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# Ambient temperature SNAD process treating anaerobic digester liquor of swine wastewater

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## HIGHLIGHTS

- SNAD process was used to treat the anaerobic digester liquor of swine wastewater.
- High $NH_4^+$-N removal efficiency (96%) was achieved under steady state conditions.
- Average COD removal efficiency of 76% was achieved under steady state conditions.
- Organic nitrogen negatively affects the performance of SNAD process.
- Fed batch feeding strategy facilitated to have stable SNAD process.

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## ABSTRACT

In present study, effluent from anaerobic digestion of swine wastewater was treated by the simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD) process using a lab scale 5 L sequencing batch reactor (SBR) under ambient temperature. The fluctuation of anaerobic digester liquor quality (COD, 387 ± 145 mg/L; TKN, 662 ± 190 mg/L; $NH_4^+$-N, 519 ± 134 mg/L) and temperature created difficulties to develop a stable SNAD process in the SBR (days 1–285). Fed batch feeding strategy was adopted to have a stable condition in the reactor and overcome the negative effects of organic nitrogen. The average total nitrogen, $NH_4^+$-N and COD removal efficiencies in the SBR under steady state conditions (days 485–523) were 80%, 96% and 76%, respectively. The results showed that presence of organic nitrogen, mode of feeding and reactor temperature affects the SNAD process.

## 1. Introduction

As one of the major livestocks, swine production shares about 20% of total agricultural products (Chang et al., 1993) in Taiwan – the world's highest swine production density (tons/km$^2$) (Yeh et al., 2011). To meet the worldwide increasing demand of pork meat, number of swine farms has grown rapidly in recent decades. However, overall amount of wastewater produced from swine farm also increases with swine production. As swine wastewater contains high concentration of organic matter, nutrients (nitrogen and phosphorus) and other hazardous compounds (Lee and Shoda, 2008), its treatment and disposal is necessary. In Taiwan, swine wastewater treatment process is a three-stage system: solid liquid separation, anaerobic digestion and activated sludge process (Sheen et al., 1994). Anaerobic digestion is mainly used to produce energy (biogas) from the swine wastewater. The anaerobic digester liquor of swine wastewater (ADLSW) contains high concentration of ammonium nitrogen and therefore, its post-treatment is necessary. The carbon to ammonium (*C*/*N*) ratio of ADLSW is reported to be very low (Bernat et al., 2012; Figueroa et al. 2012; Vazquez-Padin et al., 2009), which makes traditional nitrification and denitrification process economically unfavorable. However, novel autotrophic nitrogen removal processes based on partial-nitrification combined with anammox (anaerobic ammonium oxidation) process are regarded as the most suitable for post-treatment of such wastewaters (Vazquez-Padin et al., 2009). Single reactor system for high activity ammonium removal over nitrite (SHARON) process combined with anammox, and completely autotrophic nitrogen removal over nitrite (CANON) processes have been successfully used to treat low *C*/*N* ratio wastewaters (van Dongen et al., 2001; Fux et al., 2002; Figueroa et al. 2012; Vazquez-Padin et al., 2009). SHARON–anammox process is a two-reactor system, in which SHARON converts 50% ammonium nitrogen into nitrite by aerobic ammonium oxidizing bacteria (AOB) and ammonium and nitrite in its effluent are autotrophically converted to nitrogen gas under anoxic conditions in anammox reactor. On the other hand, in CANON system partial nitrification and anammox reactions are simultaneously carried out under low DO levels. The combined partial nitrification–anammox process is regarded as the

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most cost effective treatment processes with low aeration requirement, no need of extra carbon source, less sludge production and less or no emission of greenhouse gases (Strous et al., 1998; van Graaf et al., 1996).

However CANON process removes only ~90% of influent $NH_4^+$-N (Eq. (1)), leaving ~10% of nitrogen as nitrate (Kumar and Lin, 2010). Additionally, presence of organic carbon, which is in general present in the ADLSW, can destabilize the CANON process.

$$1NH_3 + 0.85O_2 \rightarrow 0.11NO_3^- + 0.44N_2 + 1.43H_2O + 0.14H^+ \quad (1)$$

Coexistence of AOB, anammox and denitrifiers has opened the possibilities of converting this nitrate into nitrogen gas by consuming organic carbon in a single reactor (Chamchoi et al., 2008; Chen et al., 2009; Gao et al., 2012; Lan et al., 2011; Wang et al., 2010). This novel process, with simultaneous partial nitrification, anammox and denitrification (SNAD) has successfully been used to remove nitrogen from synthetic wastewater, landfill leachate and optoelectronic wastewater (Chen et al., 2009; Wang et al., 2010; Daverey et al., 2012).

