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ORIGINAL PAPER

High-rate nitrogen removal from livestock manure digester liquor by combined partial nitritation–anammox process

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Abstract In this study, combination of a partial nitritation reactor, using immobilized polyethylene glycol (PEG) gel carriers, and a continuous stirred granular anammox reactor was investigated for nitrogen removal from livestock manure digester liquor. Successful nitrite accumulation in the partial nitritation reactor was observed as the nitrite production rate reached 2.1 kg-N/m^3/day under aerobic nitrogen loading rate of 3.8 kg-N/m^3/day. Simultaneously, relatively high free ammonia concentrations (average 50 mg-NH_3/l) depressed the activity of nitrite oxidizing bacteria with nitrate concentration never exceeding 3% of TN concentration in the effluent of the partial nitritation reactor (maximum 35.2 mg/l). High nitrogen removal rates were achieved in the granular anammox reactor with the highest removal rate being 3.12 kg-N/m^3/day under anaerobic nitrogen loading rate of 4.1 kg-N/m^3/day. Recalcitrant organic compounds in the digester liquor did not impair anammox reaction and the SS accumulation in the granular anammox reactor was minimal. The results of this study demonstrated that partial nitritation–anammox combination has the potential to successfully remove nitrogen from livestock manure digester liquor.

Keywords Livestock manure digester liquor · Immobilization · Partial nitritation · Anammox · Nitrogen removal

Introduction

Anaerobic fermentation of livestock manure is practiced world wide due to several advantages such as no oxygen requirement, low sludge production and generation of methane gas for energy recovery (Baykov et al. 1994; Hill and Bolte 2000). Direct discharge of digester liquor after anaerobic fermentation is detrimental to receiving waters as it contains high concentrations of organic carbon, nitrogen and phosphorus. In particular, the presence of high nitrogen concentrations in the digester liquor can cause adverse impacts, such as toxicity to fish and

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other aquatic organisms, dissolved oxygen (DO) depletion, eutrophication and potential public health hazard (Obaja et al. 2003).

Results of a number of studies have been reported in literature for nitrogen removal from livestock manure digester liquor (LMDL) using conventional nitrification–denitrificaiton process (Bernet et al. 2000; Obaja et al. 2003, 2005; Yang et al. 2003; Shin et al. 2005; Vanotti et al. 2007; Dosta et al. 2008; Waki et al. 2008). Amongst these, Obaja et al. (2003) achieved relatively higher nitrogen removal rate of approximately 1.03 kg-N/m^3/day at HRT of 0.87 days but had to keep C/N ratio equal to or higher than 1.7 in order to realize complete denitrification. However, Obaja et al. (2005) stressed that using internal carbon source of non-digested pig manure required long HRT for removing nutrients. It is obvious from these results that relatively longer HRT and lower nitrogen removal rate reflect limitation of conventional nitrification–denitrification process. In addition, high operational cost and large footprint were also prerequisites for achieving high nitrogen removal efficiency.

In comparison, anammox process, discovered about 14 years ago (Mulder et al. 1995), directly combines ammonium and nitrite to produce dinitrogen gas and low levels of nitrate in accordance with Eq. 1.

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

Anammox process presents considerable potential for high nitrogen removal rate (NRR) from wastewaters containing low C/N ratio or high ammonium concentration, such as LMDL, landfill leacheate and sludge digester liquor (Strous et al. 1998). Generally, partial nitritation (PN) process precedes anammox process as a pretreatment step in order to supply ammonium and nitrite to anammox biomass. Combination of PN and anammox processes would not only reduce the operational cost significantly, but also decrease the post treatment cost due to the lower sludge production rate in anammox process (3 g-COD$_{biomass}$/mol NH_4^+) (Fux et al. 2002). Ahn et al. (2004) reported successful ammonium removal from actual piggery waste digester liquor using anammox process in a lab-scale UASB reactor by adding synthetic nitrite ($NaNO_2$) attaining average nitrogen removal rate (NRR) of 0.66 kg T-N/m^3/day (0.3 kg NH_4–N/m^3/day). Hwang et al. (2005) and Yamamoto et al. (2008) also reported successful nitrogen removal from real piggery waste by combined PN–anammox processes, however the NRRs only reached 0.72 and 0.22 kg T-N/m^3/day for anammox reactors, respectively. The relatively lower NRRs reported in these two studies do not demonstrate realistic advantages of anammox process for nitrogen removal from high ammonium or low C/N ratio wastewaters. Low NRRs could be attributed to factors inhibitory to anammox bacteria, such as high levels of SS preventing contact between anammox bacteria and substrate, and organic carbon degradation resulting in proliferation of denitrifiers with concurrent decrease of anammox bacteria (Hwang et al. 2005; Chamchoi et al. 2008; Yamamoto et al. 2008; Molinuevo et al. 2009). Consequently, in order to demonstrate the realistic potential of nitrogen removal in anammox process, further efforts are required to avoid inhibition caused by high concentration of SS and organics in wastewaters with low C/N ratio or high ammonium concentration, including LMDL.

