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Piggery Waste Treatment using Partial Nitritation and Anaerobic Ammonium Oxidation

In-Su Hwang · Kyung-Sok Min[*,†] · Young-Ok Lee[**]

Office of Livestock Wastewater Treatment Plant, Sanju city
[*]*Department of Environmental Engineering, Kyungpook National University*
[**]*Division of Life Science, Daegu University*

부분질산화와 혐기성 암모늄산화를 이용한 돈사폐수처리

황인수 · 민경석[*,†] · 이영옥[**]

상주시축산폐수처리사업소
[*]경북대학교 환경공학과
[**]대구대학교 생명과학부

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Abstract

Nitrogen removal with the combined SHARON (Single reactor system for high ammonium removal over nitrite)-ANAMMOX (Anaerobic ammonium oxidation) process using the effluent of ADEPT (Anaerobic digestion elutriated phased treatment) slurry reactor with very low C/N ratio for piggery waste treatment was investigated. For the preceding SHARON reactor, ammonium nitrogen loading and removal rate were 0.97 kg NH_4-N/$m^3_{reactor}$/day and 0.68 kg NH_4-N/$m^3_{reactor}$/day respectively. In steady state, bicarbonate alkalinity consumption for ammonium nitrogen converted to NO_2-N or NO_3-N was 8.4 gram per gram ammonium nitrogen. The successive ANAMMOX reactor was fed with the effluent from SHARON reactor. The loading and removal rate of the soluble nitrogen defined as the sum total of NH_4-N, NO_2-N and NO_3-N in ANAMMOX reactor were 1.36 kg soluble N/$m^3_{reactor}$/day and 0.7 kg soluble N/$m^3_{reactor}$/day, respectively. The average NO_2-N/NH_4-N removal ratio by ANAMMOX was 2.41. Fluorescence in situ hybridization (FISH) analysis verified that *Candidatus* Kuenenia stuttgartiensis were dominate, which means that they played an important role of nitrogen removal in ANAMMOX reactor.

keywords : Combined SHARON-ANAMMOX process, Low C/N ratio, Nitrogen removal, Piggery waste

1. Introduction

The removal of nutrients such as nitrogen and phosphorus is very important issue in water environment protection. Regulatory requirements for reducing the nitrogen in the effluent have tended to be a major focus in the development of new waste treatment processes. In several years, researchers reported that anaerobic ammonium removal from the real strong nitrogenous waste, such as piggery waste, could be performed in Korea (Min et al., 2002; Ahn et al., 2004; Hwang et al., 2004). Compared with nitrification and denitrification process, the combined SHARON-ANAMMOX process has an economic advantage for nitrogen removal in wastewater treatment with an unfavorable C/N ratio. The introduction of oxygen into wastewater for the oxidation of ammonium requires a large amount of energy. Furthermore, the amount of COD present in the wastewater is often limited, making the purchase of COD in the form of methanol necessary. Some of these limitations might be circumvented by applications of developed new biotechnological process as partial nitrification of ammonia to nitrite by fast growing nitrifiers and denitrification of nitrite to nitrogen gas using ammonia as electron donor. In this way nitrogen is removed with a minimum of COD and energy (van Dongen et al., 2001).

SHARON is a process for nitrogen elimination from concentrated waste stream and operated at relatively high temperatures (35°C) and without sludge retention. In this process, nitrite oxidation is permanently prevented and denitrification with nitrite could begin. As a result, 25% of the oxygen and 40% of the carbon demand nitrification/denitrification but an external electron donor for denitrifi-

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[†] To whom correspondence should be addressed.
ksmin@knu.ac.kr

cation such as methanol as well as an effective aeration system are still necessary (Fux et al., 2002).

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

Sustainable wastewater treatment systems are being developed that minimize energy consumption, CO_2 emission with bicarbonate consumption and sludge production. However, these systems typically yield effluent rich in ammonium nitrogen and poor in biodegradable organic carbon, thereby making them less suitable for biological N removal through the conventional nitrification-denitrification sequence. Combination of the SHARON process with ANAMMOX process, which is the autotrophic, anoxic oxidation of ammonium nitrogen to nitrogen gas with nitrite nitrogen as the electron accepter, can bring about completely autotrophic nitrogen removal (Pynaert et al., 2003).