Recent researches indicated that anammox process (and therefore, its related processes such as CANON and SNAD) is very sensitive to environmental conditions (temperature, pH, salinity) and presence of inhibitors (nitrite, free ammonia, and high *C*/*N* ratio) (Vazquez-Padin et al., 2011; Dosta et al., 2007; Dapena-Mora et al., 2007; Mosquera-Corral et al., 2005; Tang et al., 2009). However, little attention has been paid on the effect of organic nitrogen on anammox process.

**Table 1**
Nature of ADLSW.

| Parameter | Average value[a] | Measurements | Range | |
|---|---|---|---|---|
| | | | Maximum | Minimum |
| COD | 387 ± 145 | 12 | 720 | 206 |
| BOD | 288 ± 229 | 8 | 812 | 78 |
| sBOD | 81 ± 69 | 8 | 208 | 1 |
| TKN | 662 ± 190 | 18 | 991 | 403 |
| $NH_4^+$-N | 519 ± 134 | 18 | 766 | 305 |
| $NO_2^-$-N | <1 | 18 | 0.3 | 0 |
| $NO_3^-$-N | 3.5 ± 2.9 | 18 | 9 | 0 |
| $PO_4^{3-}$-P | 30 ± 23 | 9 | 64 | 2.5 |
| pH | 7.4 ± 0.3 | 7 | 7.7 | 7.1 |
| Alkalinity[b] | 3036 ± 1036 | 9 | 5100 | 1900 |

[a] All units in the table are in mg/L, except pH and alkalinity.
[b] As $CaCO_3$.

Sequencing batch reactor (SBR) is considered to be the most suitable reactor for the growth of anammox bacteria due to complete biomass retention (Strous et al., 1998; van Graaf et al., 1996). Some researchers recognized feeding strategy as one of the important factors for improving treatment efficiency in SBR (Cheong and Hansen, 2008; Shizas and Bagley, 2002), which can also affect the stability and performance of anammox or its related processes (CANON and SNAD). To the best of our knowledge no report is available on the effect of feeding strategy on the stability of anammox or SNAD process in SBR.

Therefore, the aims of this work were to (1) remove nitrogen and organic matter present in ADLSW by SNAD process in a lab scale SBR under ambient temperature, (2) study the effect of organic nitrogen on the performance of SNAD process, and (3) study the effect of feeding strategy on the stability of SNAD process.

## 2. Methods

### 2.1. Wastewater

The ADLSW was collected twice a month from the swine farm located in Taiwan and kept at 4 °C until used. The nature of collected ADLSW is presented in Table 1. The average chemical oxygen demand (COD) to $NH_4^+$-N ratio, represented as COD/$NH_4^+$-N ratio was 0.75 and the COD (387 ± 145 mg/L), total Kjeldahl nitrogen (TKN, 662 ± 190 mg/L) and $NH_4^+$-N (519 ± 134 mg/L) fluctuated widely over the studied period. The maximum values of COD, TKN and $NH_4^+$-N concentration in wastewater were 720, 991 and 766 mg/L, respectively. The difference between TKN and $NH_4^+$-N values in Table 1 suggests the presence of organic nitrogen in ADLSW. The major inorganic nitrogen in the ADLSW was $NH_4^+$-N.

### 2.2. Reactor set-up and operational conditions

A 5 L SBR (Fig. 1) was used to treat the ADLSW. The SBR was inoculated with sludge collected from a landfill-leachate treatment plant located in Taiwan, known to have AOB, anammox bacteria and denitrifiers (Wang et al., 2010). An overhead stirring (~100 rpm) ensured the proper mixing of reactor contents. The SBR was operated in cycles of 24 h – consisted of 23.4 h for reaction (including feeding time), 0.45 h for settling and 0.15 h for decanting. Feeding of ADLSW was introduced into the SBR either in a short feed (5 min) batch mode (days 1–426) or a longer feed (12 h) fed-batch mode (days 427–523). Under fed-batch mode of feeding, the flow rate of peristaltic pump was adjusted in such a