Successful and stable nitrite accumulation in PN process is another key factor to ensure nitrogen removal in the overall PN–anammox process. This stability could be achieved by utilizing superior sensitivity of ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) to operational conditions such as temperature, pH, DO and inhibitors. Several researchers have reported successful nitrite accumulation by controlling these factors (Hunik et al. 1994; Jetten et al. 1997; Hellinga et al. 1999; Ruiz et al. 2003). DO control may be difficult to implement since excessive oxygen availability will cause conversion of nitrate from nitrite. Manipulation of SRT may also be an alternate to “select” specific bacterial population and although shorter SRT will wash out most NOB from the reactor, it is inevitable that some AOB will also be washed out. The unavoidable AOB population washing out impairs the capacity of nitrite accumulation and further limits total NRR of the combined PN–anammox process. Therefore, nitrite accumulation by inhibition of free ammonia (FA) and free nitrous acid (FNA) is preferable as reported by Anthonisen et al. (1976). They stated that NOB were inhibited at FNA concentrations higher than 0.2–2.8 mg-HNO_2/l or 0.1–1.0 mg-NH_3/l, while AOB by FA concentrations higher than 10–150 mg NH_3/l. Thus, it is easier to accumulate nitrite at suitable FA or FNA levels by adjusting operational temperature and pH levels.

The purpose of this study was to investigate nitrogen removal from real LMDL through combined PN–anammox process. In particular, it was our objective to examine the possibilities of long-term nitrite accumulation in PN process by controlling FA and FNA levels and to assess NRR augmentation in granular anammox reactor treating PN reactor effluent. Effects of high concentrations of COD and SS in the digester liquor on anammox bacteria were also evaluated.

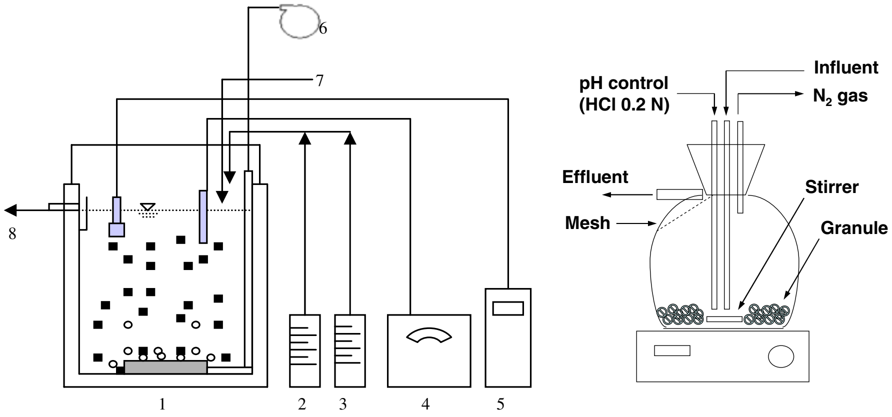
Materials and methods

Experimental setup

A column type reactor was used for the PN treatment in this study as shown in Fig. 1a. The reactor had an inner diameter of 17 cm and a height (to effluent port) of 35 cm. The volume of the reaction zone was 8.0 l and the HRT was maintained at 12 h. The temperature was controlled at about 30°C by a thermostatic water bath. The pH of the reactor was maintained between 7.5 and 8.0 by adding $NaHCO_3$ (1 N) or HCl (2 N), as required. pH controller (NPH-6900, Nissin Co. Ltd., Japan) was used for pH control. The air flow rate to the reactor was set at 8 l/min.

