Nitrogen removal from piggery waste using the combined SHARON and ANAMMOX process

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Abstract Nitrogen removal in piggery waste was investigated with the combined SHARON-ANAMMOX process. The piggery waste was characterized as strong nitrogenous wastewater with very low C/N ratio. For the preceding SHARON reactor, ammonium nitrogen loading and conversion rates were 0.97 kg NH_4-N/m^3reactor/day and 0.73 kg NH_4-N/m^3 reactor/day, respectively. Alkalinity consumption for ammonium conversion was 8.5 gr bicarbonate utilized per gram ammonium nitrogen converted to NO_2-N or NO_3-N at steady-states operation. The successive ANAMMOX reactor was fed with the effluent from SHARON reactor. Nitrogen loading and conversion rates were 1.36 kg soluble N/m^3 reactor/day and 0.72 kg soluble N/m^3 reactor/day, respectively. The average NO_2-N/NH_4-N removal ratio by ANAMMOX reaction was 2.13. It has been observed that *Candidatus* "Kuenenia stuttgartiensis" were dominated in the ANAMMOX reactor based on FISH analysis.

Keywords Nitrogen removal; piggery wastewater; SHARON-ANAMMOX process

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Introduction

The removal of nutrients such as nitrogen and phosphorus is very important issue to preserve the water environment. A stringent regulatory requirement for the nitrogen in the effluent from livestock waste has directed to the development of new waste treatment processes in Korea. Because of the high nitrogen and solids contents, piggery wastes are considered as a tough subject to meet the current effluent limitation of 60 mgTN/L in Korea.

During the last several years, we have focused to examine the feasible alternatives rather than conventional nitrification-denitrification processes to remove nitrogen from piggery wastewater (Min *et al.*, 2002; Ahn *et al.*, 2004a,b; Yun *et al.*, 2004). The combined Single reactor system for High Ammonium Removal Over Nitrite (SHARON) - Anaerobic Ammonium Oxidation (ANAMMOX) process configuration was originally developed for the nitrogen removal in reject water treatment in sewage treatment plant (van Dongen *et al.*, 2001). Compared to the conventional nitrification and denitrification processes, the combined SHARON-ANAMMOX process has an advantage for nitrogen removal at wastewater with an unfavourable C/N ratio such as seen in piggery wastewater.

The original SHARON process has been developed to achieve the nitrite nitrification at concentrated waste stream and operated at relatively high temperature (35 °C) with a chemostat-like operation. In this process, the controlled nitrite oxidation prevented an accumulation of nitrate. As a result, the nitrite denitrification could save up to 40% of

carbon demand compared to the conventional nitrate denitrification. Possible saving of up to 25% of oxygen requirement is an added benefit in SHARON process (Equation 1).

$$\text{SHARON}: \ 2NH_4^+ + 2HCO_3^- + 1.5O_2 \rightarrow NH_4^+ + NO_2^- + 2CO_2 + 3H_2O \quad (1)$$

A stable supply of nitrite-rich effluent is a prerequisite for the ANAMMOX operation because ANAMMOX reaction requires both ammonium and nitrite (2). Since no carbon energy is required in this reaction, ANAMMOX process is a suitable technology for nitrogen removal in wastewaters with low usable carbon energy.

$$\text{ANAMMOX}: NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (2)$$

With a combination of the SHARON and ANAMMOX processes, in which utilize both autotrophic organisms, an overall ammonium removal could be accomplished without an addition of organic carbon that requires for conventional denitrification (Equation 3).

$$\text{Combined SHARON/ANAMMOX}: 2NH_4^+ + 2HCO_3^- + 1.5O_2 \rightarrow N_2 + 2CO_2 + 5H_2O \quad (3)$$

The nitrogen removal is a 'bottle-neck' for the process design in piggery wastewater since nitrification step is accomplished by an aeration in which also simultaneously removes easily degradable organics. In practice, an external supply of carbon energy is often introduced to denitrify the nitrified piggery wastewater. Although the development of ANAMMOX organism was considered as a difficult task, a previous study (Ahn *et al.*, 2004b) has demonstrated that an inoculation of anaerobic granule could reduce the acclimation period to develop the ANAMMOX organism. This paper mainly discusses on the operational results of the combined SHARON - ANAMMOX process configuration for nitrogen removal in piggery wastewater treatment. The result of microbial community analysis on anaerobic granules in ANAMMOX reactor is also presented.

