Bioresource Technology 102 (2011) 5514–5519

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Development of simultaneous partial nitrification, anammox and denitrification (SNAD) process in a sequential batch reactor

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ARTICLE INFO

Article history:
Received 29 July 2010
Received in revised form 4 November 2010
Accepted 4 November 2010
Available online 12 November 2010

Keywords:
Anammox
Nitrification
Denitrification
Landfill-leachate
Nutrient removal

ABSTRACT

Simultaneous partial nitrification, anammox and denitrification (SNAD) process was developed in a sequential batch reactor (SBR) and the influence of hydraulic retention time (HRT) on the SNAD process was investigated. Around 96% NH_4^+–N removal and 87% COD removal were observed at 9 d HRT. Marginal decreases in the removal efficiencies were observed when the HRT was reduced to 3 d or the loading rate was increased by three times. On the other hand, a drastic decrease in NH_4^+–N and COD removals were observed when the DO, pH and temperature were dropped shockingly. The response of the SNAD system towards the shock in substrate loading and operating conditions was evaluated by sensitivity index. Finally, the extent of total nitrogen (TN) removal by partial nitrification with anammox and denitrification was modeled using stoichiometric relationship. Modeling results indicated a TN removal of 85–87% by anammox with partial nitrification and 7–9% by denitrification.

1. Introduction

The release of excessive nitrogen into the aquatic systems leads to acidification and eutrophication problems. At the same time, it can also impair the survival of aquatic plants and other organisms. Thus, the removal of nitrogenous compounds from wastewater systems prior to its disposal is an important issue. Nitrogen removal from wastewaters is usually accomplished through sequential nitrification and denitrification processes, i.e., conventional nitrification–denitrification process. This is recognized as the highly efficient and suitable process for the treatment of wastewater with high ammonium (NH_4^+) content and rich in biodegradable carbon (Fernandez et al., 2008). During the conventional nitrification–denitrification process, NH_4^+ is oxidized to nitrate (NO_3^-) followed by NO_3^- reduction to gaseous nitrogen (N_2). However, several novel nitrogen removal processes have been developed to reduce the energy consumption in the nitrification–denitrification process. These novel processes include single reactor system for high ammonium removal over nitrite (SHARON) (Hellinga et al., 1998; van Dongen et al., 2001), completely autotrophic nitrogen removal over nitrite (CANON) (Sliekers et al., 2002), oxygen-limited autotrophic nitrification–denitrification (OLAND) (Kuai and Verstraete, 1998) and anaerobic ammonium oxidation (anammox) (Jetten et al., 1999).

Anaerobic ammonium oxidation (anammox) process is gaining lot of importance for nitrogen removal compared to the conventional nitrification–denitrification process. Anammox is an autotrophic oxidation process, which converts NH_4^+ to N_2 using nitrite (NO_2^-) as the electron acceptor. It is well established that autotrophic anammox bacteria belongs to the category of planctomycetes. Since anammox process is an anaerobic-autotrophic process that eliminates the requirements of aeration and exogenous carbon source (Strous et al., 1997, 1999). However, the anammox process depends on the availability of both NH_4^+ and NO_2^- in the system (Eq. (1)); therefore, anammox process was coupled with partial nitrification in a single reactor system. The combination of anammox and partial nitrification decreases the overall cost of the nitrogen removal process; however, a significant quantity of NO_3^- (10%) is released from the anammox systems. This could be more than the wastewater disposal standards at times. On the other hand, combining anammox and denitrification for complete nitrogen removal has been reported (Kumar and Lin, 2010).