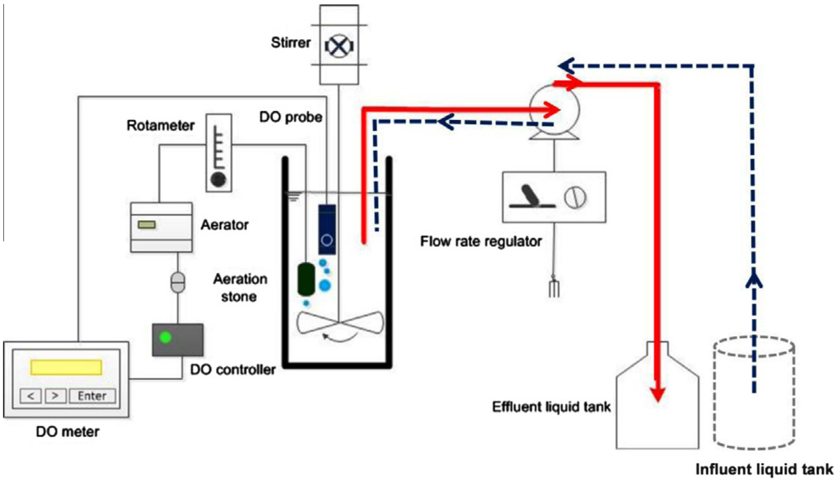

**Fig. 1.** Schematic diagram of sequencing batch reactor used to study the treatment of anaerobically digested liquor of swine wastewater.

**Table 2**
SBR operation conditions to treat ADLSW.

| Parameter | Value | | | | | |
|---|---|---|---|---|---|---|
| Duration (d) | 1–41 | 42–83 | 84–295 | 296–405 | 406–483 | 484–523 |
| Inf. $NH_4^+$-N (mg/L) | 225 | 263 | 263–395 | 263 | 438 | 370 |
| Inf. TKN (mg/L) | 225 | 331 | 331–582 | 330 | 560 | 630 |
| HRT (d) | 5 | 5 | 2.5 | 2.5 | 2.5 | 2.5 |
| NLR ($g/m^3$ d) | 45 | 53 | 105–158[a] | 105 | 175 | 153 |
| OLR ($g/m^3$ d) | 26 | 28 | 56–132[a] | 76 | 139 | 236 |

[a] Variation in NLR and OLR was due to dilutions.

way so that the feeding of wastewater took 12 h to avoid the shock loading. The temperature of reactor was not controlled and it fluctuated between 15 and 30 °C during the study under ambient condition. The pH of reactor content was maintained in a range of 7–8 and it was measured in the effluent sample using a pH meter. The DO concentration in reactor was maintained below 0.5 mg/L either manually (from day 1 to 304) using a rotameter or automatically using a DO controller (from day 338 to 523). Between days 305 to 337 reactor was kept idle for rearrangement of DO controller. DO was measured either in the effluent (from day 1 to 284) or inside the reactor (from day 285 to 523) using a DO meter. The studies on nitrogen and COD removals from ADLSW in the SBR were carried out by varying nitrogen loading rates (NLR) – either by reducing the hydraulic retention time (HRT) and/or diluting the wastewater with deionized water. Table 2 shows the operational strategy to treat the ADLSW in the SBR.

### 2.3. Batch test for specific anammox activity (SAA)

SAA tests for the sludge samples periodically taken from the reactor were performed in serum bottles (total volume of 67 ml) according to the methodology reported by Dapena-Mora et al. (2007). The sludge sample was washed twice with phosphate buffer solution (0.14 g/L of $KH_2PO_4$, 0.75 g/L of $K_2HPO_4$ and 0.5 g/L of $KHCO_3$) before starting the experiment. Subsequently, each serum bottle was filled with 53.6 ml of sludge, 1.7 ml of $NH_4Cl$ solution (2300 mg/L) and 1.7 ml of $NaNO_2$ solution (2300 mg/L) so that the concentrations of $NH_4^+$-N and $NO_2^-$-N inside the bottles were 70 mg/L, respectively. The initial pH value was ~7.7 before starting the experiments. The headspace (~10 ml) was flushed with $N_2$ to maintain anaerobic condition. The serum bottles were incubated at 125 rpm and 25 °C in a thermostatic shaker. After initial equalization to atmospheric pressure, the headspace pressure inside the bottle was measured every hour for 4 h using a pressure meter (Copal Electronics model PG-100 N, Taiwan). The SAA (g $N_2$–N/g $V_{SS}$ d) was calculated from the $N_2$ gas production rate divided by the volatile suspended solids ($V_{SS}$) concentration inside the bottle. All SAA tests were performed in triplicate.

### 2.4. Analytical methods

Analytical determination of nitrogen compounds (TKN, $NH_4^+$-N, $NO_2^-$-N and $NO_3^-$-N), suspended solids (SS), $V_{SS}$, alkalinity and COD were carried out according to the Standard Methods (APHA, 1998).

## 3. Results and discussion

### 3.1. Profiles of DO and pH

The profiles of DO and pH in the reactor are shown in Fig. 2. The DO was intended to maintain below 0.5 mg/L in the reactor. However, during startup period (day 1–100) it was fluctuated between 0.1 and ~4.0 mg/L due to slow DO consumption rate by microorganisms. Increase in DO above 0.5 mg/L was also observed due to the decrease in $NH_4^+$-N concentration in influent (days between 386 to 405 and 478 to 511), which reduced the oxygen demand (Fig. 2). Vazquez-Padin et al. (2009) also reported an increase in DO when $NH_4^+$-N concentration in the reactor was suddenly reduced.