Material and methods

Laboratory reactor

Figure 1 shows a photograph and schematic diagram of laboratory reactors. An effective volume of SHARON reactor was 1 L and operated with SBR-like, fill-and-draw feeding type at HRT of 1 day. Nitrifying sludge from a full-scale livestock wastewater treatment plant was inoculated as a seed. The SHARON reactor was operated at temperature of 30–35 °C. DO level was maintained within the range of 3–4 mg/L. The ANAMMOX reactor consisted of an upflow-type sludge bed reactor (1 L) and a separate setting tank (0.5 L). The anaerobic granule taken from a local brewery with an up-flow sludge blanket

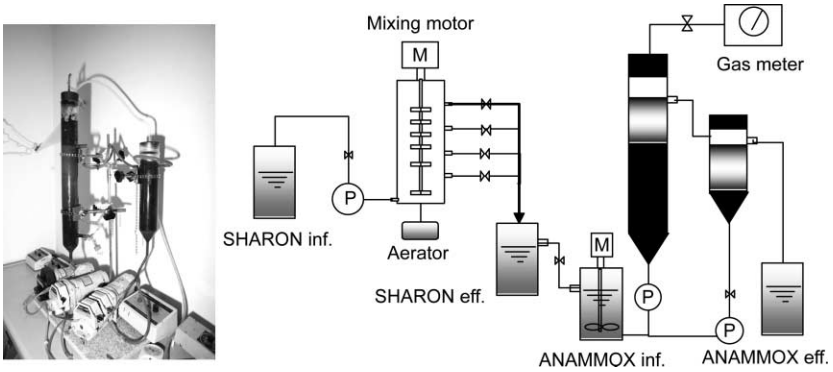

Figure 1 A photograph of granular ANAMMOX reactor (left) and the schematic diagram of combined SHARON-ANAMMOX process (right)

Table 1 Characteristics of SHARON influent

Parameters	Range	Average ± SD
pH	7.9 ~ 8.4	8.1 ± 0.1
COD	1,220 ~ 4,190	2,940 ± 1,100
SCOD	1,030 ~ 2,900	1,920 ± 580
NBDSCOD	600 ~ 1,100	870 ± 150
TKN	1,210 ~ 1,950	1,550 ± 160
NH_4-N	890 ~ 1,070	970 ± 50
NO_2-N	< 2	–
NO_3-N	< 0.4	–
Total P	70 ~ 160	93 ± 26
PO_4^{2-}	40 ~ 73	53 ± 8
Bicarbonate alkalinity	5,410 ~ 7,750	6,780 ± 580
VFA(as HAc)	960 ~ 1,980	1,450 ± 250
Ca^{++}	18.5 ~ 23.6	20.5 ± 4
Mg^{++}	6.85 ~ 10.23	8.78 ± 1.3

Note Unit: mg/L, except pH

(UASB) reactor was used as a seed for ANAMMOX reactor. The reactor was also operated as a fill-and-draw feeding system. HRT of ANAMMOX reactor was 2.5 d. Average operating temperature of 35 °C was maintained with an electronic heat controller. The settled sludge from the setting tank was recycled to the ANAMMOX reactor as a ratio of 0.5Q. In order to prevent adverse effects of DO, the SHARON effluent was stored in an effluent reservoir to remove DO prior to feeding the ANAMMOX reactor.

Wastewater

Table 1 shows the characteristics of piggery wastewater that is fed to the SHARON reactor. The influent of SHARON reactor was the effluent from an anaerobic digestion system with an elutriated phased treatment for piggery wastewater. Because of the pre-anaerobic treatment, biodegradable SCOD/NH_4-N ratio of SHARON influent is as low as 1.26. The supplemental alkalinity was added into SHARON influent for the development nitrification. The effluent of SHARON process was used as an influent of ANAMMOX reactor. Wastewater pH in ANAMMOX influent was not artificially controlled during the operation.

Analytical procedures

All water quality parameters measured in accordance to *Standard Methods* (APHA *et al.*, 1998). A visual inspection of ANAMMOX granules was conducted using a scanning electronic microscope. The gas production was monitored daily with a wet gas meter (Sinagawa Model W-NK-0.5A). A HPLC (Shimadzu Model LC-10AD, Japan) equipped with a UV detector and an organic acid analysis column (Aminex HPX-87H, Bio-Rad, Inc., U.S.A.) was used to monitor volatile fatty acids (VFAs). Calcium and Magnesium contents were measured by the optical emission spectrometer (Perkin Elmer, Optema 4300DV).

