Title

Partial nitritation and anammox of a livestock manure digester liquor and analysis of its microbial community

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Abstract

A swim-bed reactor for partial nitritation with polymeric coagulant treatment and an UASB reactor for anammox were applied to the treatment of livestock manure digester liquor. The partial nitritation was maintained for 32 days under a 1.6 kg-N/m^3/d nitrogen loading rate (NLR) with an average conversion efficiency of 51%, and achieved 1.65 kg-N/m^3/d of the maximum nitrite production rate under 2.58 kg-N/m^3/d of NLR. Although 200 mg/L of TOC remained in the effluent of the partial nitritation reactor, the anammox nitrogen removal rate was not significantly decreased and a relatively high rate of 2.0 kg-N/m^3/d was obtained under a NLR of 2.2 kg-N/m^3/d. 16S rRNA gene analysis showed that *Nitrosomonas* and KSU-1 were dominant in the partial nitritation and anammox reactor, respectively. The results of this study demonstrated that the partial nitritation-anammox process has possibility of applying to the nitrogen removal of livestock manure digester liquor.

[**Key words:** partial nitritation, anammox, livestock manure, digester liquor, microbial community]

1. Introduction

From an energy recovery view point, anaerobic methane fermentation for livestock manure has attracted attention recently (Angenental et al., 2002; Hill et al., 2000; Sanchez et al., 2005). Anaerobic methane fermentation cannot remove nitrogen and phosphorus efficiently so digester liquor is either recycled as liquid fertilizer or further treated by advanced treatment. The use of advanced treatment of anaerobic digester liquor involves investment and high operational costs, which determines that digester

liquor is mostly utilized as fertilizer in Japan. However, because there is a high concentration of nitrogen remaining in the liquor, its excessive use as fertilizer can cause groundwater pollution. Therefore, it is important to find an alternative way to treat digester liquor efficiently to make the use of methane fermentation of livestock manure in an environmentally safe manner.

Nitrification-denitrification is generally used for nitrogen removal from digester liquor. When applying this process, a large amount of oxygen, alkalinity for nitrification and supplementation by extra carbon sources such as methanol for denitrification are required. By comparison, partial nitritation-anammox, which has been developed since 2000, is a cost effective process (Fujii et al., 2002; Fux et al., 2002; Schmidt et al., 2003; van Dongen et al., 2001). The anammox reaction consists of the oxidation of ammonia to nitrogen gas using nitrite as an electron acceptor under anaerobic conditions (Strous et al., 1998). This autotrophic process produces very little sludge and does not require supplementation with carbon sources. Because only partial nitritation (PN) of around 60% conversion of ammonia to nitrite is required as a pretreatment, rather than complete nitrification, the amount of oxygen and alkalinity required for nitrification can be reduced (van Dongen et al., 2001).

The partial nitritation-anammox process is best suited for the treatment of wastewaters containing high concentrations of ammonium and can be applied to sewage sludge digester liquor, livestock wastewater, landfill leachate, and power plant wastewater (van Dongen et al., 2001). Recently, the application of this process to livestock wastewaters has been reported in steps. We applied a swim-bed reactor for PN, using a filtration system for pretreatment and a fixed-bed reactor for anammox, to treat swine wastewater digester liquor (Yamamoto et al., 2008).We reported that the PN was stable over a long period but anammox nitrogen removal efficiencies were significantly

decreased when the PN effluent was treated with a polymeric organic coagulant (Yamamoto et al., 2008). Furukawa et al. (2009) demonstrated that a PN reactor using nitrifying activated sludge entrapped in a polyethylene glycol (PEG) gel carrier and an anammox reactor using anammox sludge entrapped in the PEG gel carrier could be applied to livestock manure digester liquor. Qiao et al. (2010a) also demonstrated that a PN reactor using nitrifying activated sludge entrapped in a PEG gel carrier and a continuous stirred granular anammox reactor could be applied to livestock manure digester liquor. However, Furukawa et al. (2009) and Qiao et al. (2010a) did not apply suspended solids (SS) removal before PN. They reported that influent SS caused frequent clogging of the air diffuser, resulting in the deterioration of PN treatment efficiency.