For preparing immobilized nitrifying sludge, PEG gel carriers (Hitachi Plant Technologies Co., Ltd., Japan) were mixed with a promoter (*N*,*N*,*N*′,*N*′-tetramethylenediamine) in water and sewage sludge in a beaker. An initiator (potassium persulfate) was added to the beaker to initiate polymerization. The resulting polymerized gel carrier was cut into 3-mm cubes and treated by heat shock for 1 h at 60°C to destroy nitrite oxidizing bacteria (Isaka et al. 2008). The gel carrier contained 10% (w/v) PEG, 0.5% (w/v) promoter, 0.25% (w/v) initiator, and 2% (w/v) sewage sludge (Isaka et al. 2008). 2.0 l of immobilized nitrifying sludge by PEG gel carriers was suspended in the reactor for PN treatment. Raw LMDL was obtained from Yamaga biogas plant (Yamaga city, Japan). The characteristics of this digester liquor are shown in Table 1.

The anammox reactor had a reaction volume of 0.73 l and is shown in the schematic diagram of Fig. 1b. The initial anammox granular sludge (cultivated in our laboratory) concentration in the reactor was set at 6 g/l (dry weight) (Furukawa et al. 2001). Before feeding raw LMDL to the anammox reactor, inorganic synthetic medium (Qiao et al. 2009), mainly composed of ammonium and nitrite, was fed for biomass cultivation. The synthetic inorganic medium was also used in the batch experiment undertaken to determine reaction ratio and for cultivating anammox biomass. A magnetic stirrer was used for improving contact between the anammox sludge and the substrate at 100 rpm. The pH was initially set at less than 7.9 according to the report of Strous et al. (1999) and the temperature was set at no lower than 30°C. The influent flow rate was kept at 3.6 l/day resulting in a constant HRT of 4.8 h and the influent nitrogen concentrations were increased stepwise to elevate nitrogen loading rate (NLR). The effluent from the PN reactor was collected and adjusted with the untreated raw LMDL to get the desirable nitrogen concentration (NH_4^+–N:NO_2^-–N = 1:1) for the anammox reactor.



A PN reactor

B Anammox reactor

Fig. 1 Schematic diagrams of the PN and the anammox reactors. *1* Water jacket, *2* HCl solution, *3* $NaHCO_3$ solution, *4* pH controller, *5* DO sensor, *6* Air pump, *7* Influent, *8* Effluent

Table 1 Characteristics of the raw digester liquor from biogas plant

Parameters	Average ± SD (mg/l)	No. of analyses
pH	8.3 ± 0	10
SS	2,056 ± 534	10
Alkalinity (as $CaCO_3$)	8,652 ± 537	8
TCOD	10,976 ± 1,018	6
SCOD	7,405 ± 503	6
BOD_5	1,250 ± 200	6
NH_4^+–N	1,583 ± 62	10
NO_2^-–N	2.4 ± 3	6
NO_3^-–N	4.2 ± 4	6
TN	1,871 ± 79	8
NH_4^+–N/HCO_3^- (mol/mol)	1:1.53	

Batch experiments

Batch experiments were carried out in 500-ml Erlenmeyer flask containing 400 ml mixed liquor to determine anammox reaction ratios with the synthetic wastewater and the real LMDL. After the anammox sludge was added followed by 10 min of bubbling with argon gas, the flask was closed with butyl rubber stopper. The initial ammonium and nitrite concentrations of synthetic wastewater were set at approximately 100 mg/l while the other components were similar to those reported by Qiao et al. (2009). For real LMDL treatment, effluent from PN reactor was combined with the untreated real LMDL and diluted to desirable concentrations. The flask was incubated at 35°C and continuously stirred with mixer at 100 rpm. Samples (5 ml) were taken at regular intervals and analyzed for NH_4–N, NO_2–N, and NO_3–N. All these batch experiments for synthetic and real LMDL were carried out in triplicate and the average values were used to calculate the reaction ratios.