Microbial community analysis

In order to observe the microbial diversity in ANAMMOX reactor, fluorescent *in situ* hybridization (FISH) test was performed at the beginning and end of operation. The 16S rRNA-targeted oligonucleotide probes were used in this study (Table 2). The mean values for each order, genus and group-specific bacteria and total cell counts were calculated from the counts of 15 randomly chosen fields using epifluorescence microscope (Zeiss

Table 2 Oligonucleotide probe sequences, target organisms, formamide concentrations requires for in situ hybridization buffer and references

Probes	Probe sequence (5′ → 3′)	Target organisms	% FA[a]	NaCl (mM)[b]	References
Pla46	GACTTGCATGCCTAATCC	*Planctomycetales*	25	159	c
Kst1275	TCGGCTTATAGGTTTCGCA	*Candidatus* Kuenenia stuttgartiensis	25	159	d
NSO190	CGATCCCCTGCTTTTCTCC	Ammonia-oxidizing β- *Proteobacteria*	55	20	e
NIT3	CCTGTGCTCCATGCTCCG	*Nitrobacter* spp.	35	80	f

[a] percentage formamide in the hybridization buffer
[b] Millimolar concentration of sodium chloride in the washing buffer
[c] Neef *et al.* (1998); [d] Schmid *et al.* (2000); [e] Mobarry *et al.* (1996); [f] Wagner *et al.* (1996)

Axioplan, Germany), and the results were expressed in percentile of the number of individual group-specific bacteria to the number of total bacteria.

Results and discussion

The SHARON reactor

Figure 2 shows the nitrogen conversion behaviour in the SHARON reactor. It appears that a steady-state condition that producing a nitrite-accumulated effluent was reached after the 65 days from the start-up operation. From the start-up day to 65th day of operation, nitrite accumulation mechanism was not fully developed although a high spike of effluent nitrite concentration was observed. Operating results during the steady state condition is shown on Table 3. During the steady state operational period, the SHARON reactor could convert 58.8% of influent ammonium to NO_X-N while 15.8% of influent ammonium was mainly removed by NH_3 stripping and cell synthesis. 25.4% of influent NH_4 was not converted. Alkalinity consumption ratio was calculated as 8.5 gr $Alk._{consumed}$/gr NH_4-$N_{converted}$, which is higher value than the stoichiometric value of 7.12.

The ANAMMOX reactor

The start-up operation of the ANAMMOX reactor had begun with a feed consisted of stock nitrite solution and piggery wastewater. During the start-up operation, $NaNO_2$ was used as nitrite source. At 28th day of operation, the effluent from SHARON reactor was fed into the ANAMMOX reactor. Figure 3 shows the behaviour of nitrogen removal at

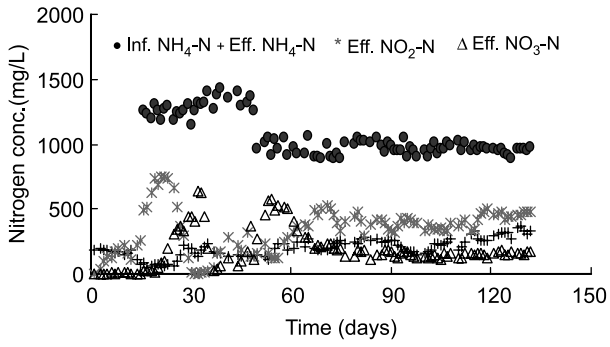

Figure 2 Nitrogen conversion characteristics in the SHARON reactor at 1-day HRT and 35 °C

Table 3 Nitrogen conversion characteristics in SHARON reactor at steady states operation

Parameter	Value	Unit
pH	8.1 ± 0.5	
influent NH_4-N	972 ± 45	mg/L
effluent NH_4-N	247 ± 53	mg/L
effluent NO_2-N	411 ± 58	mg/L
effluent NO_3-N	161 ± 33	mg/L
NH_4-N conversion to NO_x-N	58.8	%
effluent NO_2-N/NH_4-N	1.7 ± 0.9	
NH_4-N loading rate	0.97 ± 0.05	kg NH_4-N/m^3-day
NH_4-N conversion rate	0.73 ± 0.07	kg NH_4-N/m^3-day