In CANON process, partial nitrification and anoxic NH_4^+ oxidation are carried out by aerobic and anaerobic ammonia oxidizing bacteria (AOB), respectively. On the other hand, in OLAND process, AOB oxidizes a portion of NH_4^+ to NO_2^- with oxygen as electron acceptor and subsequently, NO_2^- is reduced to N_2 using the remaining NH_4^+ as the electron donor. Both these processes are autotrophic nitrogen removal process, which are operated mainly under oxygen-limited condition. These two processes, i.e., CANON and OLAND, are better suitable for the wastewaters with high NH_4^+ concentration but without organic matter. The presence of organic matter is often

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doi:10.1016/j.biortech.2010.11.024

limited the removal efficiency of the later process, i.e., OLAND. However, most of the wastewaters contain both organic carbon and nitrogen. The co-existence of these processes along with denitrification could be useful for the simultaneous removal of nitrogen and organic carbon in a single system rather than a sequential chain of treatment.

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \qquad (1)$$

Recently, simultaneous partial nitrification, anammox and denitrification (SNAD) (Chen et al., 2009) process was developed, which has the potential of treating NH_4^+ and biodegradable organics from wastewaters. The advantage of this process is the complete nitrogen removal and a reduction in the portion of chemical oxygen demand (COD). The granules capable of carrying out the SNAD process were identified in a full-scale landfill-leachate treatment plant in Taiwan (Wang et al., 2010). In the SNAD process, majority of nitrogen is removed by the anammox process. However, developing a SNAD process in the laboratory is highly difficult owing to the requirement of longer start-up time and slow growth rates of anammox bacteria (the doubling time was reported to be approximately 11 d). In addition, the reactor carrying out anammox must be efficient in retaining the biomass (Strous et al., 1998).

In several studies, the sequential batch reactors (SBRs) have been successfully applied for the enrichment of very slow-growing microbial community (Dapena-Mora et al., 2004; Lopez et al., 2008; Joss et al., 2009). The SBR provides efficient biomass retention (over 90% of biomass retention) and also reduces the doubling time compared with other nitrogen removal configurations (Vandegraaf et al., 1996; Strous et al., 1998). However, the optimum conditions of SBR for the enrichment of SNAD organisms (nitrifying, anammox and denitrifying bacteria) are not well understood. Therefore, the present study was aimed to (1) develop a SNAD process in a laboratory scale SBR using synthetic wastewater, and (2) investigate the effect of hydraulic retention time (HRT) on the performance of SNAD.

2. Methods

2.1. SNAD seed sludge

The SNAD seed sludge was collected from a biological treatment unit (aeration tank) of the full-scale landfill-leachate treatment plant, Taiwan. The operating conditions established the SNAD process in the aeration tank (384 m^3) were: DO ~0.3 mg/L, pH ~7.4, HRT ~1.26 d, and the sludge retention time (SRT) ~12 to 18 d. The fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) techniques were applied to verify the presence of anammox bacteria in the SNAD seed sludge (Wang et al., 2010). In addition to anammox bacteria the seed sludge also consists of nitrosomonas-like aerobic microorganisms and denitrifiers. The anammox bacteria are chemolithoautotrophic in nature and having a doubling time of 11 d (Strous et al., 1998). The physicochemical analyses were used to confirm the activities of nitrifiers, anammox and denitrifiers in the SNAD seed sludge. Subsequently, biotechnological analyses such as FISH and PCR were conducted to verify the presence of anammox bacteria (Wang et al., 2010). Moreover, the studies are still in progress to confirm the ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB) and denitrifiers in the system. Overall, the absence of NH_4^+, NO_2^- and glucose (as carbon source) in the influent during initial stages has not shown any activity of the SNAD sludge in the lab-scale reactors. On the other hand, the seed sludge has shown reversible inhibition to oxygen and irreversible inhibition to phosphate.