Analytical methods

Nitrate nitrogen (NO_3–N), nitrite-nitrogen (NO_2–N), chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD_5), and alkalinity were measured according to Standard Methods (APHA 1995; 4500-NO_3^-E, 4500-NO_2^-B, 5220D, 5210B, and 2320B; respectively). Ammonium-nitrogen (NH_4–N) was quantified by the method described by Kanda involving use of *o*-phenylphenol as a substitute for liquid phenol (Kanda 1995). The suspended solids (SS) concentrations were determined according to Standard Methods (APHA 1995; 2540D). The pH level was measured by the electrometric method using a pH meter (IM-22P; TOA Electronics, Ltd., Tokyo, Japan). Dissolved oxygen (DO) was measured using a DO meter (HORIBA, pH/DO meter D-55).

Results and discussion

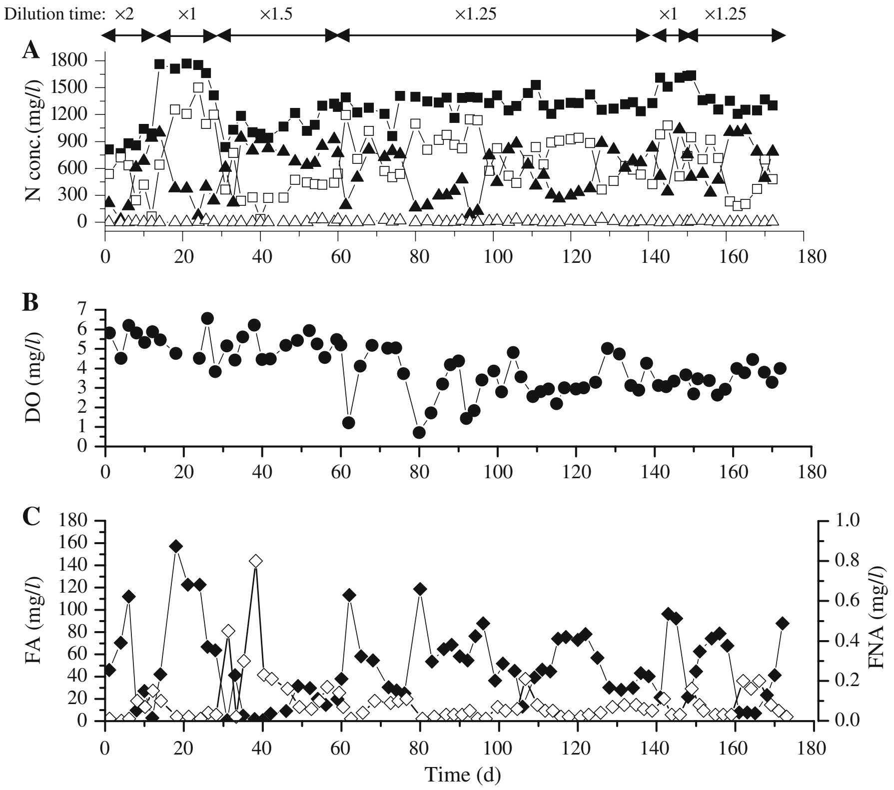
PN performance

The changes of nitrogen concentrations in the PN reactor during the operational period are shown in Fig. 2a. Accumulation of nitrite in the effluent was clearly observed during the first 10 days and the highest nitrite concentration reached nearly 1,000 mg/l. However, the subsequent high influent ammonium concentration suppressed the build-up of effluent nitrite resulting in decrease in effluent nitrite levels to about 300–400 mg/l. As the influent was diluted by 1.5 times, the accumulation of effluent nitrite was again observed and lasted for about 30 days with average nitrite concentration of 700 mg/l versus ammonium concentration of 350 mg/l. Later, high influent SS concentration caused clogging of the air diffuser several times, which resulted in the decrease in effluent nitrite while increasing effluent ammonium concentration. Resumption of effluent nitrite levels was observed after the air diffuser was replaced. From day 131, consistent build-up of effluent nitrite was confirmed and the average ratio of effluent nitrite to ammonium reached 1.72. During the operational period, the highest nitrite production rate of 2.1 kg-N/m^3/day and the highest TN loading rate of 3.8 kg-N/m^3/day were obtained. The effluent nitrate concentrations remained at very low levels as the highest concentration was only 35.2 mg/l accounting for about 2.3% of the effluent TN concentration. The accumulation of nitrite and depression of nitrate production in PN effluent was favorable for providing a suitable substrate for nitrogen removal by subsequent anammox process.