the ANAMMOX reactor. It appears that the reactor reached at steady state condition after the 66th days of operation. With an influent NO_2-N to NH_4-N ratio was 1.56, effluent NO_2-N to NH_4-N removal ratio averaged to 2.13. According to van Dongen *et al.* (2001), a stoichiometric ratio of influent NO_2-N to NH_4-N ratio for ANAMMOX reaction is 1.32 while the ratio varies from 0.5 to 4 as seen in various literatures (van de Graaf *et al.*, 1996; Strous *et al.*, 1997, 1999; van Dongen *et al.*, 2001; Fux *et al.*, 2002; Sliekers *et al.*, 2003; Ahn *et al.*, 2004b). A low nitrogen conversion between operating days in 20th to 60th probably reasoned to the higher COD concentration in SHARON effluent. With a higher COD in the influent, anoxic denitrification could be occurring rather than ANAMMOX reaction. Average operating results during the steady state period are shown on Table 4. The sum of NH_4-N, NO_2-N and NO_3-N concentration is defined as soluble N in Table 4. The soluble nitrogen loading to ANAMMOX reactor was 1.36 kg soluble N/m^3reactor-day during the steady states condition. According van Dongen *et al.* (2001), the nitrogen removal was in the range of 0.91–0.96 kgN/m^3-day (or 0.18–0.33 kgN/kg Dry Solids-day) for reject water treatment. Our data for soluble nitrogen conversion rate of 0.72 kg soluble N/m^3reactor-day is lower than that of van Dongen *et al.*(2001) report. Specific N removal based on biomass in the piggery wastewater was also slightly lower than that of the reject water treatment. It seems that the effluent from SHARON-ANAMMOX combination for piggery wastewater still requires a further removal to meet the effluent limitation of 60 mgN/L. In order to reduce the effluent N concentration, it can be considered either an increase of ANAMMOX reactor HRT or accumulate more biomass in the reactor. Since the increase of reactor HRT is an analogy of the cost-increase, the high biomass operation seems a feasible alternative. In this regards, a further research on ANAMMOX with a high-biomass operation would be required.

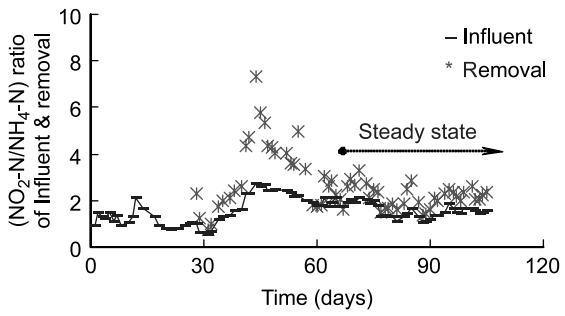

Figure 3 Nitrogen conversion behaviour in ANAMMOX reactor

Table 4 Nitrogen removal in the ANAMMOX reactor at steady state condition

Parameter	Value	Unit
steady states period	66 ~ 94	day
HRT	2.5	day
influent NH_4-N	213 ± 32	mg/L
influent NO_2-N	323 ± 34	mg/L
soluble N loading	1.36 ± 0.09	kg soluble N/m^3reactor-day
effluent NH_4-N	92 ± 14	mg/L
effluent NO_2-N	77 ± 37	mg/L
effluent NO_3-N	155 ± 32	mg/L
NO_2-N/NH_4-N removal ratio (effluent)	2.13	
soluble N conversion rate	0.72	kg soluble N/m^3reactor-day
specific soluble N removal rate	0.09	kg soluble N/kgVSS-day

Nitrogen mass balance

Results of nitrogen balances based on operating results of the combined SHARON-ANAMMOX reactor are shown in Table 5. In SHARON reactor, the nitrogen conversion due to ammonia stripping and nitrogen utilization for cell synthesis were relatively insignificant. With 1 day HRT, SHARON reactor could produce the substrate for ANAMMOX reactor stably. In overall, SHARON-ANAMMOX system for piggery wastewater could remove 66.7% of soluble nitrogen (NH_4-N + NO_2-N + NO_3-N) without an aid of external carbon energy. No ammonia gas was detected from the ANAMMOX reactor so that the possible nitrogen removal by ammonia stripping in ANAMMOX reactor could be eliminated. In ANAMMOX reactor, however, a fraction of nitrate (0.0064 g/d) was removed by anoxic denitrification according to COD balance (calculation procedure is not shown in here). The ANAMMOX reactor itself could convert 56.8% influent NH_4 to cell and N_2 gas. It is noted that a significant ammonium conversion occurred in the effluent reservoir of SHARON reactor because of the nitrification with residual DO. But nitrite and nitrate removal also occurred at the bottom of reservoir probably due to the anoxic denitrification. Hence, the reservoir for SHARON effluent removed about 5.3% of ammonium and 10.1% of NO_X-N via anoxic denitrification, which is considered as a significant contribution.