Analysis of microbial communities in PN and anammox has been carried out in previous studies. Many researchers reported that bacteria belonging to *Nitrosomonas* were detected in a nitritation reactor (Egli et al., 2003; Ganigue et al., 2009; Qiao et al., 2010b; Shinohara et al., 2009; Sliekers et al., 2002). Qiao et al. (2010b) and Ganique et al. (2009) also reported that *Nitrosomonas* were dominant in PN reactors treating wastewaters containing 500 mg/L and 2700 mg/L of NH_4-N, respectively. In addition, the analysis of microbial communities in anammox reactors for synthetic inorganic wastewater treatment showed that *Chloroflexi* bacteria coexisted with anammox bacteria (Qiao et al., 2008; Cho et al., 2010). However, until now, the analysis of microbial communities has not been attempted for PN and anammox reactors treating livestock wastewaters and it is not clear which microbes are dominant.

The objectives of our study were to apply a swim-bed reactor for PN with polymeric organic coagulant treatment and an UASB reactor for anammox to the treatment of livestock manure digester liquor, and to examine the PN and anammox bacterial

communities by 16S rRNA gene analysis.

2. Methods

2.1 Livestock manure digester liquor

Wastewater was obtained from the digester liquor storage tank of a biogas plant in the city of Yamaga, Kumamoto, Japan. In this biogas plant, mixed wastes including livestock manure (62.8 t/d), raw garbage (3 t/d), and activated sludge (2 t/d) were separated into solid and liquid fractions and the liquid waste was then treated by methane fermentation (mesophilic fermentation at 37°C). Characteristics of wastewater were pH 8.5, 2000-3000 mg/L SS, 8000-10,000 mg/L total chemical oxygen demand (COD), 1000-1500 mg/L 5-day biological oxygen demand (BOD_5), 1400-1600 mg/L NH_4-N, 1600-2200 mg/L total nitrogen (T-N), and NO_2-N and NO_3-N were negligible. The livestock manure digester liquor contained a high concentration of T-N, a large percentage (73-88%) of which was NH_4-N. Consequently, nitrogen removal is required before discharging the digester liquor into natural water bodies. Also, because a high NH_4-N concentration would cause air pollution by ammonia stripping and groundwater pollution by permeation, its concentration should be decreased before the digester liquor is used as liquid fertilizer.

2.2 Experimental setup and operational conditions for partial nitritation

The digester liquor contained a high concentration of SS that were removed using a polymeric organic coagulant (polyacrylamide, SNF, USA). The coagulant concentration, mixing speed, mixing time and standing time were 500 mg/L, 300 rpm, 5 min and over 10 min, respectively. The average characteristics of wastewater after the coagulation

treatment were pH 8.6, 950 mg/L SS, 6400 mg/L total COD, and 1600 mg/L T-N. The removal efficiency of SS was approximately 65%.

The swim-bed reactor used for PN treatment had downdraft and updraft sections in a parallel upright arrangement (Fig. 1a). The cross-sectional areas of the downdraft and updraft sections of the reactor were 115 × 115 mm and 115 × 35 mm, respectively, and the height to the effluent port was 630 mm (effective volume 10.8 L). Biofringe (acryl-fiber biomass carrier, NET, Japan) was used as a biomass carrier and set at a length of 600 mm. The settling tank had a volume of 2.5 L and a water surface area of 0.017 m^2. The settled sludge was gently mixed by a chain and returned to the swim-bed reactor with 100% recycle. The reactor was seeded with 22 g of activated sludge that had been cultivated using synthetic wastewaters (composed mainly of peptone and meat extract) by a fill-and-draw method for a long period under total oxidation conditions (Ha et al., 2005). After seeding, the PN reactor was fed with the test wastewater for about 300 days to allow sludge acclimation. Continuous-flow treatment was then begun. The influent was diluted 2 times with tap water to adjust the nitrogen loading rate (NLR) until day 12 and not diluted after day 12. The reactor temperature and hydraulic retention time (HRT) were maintained at 30-32°C and 24-15 h, respectively. The air flow rates were adjusted to 5 L/min until day 76 and 7 L/min after day 76. The pH of the reactor was controlled at 7.6-7.8 by 1 M $NaHCO_3$ and 2 M HCl.