Effect of FA on PN performance

It is well known that ammonia (NH_3) and nitrous acid (HNO_2) are the true substrates of AOB and NOB, respectively. Both these substrates have inhibitory effect on AOB and NOB since these two kinds of bacteria are very sensitive to their own substrates and more so to the substrate of the other (Sinha and Annachhatre 2007). Anthonisen et al. (1976) reported that at FA concentrations between 1.0 and 10 mg-NN$_3$/l or FNA levels between 0.2 and 2.8 mg-HNO$_2$/l, only the activity of NOB will be inhibited and the accumulation of nitrite could be expected. Notwithstanding the negative effects of FA or FNA on the activity of NOB, it was reported that both AOB and NOB were able to acclimatize to high FA levels and nitrite accumulation could not be maintained for a long time (Turk and Mavinic 1989). In our study, FNA concentrations were frequently depressed below 0.2 mg-HNO$_2$/l due to the high operational temperature and pH levels, as shown in Fig. 2c. Also, the FA levels were much higher than the inhibiting limit reported by Anthonisen and the average FA concentration was approximately 50 mg-NH$_3$/l (peak of 160 mg-NH$_3$/l). In this range (over 1–10 mg-NH$_3$/l), the activities of both AOB and NOB are supposed to be severely inhibited and low effluent nitrite levels are expected. However, successful accumulation of nitrites was achieved in our study likely due to the fact that the AOB were immobilized into the PEG carriers providing a preventive cover to the inside microorganisms. Isaka et al. (2007) also reported a high nitrification rate of 0.71 kg N/m^3/day using the same PEG gel carriers at 10°C and considered that the AOB entrapped in the gel carrier belonged to the AH group due to its high Km values. Furthermore, the bulk DO levels during the entire experimental period varied between 2.5 and 6.5 mg/l, which did not fall in the favorable DO range (below 2.0 mg/l) for PN performance reported by other studies (Garrido et al. 1997; Pollice et al. 2002; Ruiz et al. 2003). Although the actual DO and FA concentrations inside the PEG gels were not measured in this study, we assumed that the inhibiting function on the NOB activity of FA was the main factor for nitrite accumulation in the PN effluent by considering the relatively higher FA concentrations and diffusion limitation into the inner part of the PEG gels. Further

Fig. 2 Time courses of nitrogen, DO, FA and FNA concentrations in PN reactor (*Solid squares* influent NH$_4$–N, *open squares* effluent NH$_4$–N, *solid triangles* effluent NO$_2$–N, *open triangles* effluent NO$_3$–N, *solid circles* DO, *solid diamonds* FA, *open diamonds* FNA)



investigation is required to determine the actual concentration gradient from the surface to the inside of PEG gels to confirm this assumption.

Anammox reactor performance

Before the effluent of PN reactor was introduced into the granular anammox reactor, batch experiments were carried out using synthetic medium and raw LMDL in order to investigate the feasibility and the reaction ratios of anammox process. Figure 3 shows comparison of the reaction rates for ammonium and nitrite removal, and nitrate production using synthetic medium and partial nitrified LMDL as feed substrate. The ammonium and nitrite consumption rates of partial nitrified LMDL were higher than those of synthetic wastewater, while the nitrate production rate of partial nitrified LMDL showed relatively lower values than that of the synthetic medium in the batch experiments. These results implied that there were minor effects of high COD and SS concentrations on nitrogen removal for granular anammox process.

For granular anammox reactor, the average nitrogen removal rate reached 3.2 kg-N/m^3/day with synthetic medium before the effluent of the PN reactor was fed into the anammox reactor. The effluent from the PN reactor was combined with untreated raw LMDL to get the desirable nitrogen concentrations (NH_4^+–N:NO_2^-–N = 1:1) for anammox reactor. The continuous stirred granular anammox reactor was operated for about 200 days for treating partial nitrified LMDL without addition of synthetic chemicals ($NaNO_2$), as illustrated in Fig. 4 and Table 2. In the first week, satisfactory removal performance was observed with high removal rates of over 2.0 kg-N/m^3/day. However, the unbalanced effluent with accumulated nitrite from PN reactor resulted in severe inhibition of anammox activity and the removal rate decreased gradually to a minimum of 0.04 kg-N/m^3/day on day 21. During the subsequent 100 days of operation, the TN removal rates fluctuated between 0.3 and 1.0 kg-N/m^3/day. The resumption of anammox activity was confirmed with increase in TN removal rate from 0.35 to 0.64 kg-N/m^3/day just after the pH was adjusted from 7.9 to 7.5. In the following 70 days of operation (from day 122 to 192), high removal rates and satisfactory performance were observed even with the stepwise increase in NLRs. Especially between day 160 and 192, the average TN removal rate of 2.6 kg-N/m^3/day (peak of 3.12 kg-N/m^3/day on day 192) was obtained under the average NLR of 3.73 kg-N/m^3/day (maximum of 4.1 kg-N/m^3/day on day 192). On day 194, the operational temperature decreased to about 12°C due to temperature control failure. This resulted in a transitory decrease in nitrogen removal rates but soon recovered after the temperature was reinstated to about 35°C.

