Hippen *et al.* 1997) and autotrophic nitrification–denitrification (Kuai & Verstraete 1998) as well as substantial N loss in wastewater bioreactor with one oxic-stage (Helmer & Kunst 1998; Gaul *et al.* 2002; Pynaert *et al.* 2003) were reported. Thus, one likely explanation to the significant N loss was that aerobic denitrification should occur in system. Could significant N loss in solution in this research be achieved via aerobic denitrification?

It is generally accepted that denitrification in any case is primarily based on NO<sub>2</sub><sup>-</sup>-N or NO<sub>3</sub><sup>-</sup>-N accumulation by nitrification as following [Equations (7) and (8), For stoichiometric calculation, C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N would be used to represent the composition of nitrifying bacteria biomass]:

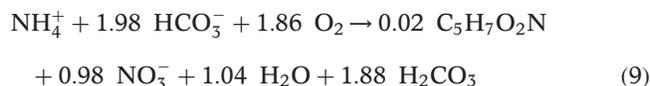
Ammonia oxidizing bacteria :



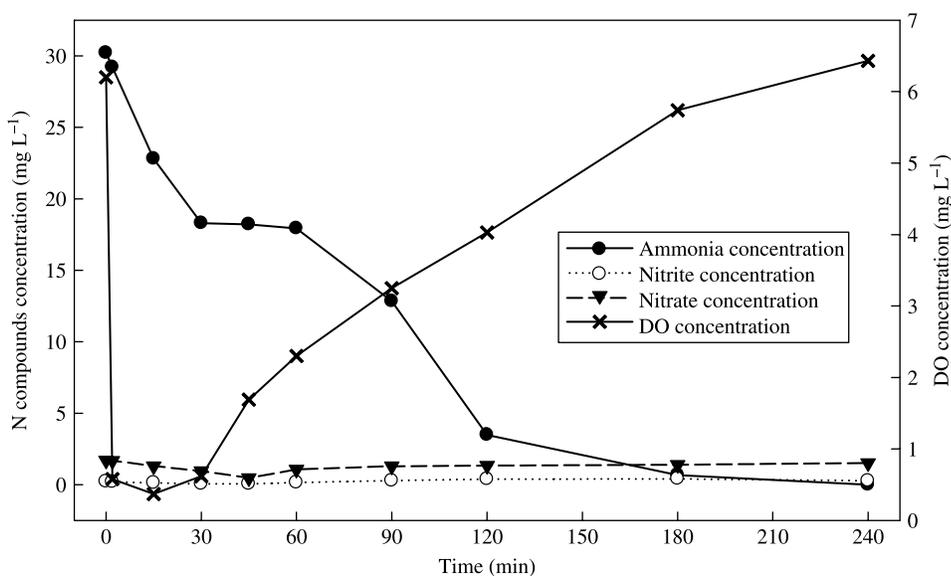
Nitrite oxidizing bacteria:



The net reaction is obtained by combining Equations (7) and (8):



Equations (7)–(9) show that about 0.98 g NO<sub>2</sub><sup>-</sup>-N or NO<sub>3</sub><sup>-</sup>-N should be accumulated when 1 g NH<sub>4</sub><sup>+</sup>-N was oxidized. However, with the decrease of NH<sub>4</sub><sup>+</sup>-N, a low amount of nitrate coupled with little nitrite accumulation (0.41 mg L<sup>-1</sup> of the maximum nitrite concentration and 1.70 mg L<sup>-1</sup> of the maximum nitrate concentration) was observed during the whole aeration in this study (Figure 2). Furthermore, just a little N<sub>Gas</sub> was detected during the aerobic phase. As shown in Table 3, a little N<sub>2</sub>O was monitored in the off-gas during the first 90 min of the



**Figure 2** | Varieties of N compounds and DO concentration during a typical aerobic cycle. Data were obtained after about 130-day steady state operation.