ANAMMOX : $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ (2)

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

The nitrogen removal is a 'bottle-neck' for the process design in piggery waste since nitrification step is accomplished by an aeration in which also simultaneously are removed easily degradable organics. In practice, an external supply of carbon energy is often introduced to denitrify the nitrified piggery waste. This paper mainly discusses on the operational results of the combined SHARON-ANAMMOX process configuration for nitrogen removal in piggery waste treatment. The result of microbial community analysis on anaerobic granules in ANAMMOX reactor as FISH is also presented.

2. Material And Methods

2.1. Laboratory experimental set-up

An effective volume of SHARON reactor was 1 liter 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. After running SHARON reactor at the mesophilic (35°C) and reaching the steady state, the ANAMMOX reactor of 1 L upflow anaerobic sludge bed reactor (UASB) and 0.5 L separate settling tank, which already had been operated with the mixture of nitrite stock solution and piggery waste for about 300 days in the previous research (Min et al., 2002; Ahn et al., 2004; Hwang et al., 2004), was incorporated (Fig. 1).

Granular sludge from a full-scale UASB reactor treating brewery wastewater had been inoculated at start-up ANAMMOX reactor. The ANAMMOX reactor was also operated using a fill-and-draw feeding system. It was operated as 2.5 day HRT at the mesophilic (35°C) condition. The settled sludge in the settling tank was recycled as ratio of about 0.5Q. In order to minimize adverse effects by remaining oxygen in ANAMMOX reaction, SHARON effluent and ANAMMOX influent tank were separated.

Fig. 1 shows a schematic diagram of laboratory reactors.

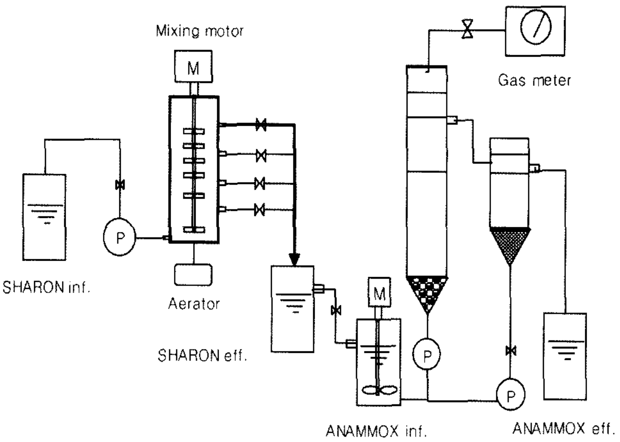

Fig. 1. Schematic diagram of the combined SHARON-ANAMMOX reactor.

2.2. Waste

The effluent of ADEPT process for hydrolysis/acidification of piggery waste, followed by a high rate methanogenic reactor such as UASB reactor was used as substrate of SHARON reactor. The influent of SHARON reactor was the effluent from anaerobic digestion system with elutriated phased treatment for piggery waste. As shown in Table 1,

Table 1. Characteristics of SHARON influent

Items	Range	Average±STD
pH	8.1~8.4	8.2±0.1
COD	1,220~4,350	1,690±770
SCOD	1,030~1,910	1,250±200
NBDSCOD	510~860	700±100
TKN	1,210~1,710	1,440±130
NH_4-N	900~1,030	970±30
NO_2-N	< 1	-
NO_3-N	<0.5	-
Total P	70~160	93±26
PO_4^{2-}	40~63	48±7
Bicarbonate alkalinity (as $CaCO_3$)	5,410~7,750	6,320±640
VFA (as HAc)	960~1,980	1,340±290
Ca^{++}	18.5~23.6	20.5±4
Mg^{++}	6.85~10.23	8.78±1.3

Note) Unit : mg/L, except pH.

because of the pre-anaerobic treatment, biodegradable SCOD/ NH_4-N ratio of SHARON influent is as low as 0.6. It was consisted of 1,250 mg SCOD/L, 700 mg NBDSCOD (non biodegradable soluble COD)/L and 970 mg NH_4-N/L. For sufficient nitrification, supplementary bicarbonate alkalinity as $NaHCO_3$ was added in SHARON influent. The effluent of SHARON process was used as influent of ANAMMOX and pH was not controlled artificially.