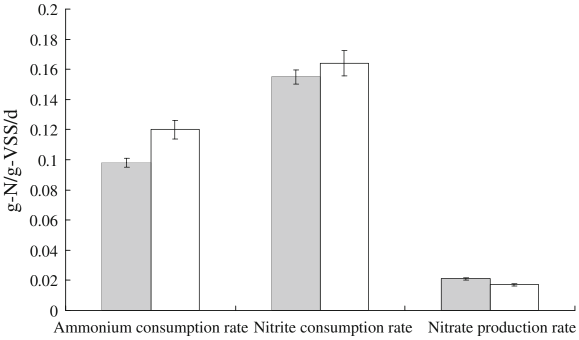


Fig. 3 Comparison of NH_4^+, NO_2^- removal and NO_3^- production rates between synthetic wastewater and partial nitrified digester liquor (*Grey bars* synthetic wastewater, *open bars* real LMDL)

Strous et al. (1999) reported that the suitable pH range for anammox reactor was between 7.0 and 8.5 and the optimum was 8.0 with the highest nitrogen removal rate. However, the relatively low pH level of 7.5 was determined to be effective for nitrogen removal from partial nitrified LMDL by anammox reactor in this study, and we found that the activity of granular anammox sludge was severely suppressed under operational pH of over 8.2. In our study, pH changes were considered to be directly related to the FA and FNA levels. The FA concentrations decreased substantially after the operating pH level was adjusted to 7.5 reducing to one-fifth of that before the pH adjustment (1.8 vs. 8.2 mg/l). Zheng and Huo (2001) reported that the inhibition constant values of FA and FNA for anammox bacteria were 75.6 and 5.3×10^{-3} mg/l, respectively. It is clear that the FA concentrations in this reactor did not reach the threshold level. In view of this, FA concentration can not be regarded as the reason for low anammox activity. The average FNA concentrations before and after the pH adjustment to 7.5 were 6.7×10^{-3} and 3.2×10^{-3} mg/l, respectively. This implied that the low anammox activity under pH level of 7.9 might be