2.2. Reactor system and the operation strategy

A sequencing batch reactor (SBR) with a working volume of 18 L was used for the establishment of the SNAD process. The schematic diagram of the SBR is shown in Fig. 1. As a precursor, the acclimation process was strated using 2 L of the SNAD seed sludge. The synthetic wastewater was used as a feed; the composition of the feed wastewater is shown in Table 1. The acclimation of the SNAD sludge was strated immediately after the inoculation of the seed sludge. The temperature of the SBR was always controlled at 35 °C by using a thermostatic water jacket, and the pH was maintained in a range of 7–8. The air flow into the reactor was controlled using a pneumatic valve. The dissolved oxygen (DO) concentration in the sample was measured outside the SBR using a DO meter. The DO concentration in the reactor was maintained around 0.5–1 mg/L and the alkalinity was maintained in a range of 250–300 mg $CaCO_3$/L. At the same time, NH_4^+ oxidation to NO_2^- (partial nitrification) was controlled by adjusting the DO concentration in the reactor. At any stage, the NO_2^-–N concentration was not allowed to exceed over 100 mg NO_2^-–N/L beyond which anammox process could be inhibited (Strous et al., 1999). By controlling the NO_2^-–N concentration in the SBR, the anammox reaction was initiated with a proper stoichimetric requirement of NH_4^+ and NO_2^-. A complete mixing inside the SBR was ensured by mixing the reactor contents via a 3-bladed mechanical stirrer at a rate of 100 rpm. After the acclimation process, the performance of the SBR for treating synthetic wastewater with ammonium (200 mg/L) and COD (100 mg/L) was investigated under three different hydraulic retention times (HRTs), i.e., 9, 4.5 and 3 d, and keeping infinite sludge retention time (SRT). For acclimation as well as studying the effect of HRT, the SBR was operated in cycles of 24 h and each cycle consists of feeding and reaction (23.4 h), settling (0.35 h) and decanting the supernatant (0.25 h).

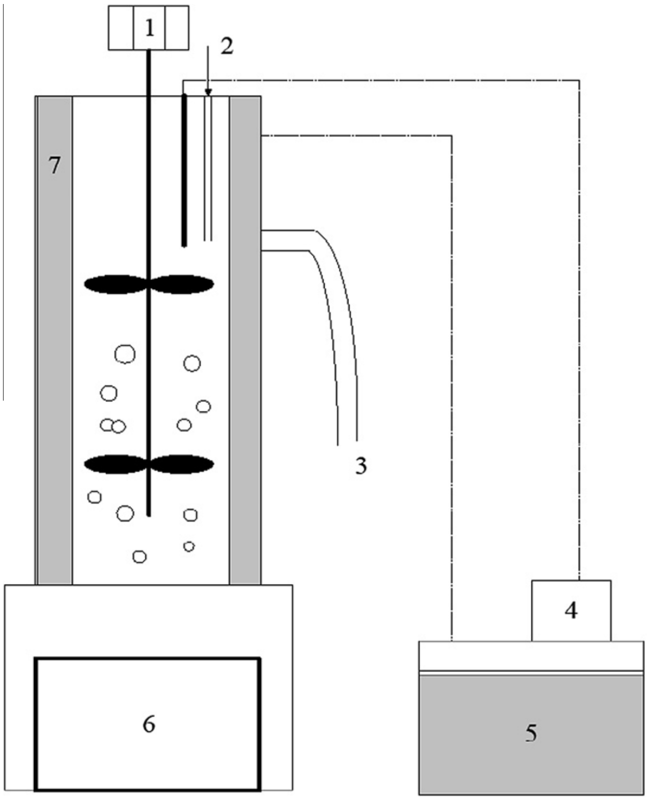

Fig. 1. Schematic representation of the lab-scale SBR (1) mechanical stirrer, (2) influent, (3) effluent, (4) DO measurement, (5) thermostat, (6) controller for mechanical stirrer, and (7) thermostatic water jacket.

Table 1
Composition of the synthetic wastewater used in this study.