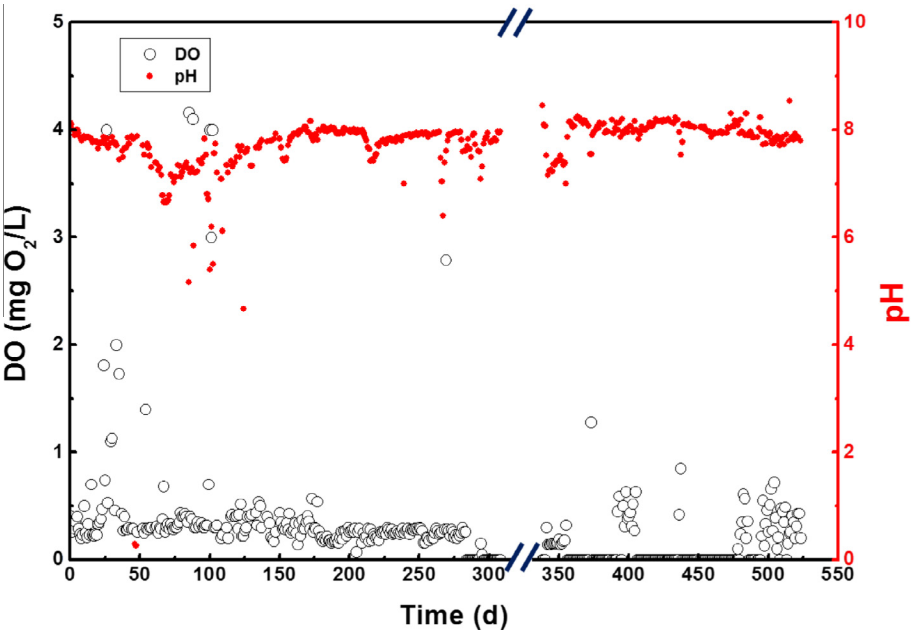

**Fig. 2.** Profiles of DO and pH in the reactor.

The pH was fluctuated between 7 and 8 during most of the operation period (Fig. 2). This pH is beneficial to suppress the unwanted growth of nitrite oxidizing bacteria (NOB) (Anthonisen et al., 1976; Vazquez-Padin et al., 2009). However, for DO level above 0.5 mg/L in the reactor, the pH reduced below 7 suggesting efficient ammonium oxidation by AOB.

### *3.2. Effect of organic nitrogen and feeding strategy on nitrogen removal*

Effect of organic nitrogen in influent and feeding strategy on the stability and nitrogen removal efficiency of SNAD process in the SBR treating ADLSW were evaluated in this study. Concentration profiles of nitrogen compounds in influent and effluent under batch mode (day 1–426) and fed-batch mode (day 427–523) of feeding are presented in Fig. 3(a and b), respectively. The concentrations of organic nitrogen did not correlate with influent $NH_4^+$-N concentrations. The percentage ratio of organic nitrogen to TKN in influent fluctuated between 0 and ~51% over the studied period.

Nitrogen removal efficiency of SBR under batch mode (day 1–426) and fed-batch mode (day 427–523) of feeding is presented in Fig. 4(a and b), respectively. For initial 83 days, the reactor was operated with influent $NH_4^+$-N concentrations between 225 and 265 mg/L at HRT of 5 d with a corresponding NLRs of 45 and 53 mg N/m$^3$ d. The $NH_4^+$-N and total nitrogen (TN) removal efficiencies were 89% and 69%, respectively on day 81. These high nitrogen removal efficiencies suggested that the reactor was started up very fast.

On day 84, HRT of the reactor was reduced at 2.5 d in order to increase the NLR. However, the TN removal efficiencies decreased to ~50% between days 84 and 110 and further decreased to below 25% after day 150 (Fig 4a). The sudden decline in HRT combined with existing high DO levels (above 1 mg/L, Fig. 2) and presence of organic nitrogen (70 mg/L between days 42 and 127) in influent had negative effects on the TN removal efficiency of the reactor.