aerobic period, and  $\text{NO}/\text{NO}_x$  as well as  $\text{N}_2$  was not detected overall. The  $\text{N}_{\text{Gas}}$  ( $\text{N}_2\text{O}$ ) was only 25.2 mg (7% of TN influent) while about 365 mg (95% of TN influent) of N loss was determined in solution. Apparently, if nitrification was the essential way to remove  $\text{NH}_4^+\text{-N}$ , either rich nitrite and/or nitrate would accumulate or significant  $\text{N}_{\text{Gas}}$  would be detected. The facts shown in Figure 2 and Table 3 strongly indicated that nitrification was not the essential pathway for  $\text{NH}_4^+\text{-N}$  removal. The accurate reason for this strong inhibition to nitrification have not been strictly investigated up till now, since the optimal pH value to the growth of nitrite bacteria was 7.4–8.3 (Villaverde *et al.* 1997; Jetten *et al.* 2001), the probable reason was that nitrite bacteria was strongly inhibited by the pH condition ( $7.0 \pm 0.1$ ) in this

research. Also because of strong inhibition to nitrification, denitrification was the subsidiary responsible for the N loss in solution and other pathways for substantial  $\text{NH}_4^+\text{-N}$  removal occurred in system rather than classic nitrification as well as denitrification. Since a little  $\text{N}_{\text{Gas}}$  was detected, and the significant N loss in solution could not be explained by the conventional denitrification even aerobic denitrification, more than ten times of cycle experiments were repeated on  $\text{N}_{\text{Gas}}$  determinations and similar results were obtained (data not shown).

### N accumulation in activated sludge

To investigate the pathway of N removal in this study, varieties of COD, TSS, and TN in wastewater as well as TN

**Table 3** | The gaseous N leaving the system during aerobic phase of a typical aerobic cycle

Aerobic time (min)	15	30	45	60	90	120	180	240	Avg	V(L)
$\text{NO}/\text{NO}_x\text{-N}_{\text{out}}$ ( $\text{mg m}^{-3}$ )	ND*	ND	35.4							
$\text{N}_2\text{O-N}_{\text{out}}$ ( $\text{mg m}^{-3}$ )	856	908	774	496	363	ND	ND	ND	485	35.4
$\text{N}_2\text{-N}_{\text{out}}$ ( $\text{mg m}^{-3}$ )	ND	ND	ND	ND	ND	ND	ND	ND	ND	35.4
$\text{N}^{\dagger}_{\text{Accu.}}$ in 50 L-water ( $\text{mg L}^{-1}$ )									0.16	50
Total $\text{N}_{\text{Gas}}$ (mg)	25.2 <sup>‡</sup>									

Data were obtained after about 130-day steady state operation.

\*ND = Not detected.

<sup>†</sup> $\text{N}_{\text{Accu.}}$  = Nitrogen accumulation in 50 L-water.

<sup>‡</sup>25.2 =  $485(\text{mg m}^{-3}) \times 35.4 \times 10^{-3}(\text{m}^{-3}) + 0.16(\text{mg L}^{-1}) \times 50(\text{L})$ , where,  $35.4 \times 10^{-3}(\text{m}^{-3})$  was calculated by the volume of the drainage in drainage collected tank.

in activated sludge were analyzed during a typical period, which were shown in Figure 3.