Fig. 1

2.3 Experimental setup and operational conditions for anammox

An up-flow glass column reactor with an internal diameter of 100 mm and a height of 380 mm (effective volume 3 L) was used for anammox (Fig. 1b). The reactor was fed with a synthetic inorganic wastewater ($(NH_4)_2SO_4$ 50-75 mg-N/L, $NaNO_2$ 50-75 mg-N/L, $KHCO_3$ 250 mg/L, KH_2PO_4 108 mg/L, T. element 0.5 ml/L (EDTA・2Na 10

g/L, $FeSO_4 \cdot 7H_2O$ 18 g/L) until the nitrogen removal rate (NRR) increased to 0.5 kg-N/m^3/d. After cultivation of anammox sludge with the synthetic inorganic wastewater, the influent was switched to the PN effluent (from day 60 until the end of experiment; total 69 days). Because approximately 480 mg/L of SS remained in the PN effluent, almost SS was removed by settling and filtration with polyester non-woven sheet (Japan Vilene, Japan). The influent was diluted 10 to 7 times with tap water to prevent inhibition of anammox activity by the NO_2-N concentration in the PN effluent of 700-800 mg/L (Strous et al., 1999; Isaka et al., 2007). The influent was mixed with synthetic inorganic wastewater, to a desired mixing rate (the ratio of PN effluent in influent) until day 73. In addition, the influent was purged with N_2 gas to maintain the dissolved oxygen (DO) below 1.0 mg/L. The reactor was inoculated with 6 g (dry weight) of granular anammox sludge, which was taken from another anammox reactor treating synthetic inorganic wastewater. The reactor temperature was maintained at 30°C during the entire operational period. The NLR was changed, depending upon NRR, by decreasing the dilution times and HRT.

2.4 Analytical methods

T-N was measured by the persulfate method, NO_2-N and NO_3-N were measured by the colorimetric method, COD was measured by the closed reflux colorimetric method, TOC was measured by the combustion-infrared method, SS was measured by drying at 105℃ on a 1 μm glass-fiber filter, and BOD was measured in accordance with the Standard Methods (APHA, 1995). NH_4-N was measured by the modified phenate method (ortho-phenyl phenol (OPP) method) (Kanda, 1995). Absorbance was measured using a spectrometer (U-2010; Hitachi, Japan). pH was measured using a pH meter (B-211; Horiba, Japan). DO was measured using a DO meter (OM-51; Horiba). Free

ammonia and free nitric acid concentrations can be estimated from equilibrium as follows:

NH_3 (mg/L) =

17 / 14 × Total ammonia as N (mg/L) × 10^{pH} / [exp (6344 / (273 + T)) + 10^{pH}]

HNO_2 (mg/L) =

47 / 14 × NO_2-N (mg /L) / [exp (-2300 / (273 + T)) × 10^{pH}]

2.5 DNA extraction and PCR amplification

DNA was extracted using ISOIL (Nippon gene, Japan) following the manufacturer's protocol. The 16S rRNA genes in the DNA were amplified by PCR with Phusion High-Fidelity DNA polymerase (Finnzaymes, Finland) and eubacterial primers of 6F (forward primer: 5'-GGAGAGTTAGATCTTGGCTCAG-3') and 1492R (reverse primer: 5'-GGTTACCTTGTTACGACT-3') (Lane, 1991; Tchelet et al., 1999). PCR was carried out according to the following thermocycling parameters: initial period of 30 sec at 98°C, 25 cycles of 10 sec at 98°C, 20 sec at 51°C, and 35 sec at 72°C, and a final period of 5 min at 72°C. The amplified products were electrophoresed on a 1% agarose gel. A band (~1.5 kb) on the agarose gel was excised, and the DNAs in that were extracted and purified using a Wizard SV Gel and PCR Clean-Up System (Promega, U.S.A.).

2.6 Cloning and sequencing of 16S rDNA

The purified DNA fragments were ligated into the EcoRV site of pBluescript II KS+ (Stratagene, U.S.A.). *E. coli* DH5α was transformed using the constructed plasmids. The plasmids were extracted from the clones carrying them by the alkaline method. The

DNA fragments were sequenced using a 3130xl genetic analyzer and BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, U.S.A.). The sequences determined in this study were compared with those in the nr database by the basic local alignment search tool (BLAST) program available on the NCBI website.

The partial 16S rRNA gene sequences determined in this study are available in the DDBJ database under Accession No. AB594203 to AB594207 (for the sequences of Operational taxonomic unit (OTU) 2, OTU 3, OTU 4, OTU 5, and OTU 6 from the anammox reactor) and AB594208 to AB594212 (for the sequences of OTU1, OTU 2, OTU 3, OTU 4, and OTU 5 from the PN reactor).