The high $NH_4^+$-N with low TN removal efficiencies in between days 84 and 245 suggest the optimal growth of AOB in reactor, which oxidized $NH_4^+$-N to $NO_2^-$N, which was further oxidized to $NO_3^-$-N by NOB. However, after day 245, the $NH_4^+$-N removal effi-

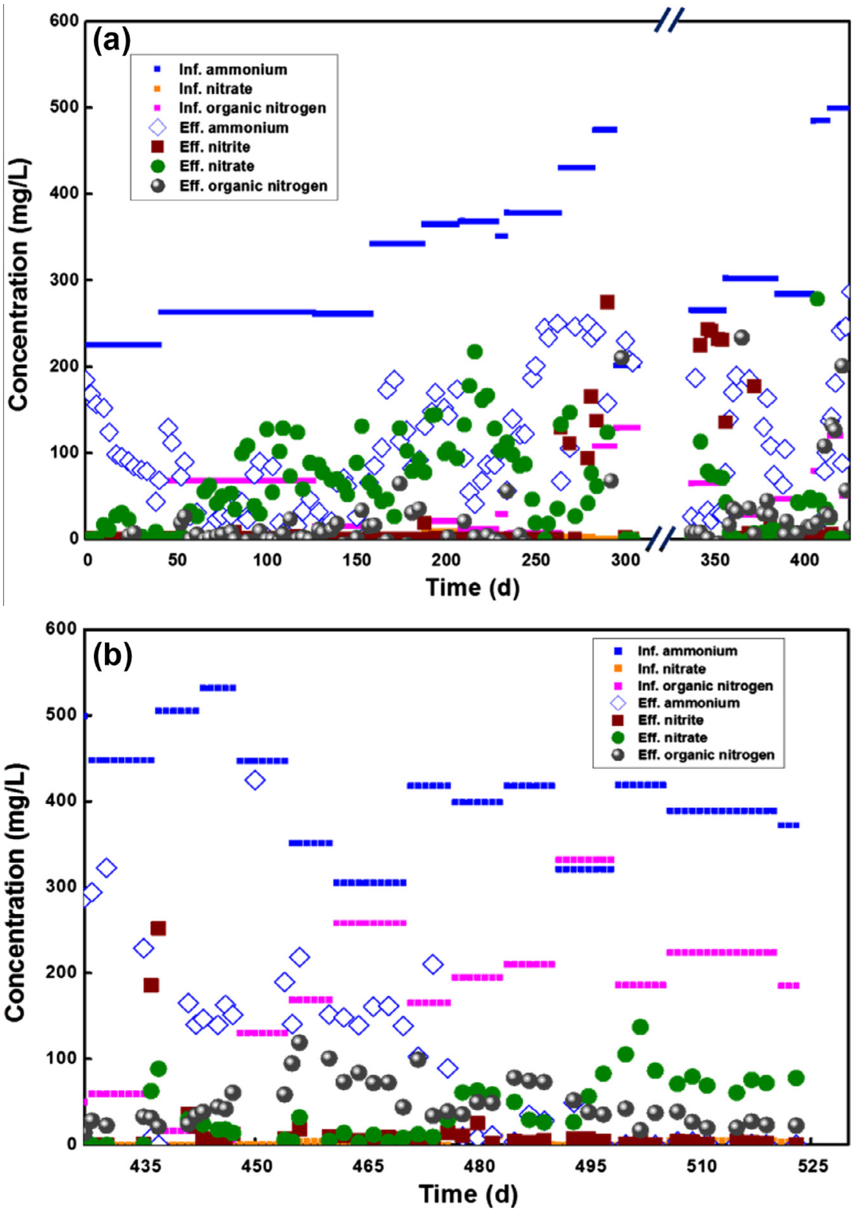

**Fig. 3.** Profiles of nitrogen compounds in influent and effluent (a) under batch mode of feeding and (b) fed batch mode of feeding.

ciency was also decreased below 50%. The percentage ratio of organic nitrogen to TKN in ADLSW was 18% (~108 mg/L of organic nitrogen) between days 284 and 295, and 39% (~129 mg/L of organic nitrogen) between days 296 and 301. This high organic nitrogen containing influent completely inhibited the TN removal efficiency of the system and nitrite was accumulated in the reactor (Fig. 4a). These results suggested that denitrification and ammonification were dominated over anammox in the reactor. Similar observation was reported by Ahn and Kim (2004) in nitrogen removal from the organic nitrogen-rich wastewater. A high concentration of nitrite is detrimental to anammox bacteria (Dapena-Mora et al., 2007; Strous et al., 1999). The effluent nitrite and nitrate concentration as measured on day 291 were as high as 275 mg/L and 124 mg/L, respectively (Fig. 3a) and as a consequence, a very low and unstable TN removal efficiency was observed (Fig. 4a). After day 357, percentage ratio of organic nitrogen to TKN reduced to 8% and the TN and $NH_4^+$-N removal efficiencies increased up to 90% and 96%, respectively on day 391 and maintained at these high levels for more than 15 days.