Composition of synthetic wastewater	mg/L	Composition of trace element solution	mg/L
NH_4^+–N (supplied from $(NH_4)_2SO_4$)	200	EDTA	1500
NO_3^-–N (supplied from $NaNO_3$)	17	$ZnSO_4{\cdot}7H_2O$	430
$KHCO_3$	2000	$CoCl_2{\cdot}6H_2O$	240
NaH_2PO_4	100	$MnCl_2{\cdot}4H_2O$	990
$CaCl_2{\cdot}2H_2O$	100	$CuSO_4{\cdot}5H_2O$	250
$MgSO_4{\cdot}7H_2O$	58	$NaMoO_4{\cdot}2H_2O$	220
$FeSO_4{\cdot}7H_2O$	18	$NiCl_2{\cdot}2H_2O$	190
EDTA-2Na	20	$NaSeO_4{\cdot}10H_2O$	210
COD[a] (supplied from glucose)	100	H_3BO_4	14
Trace element (mL/L)	1		

[a] COD is supplied as glucose ($C_6H_{12}O_6$), and 1 g of glucose produces 1.06 g of COD.

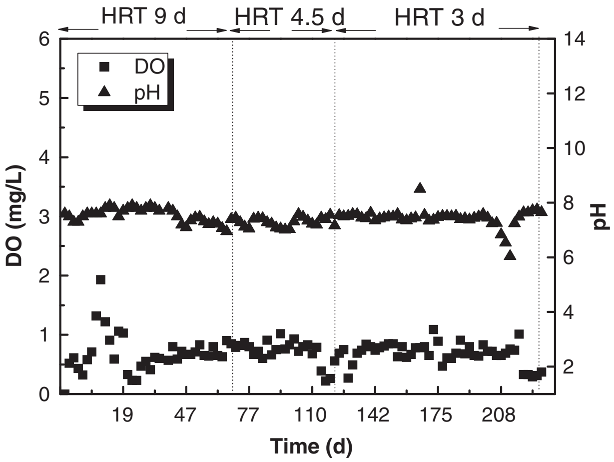

Fig. 2. Profiles of DO and pH during the SBR operation under various HRTs.

The operating conditions of the SBR under various HRTs are shown in Table 2. The organic loading rate (OLR) and nitrogen loading rate (NLR) to the SBR under various HRTs were worked out, and are also shown in Table 2. However, the NH_4^+–N and COD concentrations were kept constant under all HRTs and the ratio of influent COD/TN was maintained at a constant level (0.5).

2.3. Analytical methods

The concentrations of nitrogen compounds, suspended solids (SS), volatile suspended solids (VSS), mixed-liquor suspended solids (MLSS), mixed-liquor volatile suspended solids (MLVSS) and alkalinity were measured according to the Standard Methods (APHA, 1998). The NH_4^+–N, NO_2^-–N and NO_3^-–N concentrations were determined spectrophotometrically, and the organic matter content in the synthetic wastewater was expressed as COD. The pH was determined potentiometrically with a digital pH meter (SUNTEX SP-701, Taiwan) and the DO was monitored with a digital DO meter (YSI 5100, Taiwan).

3. Results and discussion

3.1. Profiles of pH and DO

Fig. 2 shows the profiles of pH and DO concetration in the reactor under various HRTs investigated. The pH profile was fairly constant over the HRTs except the final days of operation at 3 d HRT owing to the malfunction of the aerators. The decrease in pH at any point of time was componsated by the addition of alkalinity to the reactor. The DO concentration in the reactor was varying alot in the intitial days of operation, i.e., 9 d HRT. The activity of anammox bacteria and denitrifiers in the SNAD system relies on partial nitrification becuase the later supplies NO_2^-–N to anammox and denitrification. Moreover, anammox bacteria and denitrifiers prefer anoxic/anaerobic environment. Therfore, there was some difficulty in controlling the air flow rate to the system in the intital days of SBR operation (0–19 d, Fig. 2). After this stage, the airflow was adjusted in such a way to maintain the DO of the reactor at a constant level.