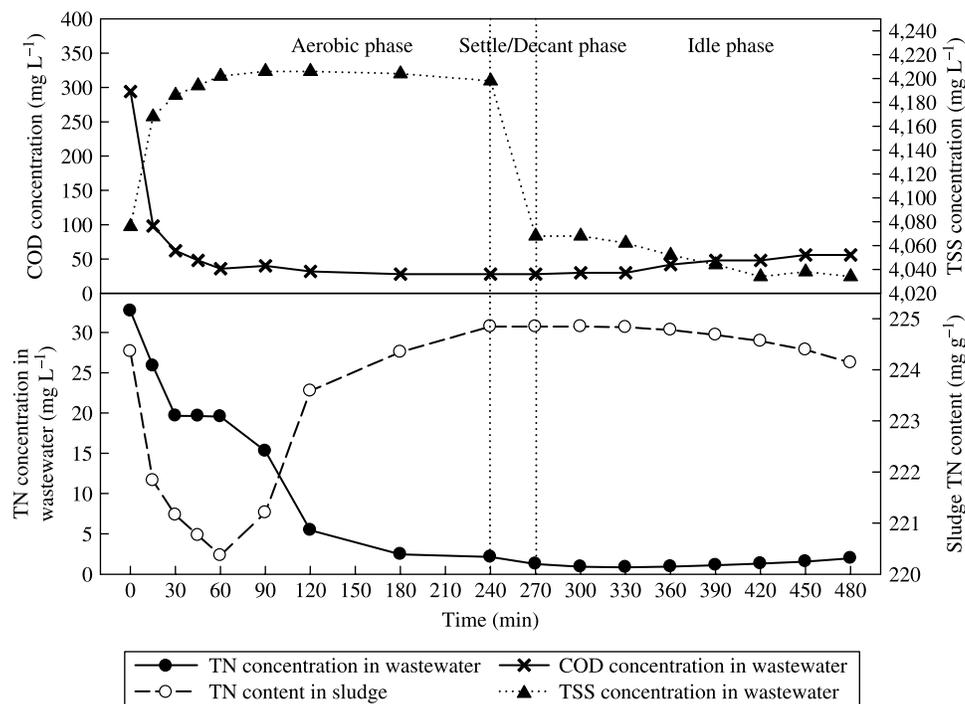
As shown in Figure 3, a rapid COD decrease was associated with a fast TSS increase, and a high amount of TN in wastewater as well as an obvious TN content in sludge decrease was observed at the first 30 min in system, that may be the results of swiftly reproduction by microorganisms. After 30 min, a slow COD decrease and a soft TSS increase were also observed. TN concentration had little changes from 30 to 90 min but a quick decrease after 90 min of aeration. TN in sludge was rapidly increased from 60–90 min of aerobic phase. At the end of aerobic period, COD, TSS, TN in wastewater as well as TN in sludge were  $28 \text{ mg L}^{-1}$ ,  $4192 \text{ mg L}^{-1}$ ,  $2.53 \text{ mg L}^{-1}$  and  $225 \text{ mg g}^{-1}$ , respectively. The facts indicated that about 322 mg N (84% of TN influent) was accumulated in sludge. Comparing with 110–120  $\text{mg N g}^{-1}$  TSS in activated sludge reported by previous publications (Pichtel & Anderson 1997; Jouraiphy *et al.* 2005; Perez-Murcia *et al.* 2006), a much higher TN was accumulated in activated sludge in this

study. Also the experiments were repeated on  $N_{\text{Slud}}$  determinations over more than ten cycles and similar results were obtained (data not shown).

### N mass balance and the essential pathway for N loss in solution

Equations (1) shows the N mass balance calculated in this study. For a typical SBR cycle,  $32.68 \text{ mg L}^{-1}$  of TN in influent coupled with  $2.53 \text{ mg L}^{-1}$  in effluent was detected (Figure 3), meanwhile, 322 mg N accumulation in sludge (Figure 3) as well as 25.2 mg N (Table 3) leaving the system was monitored. Therefore,  $N_{\text{Unacc}}$  during the typical cycle could be calculated by:

$$\begin{aligned} N_{\text{Unacc}} &= N_{\text{Inf}} - (N_{\text{Eff}} + N_{\text{Slud}} + N_{\text{Gas}}) \\ &= 32.68 \text{ mg L}^{-1} \times 12 \text{ L} - (2.53 \text{ mg L}^{-1} \\ &\quad \times 12 \text{ L} + 322 \text{ mg} + 25.2 \text{ mg}) \\ &= 392.16 \text{ mg} - (30.36 \text{ mg} + 322 \text{ mg} \\ &\quad + 25.2 \text{ mg}) \\ &= 14.6 \text{ mg} \text{ (3.7\% of TN influent)} \end{aligned} \quad (10)$$



**Figure 3** | Varieties of COD, TSS, TN in wastewater and TN in activated sludge during a typical cycle. About  $120 \text{ mg L}^{-1}$  TSS was discharged from the system at the end of the aerobic period. Data were obtained after about 130-day steady state operation.