The ADLSW was treated in fed batch mode from day 427 to overcome the negative effect of high organic nitrogen on partial-nitrification and anammox process. However, the influent organic nitrogen fluctuated a lot (from 6 to 258 mg/L) between days 427 and 483 (Fig. 3b) and steady state could not be attained in the reactor. The ADLSW, which was used from day 484 onwards, had low $NH_4^+$-N (384 ± 35 mg/L), and was used without dilution (NLR of ~153 g/m$^3$ d). Even though the organic nitrogen concentration and percentage ratio of organic nitrogen to TKN in influent fluctuated between 185–332 mg/L and 30–51%, respectively, consistent performance of SBR in treating ADLSW was evident during this stage (Figs. 3b and 4b). In between of days 485 and 523, the average TN and $NH_4^+$-N removal efficiencies were 80% and 96%, respectively (Fig. 4b). The average $NH_4^+$-N and total inorganic nitrogen concentrations in effluent, and average nitrogen removal rate

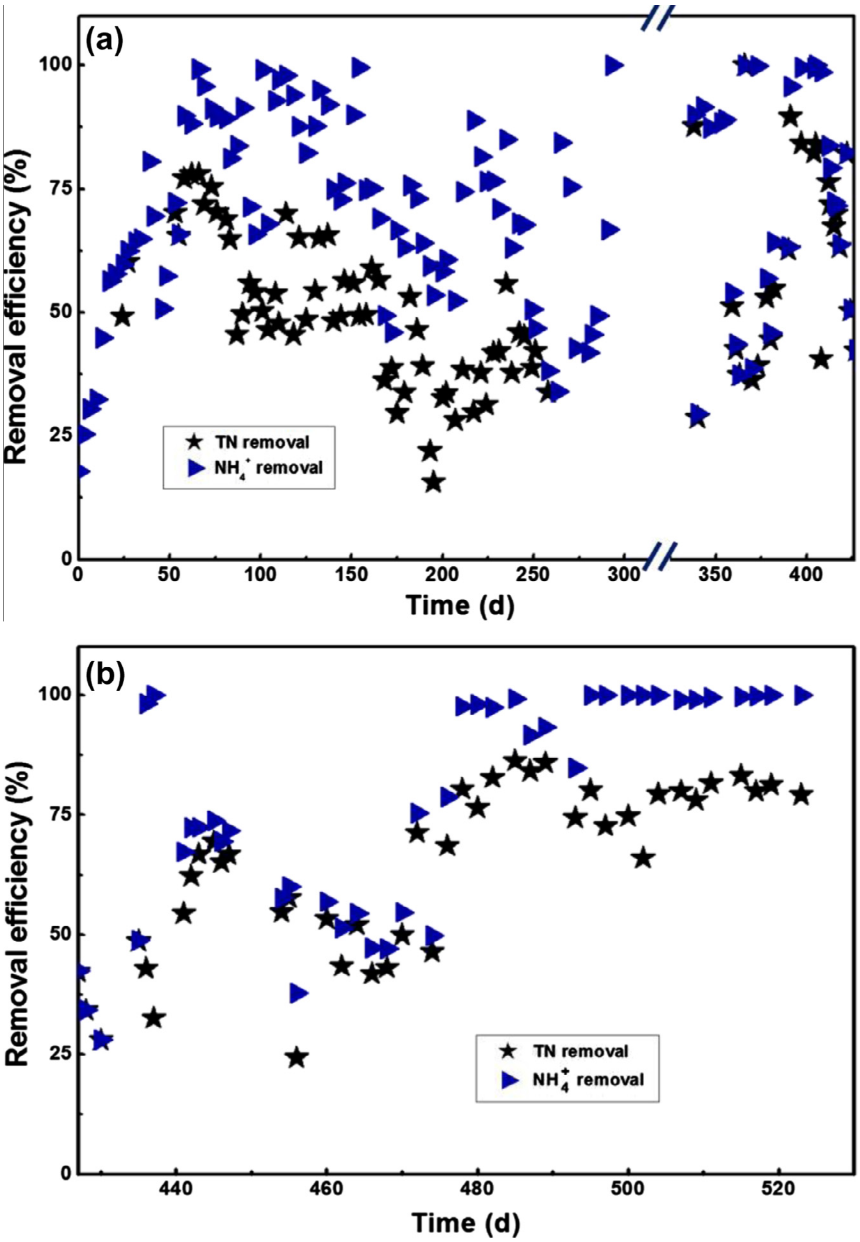

**Fig. 4.** Profiles of nitrogen removal efficiencies (TN removal, $NH_4^+$-N removal) (a) under batch mode of feeding and (b) fed batch mode of feeding.