3.2. Nitrogen and COD removals under various HRTs

At 9 d HRT, the SBR was operated with influent NH_4^+–N and COD concentrations of 200 and 100 mg/L, respectively, corresponding to the NLR of 22.2 g/m^3-d and OLR of 11.1 g/m^3-d. The influent and effluent profiles of nitrogenous matter and organics are shown in Figs. 3 and 4, respectively. In the first 40 d of operation, a consistent NH_4^+–N removal was observed and small quantities of NO_2^-–N and NO_3^-–N accumulation were found in the SBR. However, the COD concentration in the SBR displayed a very poor removal. In the subsequent days (40–65 d), the removal efficiencies increased gradually and have shown a stable NH_4^+–N and COD removal efficiencies of 96% and 87%, respectively.

In order to find the effect of loading rate on the SNAD process, the NLR and OLR were progressively increased by decreasing the HRT from 9 d to 4.5 d, and operated for 47 d (Table 2). Despite the higher influent NLR and OLR, a stable conversion of NH_4^+–N, and no NO_2^-–N/NO_3^-–N accumulation was observed in the SBR. The increases in the NLR (44 g/m^3-d) and OLR (22 g/m^3-d) have decreased the COD removal efficiency from 87% to 78%, whereas the NH_4^+–N removal efficiency was maintained in the same level, i.e., 95%. This reveals that the increases in OLR and NLR have no significant effect of the SNAD system. Table 2 shows the steady-state concentrations of NH_4^+–N, NO_2^-–N, NO_3^-–N and COD under various

Table 2
Characteristics of the synthetic wastewater before and after treatment.

HRT (d)	NH_4^+–N (mg/L)		NO_2^-–N (mg/L)		NO_3^-–N (mg/L)		COD (mg/L)		Inf. COD/TN[c]	OLR (g/m^3-d)	NLR (g/m^3-d)	Eff. TN (mg/L)[c]	Removal (%)		Remarks
	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.					NH_4^+–N	COD	
9	200	9	0	2.1	17	1	100	14	0.5	11.1	22.2	12.1	96	87	–
4.5	200	10	0	0.4	17	0.1	100	16	0.5	22.2	44.4	10.5	95	78	VFR increased by two times[d]
3[a]	200	14	0	2	17	0	100	29	0.5	33.3	66.7	16	93	72	VFR increased by three times[d]
3[b]	200	94	0	0.4	17	0.5	100	12	0.5	33.3	66.7	107	52	86	Zone of aerator problem (last for 29 days)

[a] VFR increased by three times, and without aerator and water jacket problems.
[b] VFR increased by three times, and with aerator and water jacket problems.
[c] TN is the sum of NH_4^+–N, NO_2^-–N and NO_3^-–N.
[d] VFR represents volumetric flow rate and the increases, i.e., two and three times, based on 9 d HRT.

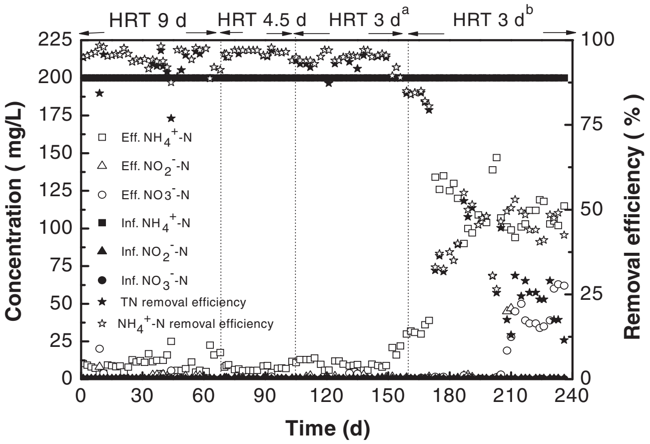

Fig. 3. Profiles of influent and effluent nitrogen concentrations, and their removal efficiencies.