3. Results and discussion

3.1 Performance of partial nitritation

Figure 2a shows the time courses of NH_4-N, NO_2-N, NO_3-N, and Figure 2b shows the time courses of NLR and nitrite production rate (NPR). When the reactor was started with 800-1100 mg/L of influent NH_4-N (2 times dilution), nitrite was immediately produced because the sludge in the reactor had been acclimatized for about 300 days with test wastewater. At 2 times dilution (day 5-12), the effluent NO_2-N concentrations were 750-850 mg/L and the average conversion efficiencies of ammonia to nitrite were about 75%. Influent dilution was then stopped. Although the influent NH_4-N concentration increased to 1550 mg/L, the effluent NO_2-N concentrations were almost constant and PN was achieved with 51% of the average conversion efficiency. In addition, the PN was maintained for 32 days (during days 26-62) at a NLR of 1.6 kg-N/m^3/d. However, mechanical trouble with the heater and clogging of the air diffuser caused a decrease in NPR at day 64. Additionally, the pH controller was out of order

during days 67-82. After restoration of the reactor, the aeration rate was increased from 5.0 to 7.0 mL/min and NLR was also increased stepwise with decreasing HRT. Finally, 1.65 kg-N/m^3/d of the maximum NPR was achieved under a NLR of 2.58 kg-N/m^3/d. Effluent NO_3-N concentrations were below 30 mg/L (the conversion efficiencies of ammonia to nitrate were about 2%) during the whole operational period, except during initial start-up, which indicates the conversion of nitrite to nitrate was successfully prevented.

Furukawa et al. (2009) and Qiao et al. (2010a) applied the PN system using a nitrifying gel carrier to the livestock manure digester liquor and reported that the PN was unstable without influent dilution because of air diffuser clogging. In contrast, our PN treatment using the swim-bed system was relatively stable for 32 days without influent dilution when SS were removed by polymeric organic coagulant. However, it was not possible to completely prevent clogging of the air diffuser in this study. Hence, influent dilution or improvement of the air diffuser are required for influent wastewaters with high SS concentrations and viscosity, such as livestock manure digester liquor.

Figure 2c shows the time courses of free ammonia and free nitric acid during the experimental period. Because pH was controlled at 7.8, free nitric acid concentrations were at a low level except during the period where there was trouble with the pH controller. Thus, we mainly discussed free ammonia inhibition. Anthonisen et al. (1976) reported that ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) exhibited evidence of inhibition above 10 mg/L and 0.1 mg/L of free ammonia concentration, respectively. Furthermore, Vadivelu et al. (2007) summarized the inhibition level of free ammonia for AOB (*Nitrosomonas*) and NOB (*Nitrobacter*) and reported that the anabolism of the AOB was not inhibited at up to 16 mg/L of free ammonia concentrations, whereas the anabolism of NOB was inhibited completely

above 6 mg/L. In this study, free ammonia concentrations were kept at approximately 10-60 mg/L (maximum 110 mg/L) except during the start-up period and the period of trouble with the pH controller. Therefore, it is assumed that NOB were inhibited, which may have prevented the conversion of nitrite to nitrate. In a previous study, NOB was shown to acclimate to the inhibition of free ammonia (Turk and Mavinic, 1989) and Wong Chong and Loehr (1978) reported that *Nitrobacter*, which adapted to free ammonia, could resist 40 mg/L of free ammonia. Although the reactor had been operated for a long period (150 days), NOB did not adapt to free ammonia and the conversion of nitrite to nitrate was almost completely prevented in this study. This phenomenon might be responsible for the relatively high concentration of free ammonia (10-60 mg/L, max. 110 mg/L). On the other hand, because AOB are also inhibited by extremely high concentrations of free ammonia, the optimal concentration of free ammonia to achieve a high rate of PN should be investigated.