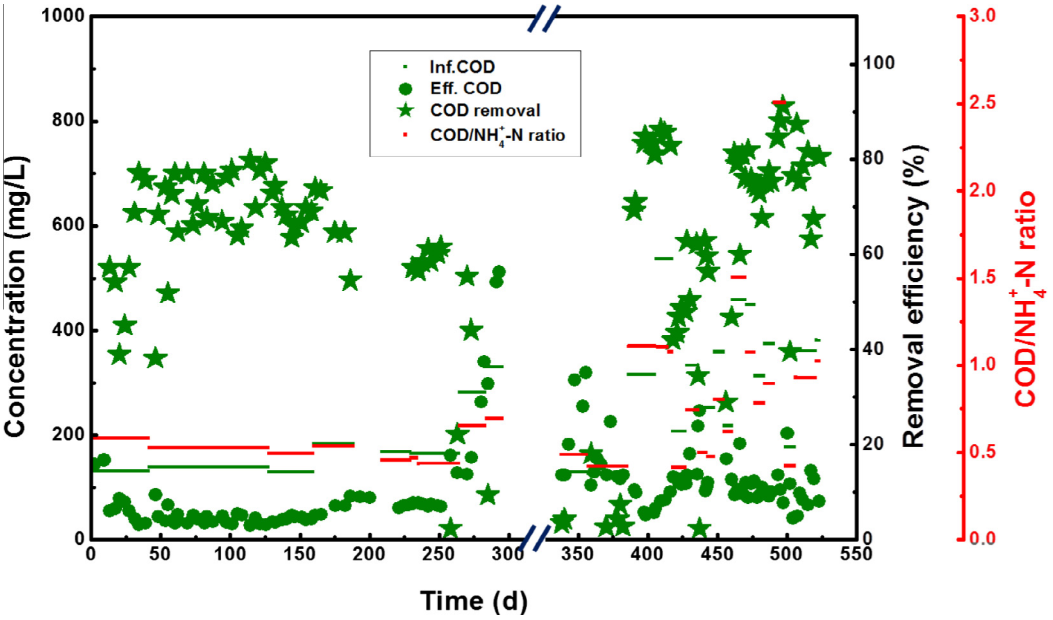

**Fig. 5.** Profiles of influent and effluent COD, COD/NH$_4^+$-N ratio and removal efficiencies of organic nitrogen and COD.

**Table 3**
SAA of sludge samples at various COD/NH$_4^+$-N ratio.

| Period (d) | COD/ NH$_4^+$-N ratio | TN removal efficiency (%) | NH$_4^+$-N removal efficiency (%) | SAA[a] (g $N_2$/ g $V_{SS}$ d) |
|---|---|---|---|---|
| 418–427 | 0.42 | 59 | 46 | 0.0437 |
| 428–436 | 0.74 | 38 | 52 | 0.0224 |
| 437–454 | 0.64 | 59 | 67 | 0.0341 |
| 455–476 | 1.06 | 51 | 59 | 0.0408 |
| 477–483 | 0.78 | 83 | 97 | 0.0499 |
| 491–498 | 2.56 | 74 | 85 | 0.0534 |
| 499–505 | 0.42 | 79 | 100 | 0.0405 |
| 506–520 | 0.93 | 80 | 99 | 0.0401 |

[a] Average values of three tests are reported.

under steady state (between days 485 and 523) were ~8 mg/L, ~80 mg/L and 0.123 kg N/m$^3$ d, respectively. The average nitrogen removal rate were 0.123 kg N/m$^3$ d. These results indicate that fed batch mode of feeding can overcome the negative effect of organic nitrogen in wastewater.

### 3.3. COD removal

Fig. 5 shows the removal efficiencies of COD in SBR. Despite of the fluctuation in nitrogen removal (TN and NH$_4^+$-N) efficiencies, the COD removal was very high (~64%) during initial 250 days. This suggests the existence of heterotrophic denitrifying microorganisms in the reactor, which utilize organic carbon and nitrite/nitrate as electron donor and acceptor, respectively. Recently, coexistence of anammox and denitrifiers has also been reported in literature (Chamchoi et al., 2008; Daverey et al., 2012; Gao et al., 2012; Wang et al., 2010).

However, the COD removal efficiency severely decreased after day 250, probably due to insufficient organic nitrogen in feed and poor nitrifying activity of AOB, which provides electron donor for denitrifiers at higher COD/NH$_4^+$-N (0.67). Mosquera-Corral et al. (2005) also observed inhibition of nitrifying activity at $C/N$ ratio of 0.6 g COD/g N. After day 338, the COD removal efficiency slowly increased and varied between 69% and 83% during days 390 and 416. However, due to fluctuations in influent COD/NH$_4^+$-N (0.47–0.8) between days 418 and 460, COD removal efficiency reduced and the average value was found to be 49% (Fig. 5). The COD removal efficiency was almost consistent thereafter with an average value of 76%.