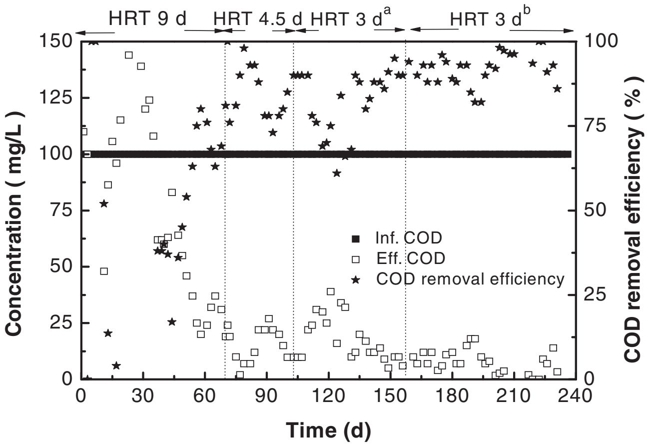

Fig. 4. Profiles of influent and effluent COD, and their removal efficiencies.

HRTs. Following to the steady-state condition at 4.5 d HRT, the reactor NLR and OLR were increased to 66 and 33 g/m^3-d, respectively. The decrease in the HRT to 3 d has decreased the NH_4^+–N and COD removals in the system. An increasing trend in the effluent NH_4^+–N concentration can be noticed in Fig. 3. This indicates that the increases in NLR and OLR (at 3 d HRT) have produced slight inhibition/toxicity to the partial nitrifiers; as a result, insufficient NO_2^-–N was produced in the system. As a result, the performance of anammox and denitrification were deprived and an overall decrease in the TN and COD removals were observed. However, an improvement in the COD removal was observed in the subsequent period (120–150 d) and reached a stable COD removal of 72%.

Unexpectedly, aerator and water jacket were went out-of-order under this recovery stage, which drastically decreased the reactor performance. Under this stage, the DO in the SBR has went down to below 0.2 mg/L, pH drop down to less than 6 and the temperature decreased by 5–8 °C. It can be noticed in Table 2 that only 52% of the NH_4^+–N was removed in the reactor, and interestingly, around 86% of the COD was removed in the reactor. Under this situation, it is hypothesized that anammox bacteria might be inactive and the NO_2^-–N produced as a result of partial nitrification could have been utilized only by denitrifiers. These observations and hypothesis indicate that high DO concentrations (>2 mg/L) could result complete nitrification in the SNAD system, whereas low DO concentration (<0.5 mg/L) could reduce the rate of nitrification and overall performance of the reactor. Moreover, these data reveal that SNAD process is more resistant to substrate shock loading compared to sudden change in aeration rate and temperature.

3.3. Model based evaluation of the SNAD system

The consumption of nitrogen compounds in partial nitrification, anammox and denitrification are modeled using the stoichiometric equations and the experimental data. Generally, the presence of organic carbon is inhibitory to anammox bacteria. For example, the presence of methanol is found to have irreversible inhibition at concentration as low as 0.5 mM. However, a recent study indicated that anammox bacteria were successful in the oxidation of propionate, and the presence of glucose, formate and alanine had no effect on the anammox process. Moreover, anammox bacteria can be competitive with heterotrophic denitrifiers for the utilization of organic matter, i.e., propionate. But, the rate of propionate utilization by anammox bacteria was 0.6 mM/mg of protein/d, which is far less than the utilization rate by denitrifiers in real-time wastewater systems. The following stoichiometric relationships are used for modeling: (i) the molar ratio of NH_4^+–N/NO_2^-–N in partial nitrification is 1:1, (ii) the stoichiometric consumption (molar ratio) of NH_4^+–N/NO_2^-–N in anammox process is 1:1.32, and produces 0.26 mol of NO_3^-–N, subsequently that can be utilized in denitrification, (iii) 1 mg/L of NO_3^-–N is used for consuming 1.74 mg/L COD in denitrification. The TN removal in partial nitrification with anammox and denitrification under all the HRTs based on the stoichiometric modeling are shown in Table 3. Moreover, the detailed modeling concept and the outcomes for 3 d^a HRT based on the average influent and effluent data are shown in Fig. 5.