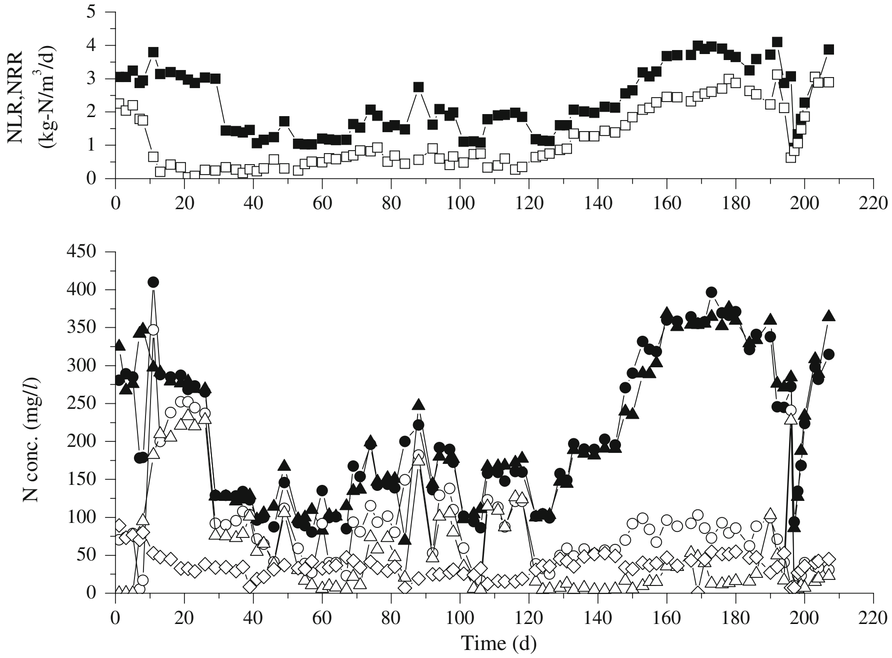


Fig. 4 Time courses of N concentrations in anammox reactor (*Solid squares* Nitrogen loading rate, *open squares* Nitrogen removal rate, *solid circles* influent NH_4–N, *solid triangles* influent NO_2–N, *open circles* effluent NH_4–N, *open triangles* effluent NO_2–N, *open diamonds* effluent NO_3–N)

Table 2 Treatment performance of granular anammox reactor

Parameters	Average ± SD (mg/l) of whole period		Average ± SD (mg/l) of stable period (between day 160 and 190)	
	Influent	Effluent	Influent	Effluent
SS	183 ± 155	179 ± 143	428 ± 74	401 ± 55
TCOD	2,491 ± 994	2,153 ± 933	3,728 ± 195	3,042 ± 461
NH_4^+–N	210 ± 94	91 ± 64	350 ± 36	86 ± 12
NO_2^-–N	212 ± 94	57 ± 65	349 ± 25	35 ± 25
NO_3^-–N	7 ± 4	37 ± 16	7 ± 5	41 ± 15
TN	462 ± 193	229 ± 138	752 ± 43	230 ± 60

related to inhibition caused by FNA. However, further investigation is required to establish the exact reason.

Comparison of nitrogen removal rate with other studies

A comparison of treatment performances of LMDL by granular anammox process by various researchers is shown in Table 3. All of the studies reported in Table 3 applied PN technique as the pre-treatment for anammox process. The TN removal rate attained in this study was approximately 3.6–12 times higher in comparison to the other studies. The reaction ratio determined in this study was almost similar to that reported by Strous et al. (1998), however the ratios reported by other studies were significantly different. The relatively higher nitrite/ammonium ratio and lower nitrate/ammonium ratio in Table 2 might be related to heterotrophic denitrification. However, Ruscalleda et al. (2008) considered that anammox bacteria could coexist with denitrifiers and play an important role in treating low C/N ratio wastewater with high quantities of slowly-biodegradable organic carbon, such as digested liquor and landfill leachate. In treating such wastewaters, conventional heterotrophic denitrification is limited by the low availability of easily biodegradable organic matter. Consequently, slight proliferation of denitrifiers would not have a severe impact on anammox reaction.

Table 3 Comparison of treatment performance of LMDL manure by anammox process

Wastewater	NLRs (kg-N/m^3/day)	NRRs (kg-N/m^3/day)	Reaction ratio (NH_4^+:NO_2^-:NO_3^-)	References
Synthetic			1:1.32:0.26	Strous et al. (1998)
LMDL	0.39	0.22	1:1.67:0.53	Yamamoto et al. (2008)
	1.02	0.66	1:1.65:0.00	Ahn et al. (2004)[a]
	1.36	0.72	1:2.03:0.09	Hwang et al. (2005)
	3.73	2.60	1:1.20:0.22	This study

[a] With addition of synthetic nitrite

In the study of Yamamoto et al. (2008), a large amount of influent SS contained in PN LMDL was reported to be attached to the nonwoven materials, which soon covered the anammox biomass attached on nonwoven carriers completely. This condition caused the decrease in anammox activity and ultimately became the main reason responsible for the unsatisfactory performance of anammox treatment. Although usage of a flocculant improved the settleability of influent SS and reduced their accumulation inside the reactor, the flocculant impaired anammox activity by attaching on the surface of the nonwoven carriers. In order to prevent this inhibition, the up-flow mode was substituted by continuous stirring mode in this study. This complete mixing condition resulted in low SS accumulation in the anammox reactor and the average effluent SS concentration was only 4.87 mg/l during the entire experimental period as illustrated in Fig. 5. Considering that the seed sludge concentrations in the study of Yamamoto et al. and our study were similar (5,000 vs. 6,000 mg/l), low accumulation of SS in granular anammox reactor was considered to be one of the important factors for obtaining successful anammox treatment performance in our study. Our attained nitrogen removal rates were about 12 times higher than the results reported by Yamamoto et al. (2008).