In addition, the ratio of  $N_{\text{Slud}}/N$  loss in solution during the typical cycle could be calculated by:

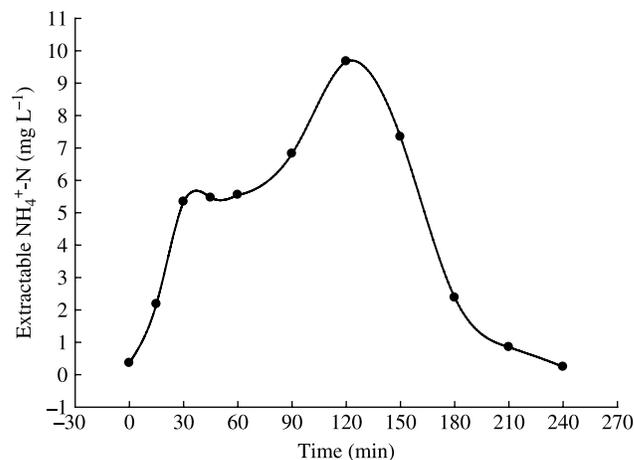
$$\begin{aligned} (N_{\text{Slud}}/N \text{ loss}) &= N_{\text{Slud}}/(N_{\text{Inf}} - N_{\text{Eff}}) \\ &= 322 \text{ mg}/(392.16 \text{ mg} - 30.36 \text{ mg}) \\ &= 0.89 \end{aligned} \quad (11)$$

$N_{\text{Unacc}}$  (N14.6 mg) calculated in Equations (10) might be the result of a little ammonia volatilization or experimental errors. However, in contrast to  $N_{\text{Inf}}$ , only 3.7% of TN influent was not accounted, therefore, the N mass balance between theory and experiment were considered to match well in this study. Furthermore, 89% of N loss in solution was accumulated in activated sludge which suggested that the essential pathway of N loss in this study was the substantial N accumulation in activated sludge.

## DISCUSSION

### The probably reason for excess N accumulation in this study

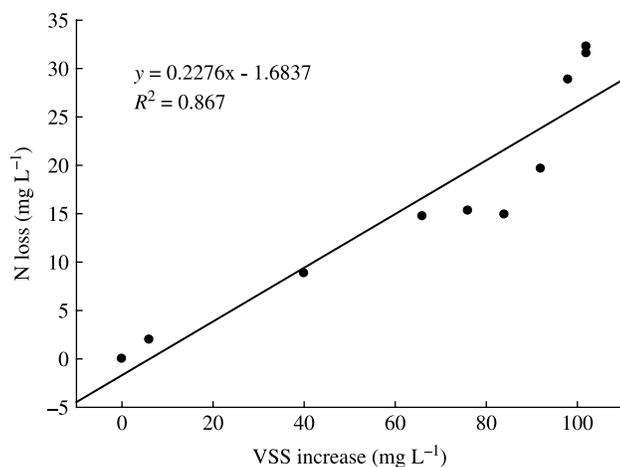
Surprisingly, 225 mg TN  $g^{-1}$  TSS which is much higher than that conducted in activated sludge by previous investigations was obtained in this study. As we known that, some of physico-chemical processes such as settling process or sludge adsorption could result in N increase in sludge (Nielsen 1996; Connolly *et al.* 2004). Could those approaches were main responsible for the substantial N accumulation in sludge? In this study, it was obvious that there was little flocculant needed by settling process, thus we eliminated this possibility. From Figure 4, it can be clearly observed that  $NH_4^+$ -N adsorption did occur in system, and the maximum adsorption was  $9.67 \text{ mg L}^{-1}$  at 120 min of aeration. However, an interestingly observation was,  $NH_4^+$ -N adsorption in activated sludge decreased rapidly after 120 min of aeration and only  $0.24 \text{ mg L}^{-1}$  (0.89% of TN in sludge) extractable  $NH_4^+$ -N was detected at the end of aeration. Accordingly,  $NH_4^+$ -N adsorption by activated sludge had little effect on N accumulation obtained in this study.



**Figure 4** | Extractable  $NH_4^+$ -N during a typical aerobic cycle. Data were obtained after about 130-day steady state operation.

It is well known that, not only heterotrophic biomass but also autotrophs need to take C, N, P and other nutrients for their assimilation to synthesize their cellular components and maintain their growth and reproduction. Hence it was worthwhile to discuss the possibility if normal anabolism was the essential pathway for the substantial N accumulation. However, N incepted by microorganism normal growth and metabolism was finite and was associated with COD concentration. Generally, the COD/N rate by biomass assimilation was  $100 \text{ mg COD}/5 \text{ mg N}$  (Zhou & Gao 2000). Moreover,  $C_5H_7O_2N$  would be used to represent the composition of biomass by many researchers (Cannon *et al.* 2000; Chuang & Ouyang 2000; Kim *et al.* 2005; Sun *et al.* 2005), which indicated that the sludge N content by biomass normal assimilation should be less than  $124 \text{ mg N g}^{-1}$  TSS. Furthermore, good correlation ( $R^2 = 0.867$ ) between VSS (biomass) increase and N loss in solution was observed in a typical cycle (Figure 5). The facts suggested that excess  $NH_4^+$ -N uptake by microorganisms occurred in this study.