Microorganism in the ANAMMOX reactor

The fractions of microorganism were estimated with FISH using the 16S rRNA gene probes Pla46, Kst1275, NSO190 and NIT3 in the granule of ANAMMOX reactor (Figure 4). They specify *Planctomycetales*, *Candidatus* "Kuenenia stuttgartiensis," ammonia-oxidizing β-subclass proteobacteria and *Nitrobacter* spp., respectively. Fractions of very little ammonia-oxidizing β-subclass proteobacteria and *Nitrobacter* spp.

Table 5 Nitrogen mass balance in steady states condition (unit: g/d)

	SHARON				ANAMMOX		
	Influent	conversion	Effluent	→	Influent	conversion	Effluent
NH_4-N	0.3892		0.0900		0.0852		0.0368
NO_2-N	0		0.1548		0.1292		0.0308
NO_3-N	0		0.0576		0.0576		0.0620
		NH_3 stripping	0.0827			**Cell synthesis**	
						in denitrification	0.0002
		Cell synthesis				in ANAMMOX	0.0004
		in nitritation	0.0029				
		in full nitrification	0.0012			**Nitrogen gas**	
						by denitrification	0.0066
						by ANAMMOX	0.1352

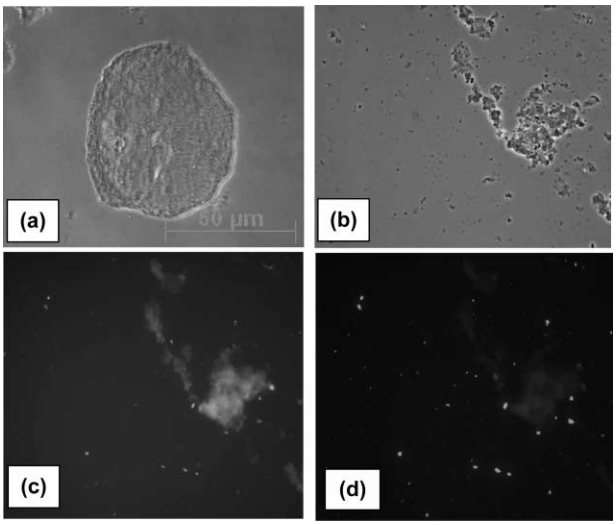

Figure 4 Microphotographs of ANAMMOX organism: (a) phase contrast micrograph of a granule in ANAMMOX reactor, (b) Disintegrated granule. (c) *In situ* hybridization with FITC-labelled probe Pla46. Cells of *planctomycetales* are shown, (d) *In situ* hybridization with CY3-labeled probe Kst1275. Cells of *Candidatus* "Kuenenia stuttgartiensis" are shown

were detected by FISH (data not shown). But estimates from FISH analysis of the fractions of *Planctomycetales* and *Candidatus* "Kuenenia stuttgartiensis" were 77% and 59%, respectively. This means that ANAMMOX bacteria were very active for nitrogen conversion in the ANAMMOX reactor with piggery wastewater.

Conclusions

The combined SHARON-ANAMMOX process was applied for nitrogen removal in piggery wastewater with very low C/N ratio. The fill-and-draw operated SHARON reactor produced an effluent for ANAMMOX reactor. The alkalinity consumption in SHARON reactor stably for piggery wastewater was 8.5 g alkalinity per g NH_4-N converted. The reactor inoculated with granules from UASB reactor successfully adopted the ANAMMOX reaction. In the ANAMMOX reactor at combined SHARON-ANAMMOX process, nitrogen conversion rate and specific nitrogen removal rate were 0.72 kg soluble N/m^3reactor-day and 0.44 kg soluble N/kgVSS-day, respectively at loading rate of 1.36 kg soluble N/m^3reactor-day. Effluent NO_2-N to NH_4-N ratio for nitrogen removal averaged to 2.13 in the ANAMMOX reactor. The microbial community analysis by FISH indicated that the ANAMMOX bacteria (*Candidatus* "Kuenenia stuttgartiensis") played great role for nitrogen conversion in ANAMMOX reactor with piggery wastewater.

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