Effect of organic carbon on anammox biomass

Organic carbon is generally considered to inhibit anammox activity (van de Graaf et al. 1996). It has also been reported that anammox activities were severely inhibited by methanol and ethanol at concentrations below 1 mM (Güven et al. 2004, 2005). During PN treatment in this study, the average total COD concentrations in the influent and the effluent were 8,390 and 7,230 mg/l, and soluble COD concentrations were 5,880 and 5,040 mg/l, respectively. Hence, removal efficiencies for total and soluble COD were only 8.6 and 8.5%, respectively. These low removal efficiencies were attributed to the presence of non-biodegradable or slowly biodegradable organic matter in the LMDL. Since the easily biodegradable organics are degraded during anaerobic digestion, the remaining organics were mainly the refractory organics such as humic acid. It was difficult to remove these kinds of organic materials under relatively short HRT of 12 h in a single-stage PN tank.

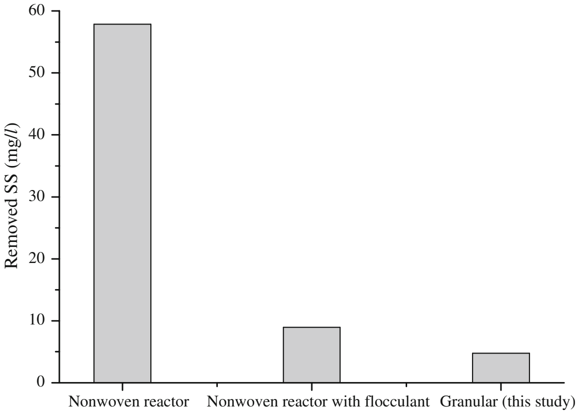


Fig. 5 Comparison of SS removal between granular anammox reactor (this study) and nonwoven carriers reactor (Yamamoto et al. 2008)

Although the results of batch experiments demonstrated feasibility of nitrogen removal from LMDL, the high average influent total COD concentration of 2,400 mg/l even after dilution had the potential to affect anammox activity of anammox biomass. Chamchoi et al. (2008) reported that COD concentration of over 300 mg/l would inactivate or eradicate anammox communities. Later, Molinuevo et al. (2009) determined more detailed threshold values of COD inhibition on anammox biomass, which were 237 mg/l (loading rate of 112 mg COD/l/day) for post-digested pig manure and 290 mg/l (loading rate of 136 mg COD/l/day) for partially oxidized pig manure. Both these values suggested that anoxic denitrifiers could compete with anammox bacteria at a higher COD concentration. In order to avoid this possibility, the HRT of the anammox reactor was set at a short duration of 4.8 h while varying the dilution rate of the actual LMDL to increase the NLR. The average effluent total COD concentration of the anammox reactor was about 2,250 mg/l and the removal efficiency was only 6.3%, which implied that little denitrification occurred in the anammox reactor under such short HRT conditions. High nitrogen removal performance of our granular anammox reactor proved that there were minor effects of high concentration of refractory organics on the activity of anammox sludge under short HRT conditions.

Conclusions

The results of this study demonstrated that stable and high treatment performance was attained with the combined PN–anammox process for nitrogen removal from the actual LMDL. The average and the maximum nitrite production rates were 1.2 and 2.1 kg NO_2^-–N/m^3/day in the PN reactor using PEG immobilized nitrifiers. High FA levels achieved (average of 50 mg-NH_3/l) were considered to be responsible for the inhibition of NOB activity. During about 200 days of continuous operation, low effluent nitrate concentrations were observed suggesting that there was no accumulation of nitrates in the PN reactor. High nitrogen removal rate of 3.12 kg-N/m^3/day was observed at a NLR of 4.1 kg-N/m^3/day and a constant HRT of 4.8 h after the effluent of the PN reactor was fed into the continuous stirred granular anammox reactor. Accumulation of SS in the granular anammox reactor was negligible. Presence of refractory organics was considered to impart minimal impact on the activity of the granular anammox sludge. The overall results of this study suggest that PN–anammox treatment can be successfully used for nitrogen removal from livestock manure digester liquor.

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