Question might be raised would be why microorganisms in this research had the capability of excess  $NH_4^+$ -N uptake and why this peculiar phenomenon could not be observed in other single-stage oxic process? In this study, external substrates were fed to reactor during the first 2 min of aerobic period (detailed in 2.1) and was almost rapidly depleted during 120 min of aeration (varieties of COD in Figure 3 can support this deduction), in addition, 210 min of idle zone was followed by decanting period.



**Figure 5** | Relationship between the VSS increase and the N loss during a typical aerobic cycle. Data were obtained after about 260-day steady state operation, VSS increase is considered to represent biomass increase.

This operational process may result in “external substrates feast - external substrates famine - idle starvation” regime in a SBR cycle. In contrast, conventional activated sludge processes which usually consist of 3–4 h aerobic period, 0.5–1 h settling/decanting period and short idle zone (e.g. 0.5 h), also result in “external substrates feast - external substrates famine” regime which induce some internal storage compounds such as poly- $\beta$ -hydroxyalkanoates (PHA), glycogen etc. accumulation in “external substrates feast” period (Carucci *et al.* 2001) and oxidization to provide carbon source as well as energy for cell growth and maintenance in “external substrates famine” period and settling/decanting/idle periods (Lu *et al.* 2007). Since short idle zone is usually operated in conventional activated sludge processes, enough energy can be generated by easy degradable internal storage (e.g. PHA) and other storage is seldom observed. Nevertheless, 210 min of “idle starvation” period was operated in this study which indicated extra energy might be required for cell maintenance. This operation might induce excess  $\text{NH}_4^+$ -N and carbon sources uptake for other internal storage (e.g. protein) by microorganisms if bacteria was under rich- $\text{NH}_4^+$ -N condition in “external substrates feast” period (most of  $\text{NH}_4^+$ -N was removed during 120 min of aeration and could support the hypothesis), and could provide carbon and nitrogen sources as well as energy for subsequent cell maintenance during “idle starvation” period. In our previous study, excess uptake of another

energy storage compound (polyphosphate) by bacteria under rich-phosphate condition was also observed in such a single-stage oxic process (Wang *et al.* 2008).

### Further investigations

A significant N loss in solution as well as a high TN removal efficiency obtained in such a single-stage oxic process was amazing. To our knowledge, this peculiar phenomenon has never been reported before. Through experiments repeated six times and more than 300 d steady-operation, we confirmed that it did occur. Further investigations are worth being considered: (1) the reason for excess  $\text{NH}_4^+$ -N uptake by microorganisms should be analyzed accurately; (2) since the substantial N accumulation in sludge was observed, the elemental compositions of sludge cultivated in this study should be determined systematically; (3) microbiological analytical techniques such as Fluorescence in situ hybridization (FISH) and Polymerase chain reaction (PCR) need to assess the biomass activities exactly.

Although there are several points that needed to further investigate, our results suggested the existence of a significant, yet previously unrecognized, microbial response to nutrient limitation and could also form the basis of a potentially novel strategy for the ‘one-step’ removal of N from effluents.

### CONCLUSIONS

The results of this study showed that good performance of N removal (79.05–89.63% of  $\text{NH}_4^+$ -N removal efficiencies) could be achieved in SBR with a single-stage oxic process, and N losses in solution and gaseous N increases paradox indicated the nitrifiers were strongly inhibited and the high efficiency of N removal in this study was not conducted by classic nitrification and denitrification. Further researches showed that 322 mg N (89% of N loss in solution) accumulated in activated sludge during a typical cycle, which implied the essential pathway of N loss in solution was the excess N accumulation in sludge. The results indicated N removal in this study was mainly achieved not by traditional nitrification/denitrification approach but by the discharge of rich-nitrogen sludge.

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