Table 3 indicates that around 85–87% of the TN removal is by the combination of anammox and partial nitrification. The NO_3^-–N produced in anammox process is utilized in denitrification along with COD, which is responsible for a TN removal of 7–9%. These observations indicate that under steady-state condition all three processes in the SBR, i.e., partial nitrification, anammox and denitrification, synchronize each other and establish a firm relationship within the reactor irrespective of the NLR and OLR. However, the shock in the operating DO, pH and temperature of the SNAD

Table 3
Performance of the SBR under various HRTs.

HRT (d)	TN removal (%)		Biomass produced (g/d)	Sensitivity Index (SI)[c,d]			
	Partial nitrification + anammox (%)	Denitrification (%)		NH_4	NO_2^-	NO_3^-	COD
9	85.7	8.7	0.067	– {9}	– {2.1}	– {1}	– {14}
4.5	87.3	7.8	0.259	0.4 (13)	0.1 (2.3)	0.8 (1.8)	0.9 (27)
3[a]	85.5	7.3	0.357	1.3 (21)	0.7 (3.6)	2.6 (3.6)	1.8 (39)
3[b]	41.9	8.7	–	14 (135)	0.1 (2.2)	0.6 (1.6)	2.6 (50)

[a] VFR increased by three times, and without aerator and water jacket problems.
[b] VFR increased by three times, and with aerator and water jacket problems.
[c] Sensitivity index based on the species concentration at 9 d HRT.
[d] The values within "{}" and "()" indicates average and maximum concentrations in mg/L, respectively.