### 3.4. Influence of COD/NH$_4^+$-N ratio on anammox activity

To evaluate the influence of organic nitrogen and carbon compounds on anammox activity, SAA test was carried out at different COD/NH$_4^+$-N ratios (Table 3). It is clear from the table that COD/NH$_4^+$-N ratio influenced the anammox activity. An increase in COD/NH$_4^+$-N ratio from 0.42 to 0.74 reduced the SAA by approximately 50% (Table 3). Molinuevo et al. (2009) also reported that COD negatively affects the anammox process and decreased the anammox bacterial population. The complete suppression of anammox activity was observed by Chamchoi et al. (2008) at COD concentration over 300 mg/L or COD/NH$_4^+$-N ratio over 2.0. However, negative effect of high COD/NH$_4^+$-N ratio (>0.7) on anammox activity was overcome with a fed batch feeding mode. Under steady state, the maximum COD/NH$_4^+$-N ratio and COD concentrations were ~2.5 and 800 mg/L, respectively in the influent. The TN and NH$_4^+$-N removal efficiencies were 74% and 85%, respectively at this COD/NH$_4^+$-N ratio.

### 3.5. Effect of temperature

In this study performance of the SBR for treating ADLSW was evaluated under ambient temperature. Fig. 6 shows that temperature was fluctuated under the influence of ambient temperature between 15 and 30 °C. The temperature varied between 15 and 20 °C between days 265 and 356 (in winter), and we observed very low TN (<50%) and COD removal (<20%) efficiencies during this

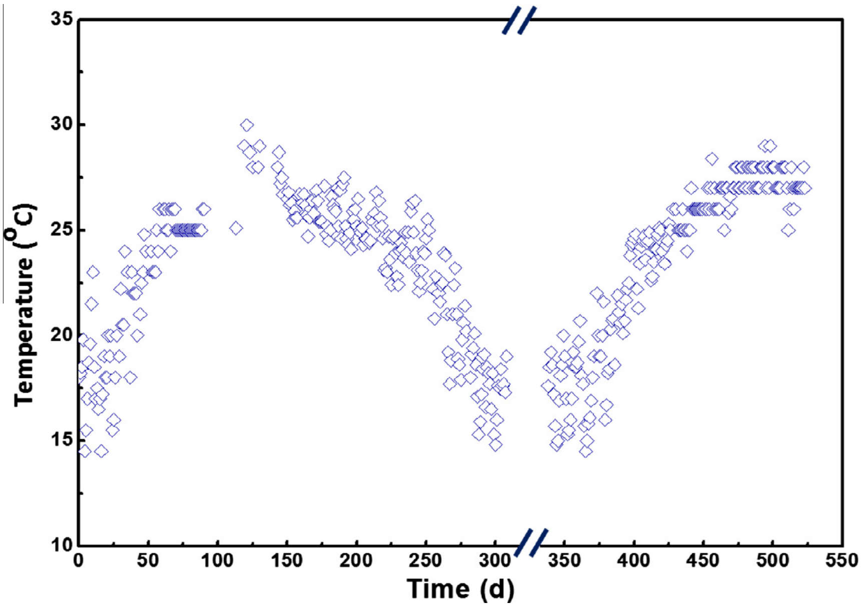

**Fig. 6.** Profile of temperature in the reactor.

period. On the other hand, between days 472 and 523 (in summer), the average temperature was 27 °C and the average values of $NH_4^+$-N, TN and COD removal efficiencies were 93%, 79% and 77%, respectively (Figs. 4 and 5). The optimum temperature for AOB, anammox and denitrifiers is believed to be approximately 35 °C (Gu et al., 2012; Strous et al., 1999; Lalucat et al. 2006). Though, they all can grow between 15 and 30 °C, their specific growth rate and activities are reported to be higher at higher temperatures. The maximum specific growth rate of NOB (undesirable) is higher than AOB at temperature below 25 °C. On the other hand, denitrifiers grown at 20 °C exhibited 75-fold less levels of *cnorB* gene expression compared to grown at 30 °C (Saleh-Lakha et al., 2009). Therefore, fluctuations in the reactor temperature and wastewater composition (COD concentrations and organic nitrogen) had synergistically negative effect on SNAD process in our study.

## 4. Conclusions

The average values of TN and COD removal efficiencies by SNAD process under batch mode of feeding in SBR for treating ADLSW with fluctuating influent quality were 52% and 57%, respectively. When SBR was operated under fed batch feeding mode, the average TN and COD removal efficiencies improved to 65% and 68% (80% and 76% under steady state), respectively. This study suggested that to achieve a stable performance of SNAD process for treating real wastewater, temperature must be controlled to above 20 °C and fed batch feeding mode should be adapted to feed wastewater into the reactor.

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