Fig. 2

3.2 Microbial community in the partial nitritation reactor

Table 1 shows the main results of homology search for 16S rRNA gene sequences in the community existing in the PN reactor (day 136). Five and two of them had 98% sequence identities with *Nitrosomonas* sp. clone74 and *Nitrosomonas* sp. IWT514, respectively. Thus, 15% (7 clones/46 clones) of bacterial members in the community belonged to the genus of *Nitrosomonas*, which was dominant in the PN reactor. In previous studies, many researchers reported that the bacteria belonging to *Nitrosomonas* were detected in the nitritation reactor (Egli et al., 2003; Ganigue et al., 2009; Qiao et al., 2010b; Shinohara et al., 2009; Sliekers et al., 2002). Qiao et al. (2010b) reported that *Nitrosomonas* sp. clone74 were dominant in the PN reactor for high ammonium containing wastewater from the magnesium ammonium phosphate process for methane

fermentation digester liquor (NH_4-N: 500 mg/L). Additionally, Ganique et al. (2009) applied PN to landfill leachate (NH_4-N: 2700 mg/L) and reported that *Nitrosomonas* sp. IWT514 were dominant. Although in this study PN was applied to the livestock manure digester liquor (NH_4-N: 1500 mg/L), the microbial community in the PN reactor appears to be consistent with the above findings, as the dominant bacteria belonging to *Nitrosomonas* might be the same in the strain level as the previous reports. Other clones seemed to belong to δ-*Proteobacterium* (5 clones), γ-*Proteobacterium* (2 clones), *Bacteroidetes* (2 clones). Because these clones had relatively low sequence identity with the BLAST database, it was difficult to identify the genus of these clones. It is assumed that the difficulty of identification might be caused by the specific conditions (PN of livestock manure digester liquor) in this study.

Table 1

3.3 Performance of anammox

After the NRR of 0.5 kg-N/m^3/d was achieved with synthetic inorganic wastewater treatment at day 60, feeding of PN effluent to the anammox reactor was begun. Figure 3 shows the time courses of NLR and NRR in the anammox reactor during days 60 to 129. The anammox reactor was operated initially at a HRT of 3 h and a 30% mixing rate, and the mixing rate was then increased from 30 to 60%, and 100% under constant HRT. Although the mixing rate was 100%, the NRR was not decreased and remained at about 0.7 kg-N/m^3/d. The dilution rate was then decreased stepwise from 10 to 7 times. When the dilution rate was 7 times, the NRR finally reached 2.0 kg-N/m^3/d under a NLR of 2.2 kg-N/m^3/d. Previously, we applied PN and anammox to the digester liquor of swine wastewater and reported that the NRR of the fixed-bed anammox reactor was 0.22 kg-N/m^3/d when PN effluent was treated with the polymeric organic coagulant (Yamamoto et al., 2008). We assumed that the polymeric organic coagulant remained in

the water phase attached to the anammox biofilm. This caused the decrease in anammox activity and resulted in the low NRR (Yamamoto et al., 2008). In contrast, a relatively high NRR of 2.0 kg-N/m^3/d was achieved in this study when the PN effluent was treated by settling and filtration. In addition, although polymeric organic coagulant was added to PN influent, the accumulation of residual polymeric organic coagulant was not observed during about 70 days. Therefore, the addition of polymer coagulant for SS removal should be carried out before PN treatment and the physical SS removal might be appropriate for PN effluent.

Furukawa et al. (2009) reported that the continuous stirred anammox reactor with the PEG gel carrier achieved NRR of more than 4.0 kg-N/m^3/d. In addition, Qiao et al. (2010a) reported that the continuous stirred granular anammox reactor achieved NRR of 3.1 kg-N/m^3/d. Although their reactor demonstrated high NRR comparing with our results, there are presumably some problems. The continuous stirred anammox reactor with the PEG gel carrier takes high initial costs for preparation of PEG gel carrier. Because the granule was generally formed in the UASB or Sequencing batch reactor (SBR), the start-up of continuous stirred granular anammox reactor seems to be difficult without enough amount of seed anammox granule. Therefore, the results of this study suggested the valuable information which the practical anammox reactor (UASB) can be applied to the treatment of livestock manure digester liquor with relatively high NRR of 2.0 kg-N/m^3/d.

Table 2 shows the treatment performances at a dilution rate of 7 times (days 117-129). The influent and the effluent TOC concentration were almost at the same level and the TOC removal rate was negligible. Because the test wastewater was treated by anaerobic methane fermentation and followed by aerobic PN treatment before anammox treatment, this wastewater might have included non-biodegradable or slowly

biodegradable organic matter instead of biodegradable matter. Thus, it seems to be difficult to remove TOC in the anammox reactor. Although organic matter is normally considered to be the inhibiting factor for anammox reaction, NRRs were not significantly decreased and a satisfactory level of 2.0 kg-N/m^3/d was achieved with 200 mg/L TOC (van de Graaf et al, 1996; Guven et al., 2005). This phenomenon might be caused by the influent containing mainly non-biodegradable organic carbon. Because the growth rate of anammox bacteria is extremely slow (11 days doubling time), it is clearly important to maintain biomass in the anammox reactor (Strous et al., 1998). In this study, the effluent SS concentrations were relatively low at about 10 mg/L, which indicates that the biomass in the anammox reactor was adequately maintained even at a HRT of 3 h. However, the influent SS partly accumulated in the reactor, so the effect of influent SS on long-term stability of anammox treatment should be further investigated.