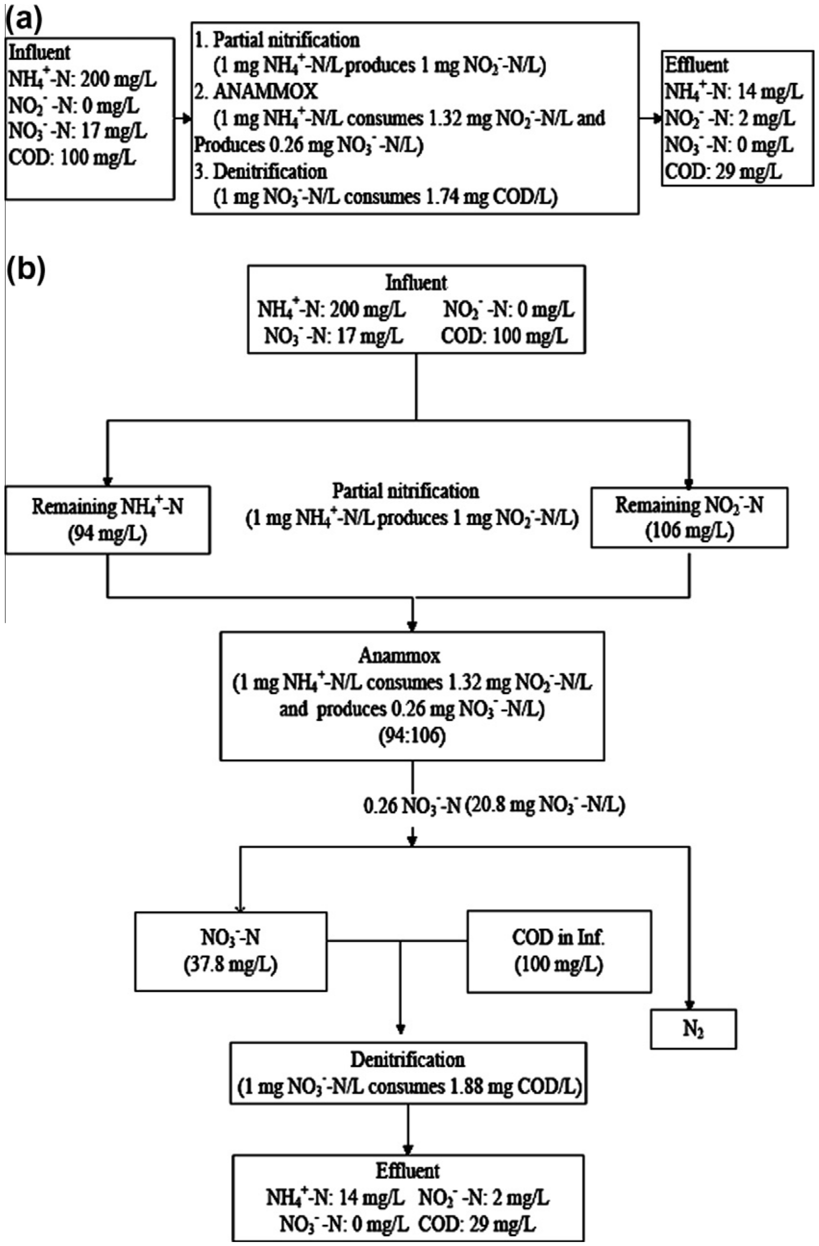

Fig. 5. Model based evaluation of the SNAD system.

system greatly affected the relationship of these processes. This can be evidenced from the poor NH_4^+–N removal efficiency of the system (52%). However, the overall TN removal efficiency of the SNAD system was maintained around 50.7% owing to the consumption of NO_2^-–N and/or NO_3^-–N in denitrification. The stoichiometric modeling results also indicate that the decrease in the HRT of the system (from 9 to 3 d) could facilitate the increase in the production of anammox bacteria (from 0.067 to 0.357 g/d). This approach could be useful to enrich the slow growing anammox bacteria in the real-time conditions. However, a very high volumetric flow rate (VFRs) could wash out the anammox bacteria from the system.

Alternatively, the sensitivity of the SNAD system to the change in VFR was evaluated based on sensitivity index (SI) as shown in Eq. (2) (Jing et al., 2009).

$$SI = \frac{O_{max} - O_s}{O_s} \qquad (2)$$

where, O_{max} is the maximum concentration of substrate in the effluent at 4.5 and 3 d HRTs (mg/L), and O_s is the average concentration of substrate in the effluent at 9 d HRT (mg/L). The values of SI for all nitrogen species and COD are shown in Table 3. The SI values indicate that the SNAD process is not greatly affected by the change in VFR of the system compared to the shock in the operating DO, pH and temperature conditions. Under the shocking DO, pH and temperature conditions, the SI values increased by 14 and 2.6 times for NH_4^+–N and COD, respectively. As indicated before, the anammox bacteria might be inactive under the shocking condition and the NO_2^-–N produced as a result of partial nitrification could have been utilized only by denitrifiers. This reveals that the SNAD system has the capability of acting as shortcut nitrification–denitrification (SND), i.e., NH_4^+–N is oxidized to NO_2^-–N in nitritation, and subsequently, the NO_2^-–N is reduced to N_2 gas. However, the removal efficiency of the SND system (under shocking condition) is far less than the efficiency observed in the SNAD system.

4. Conclusions

The SNAD process was successfully developed in the SBR and the effect of HRTs on the performance of SNAD system were investigated. Around 96% NH_4^+–N removal and 87% COD removal were achieved under the NLR and OLR of 22.2 and 11.1 g/m^3-d. The increases in NLR and OLR up to 66.7 and 33.3 g/m^3-d have produced little impact on the performance of the reactor; whereas, the sudden reduction/shock in the operating DO, pH and temperature has produced a major drop in the SNAD performance. The removal of nitrogenous compounds in each of the SNAD process was modeled using the stoichiometric relationship.

Acknowledgement

We thank National Science Council (NSC), Taiwan, ROC, for the financial support (NSC 98 -2221-E-009-022-MY3).

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