Fig. 3

Table 2

3.4 Microbial community in the anammox reactor

A sample was collected from the anammox reactor operated at a 1.5 kg-N/m^3/d NLR for 30 days after switching the PN effluent from synthetic wastewater (day 90). Table 3 shows the main results of homology search for 16S rRNA gene sequences. Twenty four clones and 6 clones had 100% and 92-99% sequence identity with KSU-1, respectively. Thus, 30 clones (59% of 51 clones) were identified as belonging to KSU-1 in the anammox reactor. KSU-1 was found in an anammox reactor treating synthetic inorganic wastewater and recognized as an anammox bacterium (Fujii et al., 2002). In addition, KSU-1 was detected in various anammox reactors seeded with anammox sludge which had been cultivated in our laboratory (Hoa et al., 2006a, b; Qiao et al., 2008). Furthermore, KSU-1 was also detected from a SNAP (Single-stage Nitrogen removal

using Anammox and Partial nitritation) reactor and from an anammox reactor containing approximately 3% salinity in our laboratory (Furukawa et al., 2006; Liu et al., 2009). From these observations, it is easily assumed that KSU-1 was detected in the anammox reactor treating the livestock manure digester liquor; however, it is interesting that KSU-1 was maintained as the dominant species even at the influent including relatively high concentration of TOC in this study. This phenomenon also might suggest that the influent contains mainly non-biodegradable organic carbon.

Three clones had 94% sequence identity with Candidatus Brocadia (genus) bacteria. Because Candidatus Brocadia bacteria were also recognized as anammox bacteria, 65% of the total clones were considered anammox bacteria. Two clones had 97% sequence identity with *Chloroflexi* (Class) bacteria. Qiao et al. (2008) reported that *Chloroflexi* bacteria coexisted with KSU-1 in a fixed-bed anammox reactor treating synthetic inorganic wastewater. In addition, Cho et al. (2010) investigated the microbial community in an anaerobic up-flow granular bed anammox reactor treating synthetic inorganic wastewater and reported that *Chloroflexi* bacteria was presented on the surface of anammox granules. Although the anammox reactor was applied to livestock manure digester liquor, the microbial communities of this and the above studies appear to have similarity in the coexistence of anammox and *Chloroflexi* bacteria. Other clones belonged to *Bradyrhizobium*, *Rhizobiales*. Although the anammox reactor was operated under light shielding and the influent mainly consisted of ammonia and nitrite probably without biodegradable organic carbon, various bacteria were detected in the anammox reactor. Thus, the function of *Chloroflexi* bacteria and other bacteria in anammox reactor should be investigated.

Table 3

4. Conclusions

The PN was stably maintained for 32 days under a NLR of 1.6 kg-N/m^3/d with an average conversion efficiency of 51%, and the maximum NPR was 1.65 kg-N/m^3/d under a NLR of 2.58 kg-N/m^3/d. The NRR by anammox was satisfactorily achieved at 2.0 kg-N/m^3/d under a NLR of 2.2 kg-N/m^3/d with 200 mg/L TOC. 16S rRNA gene analysis showed that *Nitrosomonas* spp. and KSU-1 were dominant in the PN and anammox reactor, respectively. The results of this study demonstrated that the partial nitritation-anammox process has possibility of applying to the nitrogen removal of livestock manure digester liquor. In future research, the applicability of this process should be investigated using scaled up reactor (pilot scale reactor).

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Figure Captions

Fig. 1 Schematics of the partial nitritation (a) and anammox (b) reactors.

Fig. 2 Time courses of nitrogen concentrations (a) nitrogen loading rate (NLR) and nitrite production rate (NPR) (b) free ammonia and free nitric acid (c) in the partial nitritation reactor.

Fig. 3 Time courses of nitrogen loading rate (NLR) and nitrogen removal rate (NRR) in the anammox reactor.

Tables