2.3. Analytical procedures

Nitrogen was measured in according to Standard Methods (APHA et al., 1998). Visual inspection of granules was conducted using a scanning electronic microscope. Gas production (Wet-test gas meter, Sinagawa Model W-NK-0.5A, Japan) were monitored daily. Volatile fatty acids (VFAs) were measured using 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.). Calcium and Magnesium were measured by optical emission spectrometer (Perkin Elmer, Optema 4300DV).

2.4. Fluorescence *in Situ* Hybridization

In order to evaluate the dynamics of ANAMMOX over the course of experiment, FISH was performed at the beginning and end of the experiment. The 16S rRNA-targeted oligonucleotide probes used in this study are summarized in 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 Axioplan, Germany), and the results were expressed in ratio (%) of the number of individual group-specific bacteria to the number of total bacteria.

3. Results and Discussion

3.1. The SHARON reactor

SHARON reactor was operated at 35°C and 1 day HRT. About 65 days after start-up, the SHARON reactor was reached steady state that produces a nitrite accumulated effluent. In the steady state, the SHARON reactor could convert 67.2% of influent ammonium to NO_x-N while NH_4-N removal rate by ammonia stripping and cell synthesis was about 2.5%. 30.2% of influent NH_4-N was not converted. The NO_2-N/NH_4-N ratio of SHARON effluent was about 1.5.

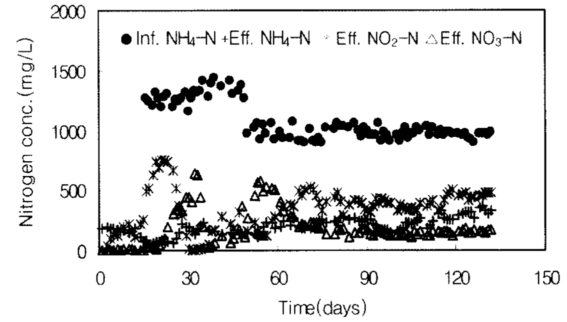

Fig. 2. Nitogen conversion in the SHARON reactor at 1 day HRT and 35°C.

Bicarbonate consumption per gram of NH_4-N converted to NO_2-N or NO_3-N except stripping was 8.4 gram. This value was a little higher than the need of bicarbonate for nitrate nitrification while it was decreased as time increased. This means that the requirement of alkalinity for nitrification is higher than 7.14 because of bicarbonate stripping. The acid equivalents released during the oxidation of ammonium are buffered with bicarbonate according to (Eq.4).

$$H^+ + HCO_3^- \rightarrow CO_2 + H_2O \qquad (4)$$

When bicarbonate is stripped from wastewater before the reaction starts, the pH increases. Because of this, the same of ammonium can be still converted. It does not matter whether bicarbonate is stripped before or after the reaction $NH_4^+ \rightarrow NO_2^-$ (van Dongen et al., 2001). Results of the nitrogen conversions in the SHARON reactor during stable state are represented in Table 3.

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]	Reference
Pla46	GACTTGCATGCCTAATCC	*Planctomycetales*	25	159	Neef et al., 1998
Kst1275	TCGGCTTATAGGTTTCGCA	*Candidatus* Kuenenia stuttgartiensis	25	159	Schmid et al., 2000
NSO190	CGATCCCCTGCTTTTCTCC	Ammonia-oxidizing β - *Proteobacteria*	55	20	Mobarry et al., 1996
NIT3	CCTGTGCTCCATGCTCCG	*Nitrobacter* spp.	35	80	Wagner et al., 1996

Note) [a] Percentage formamide in the hybridization buffer
[b] Millimolar concentration of sodium chloride in the washing buffer

Table 3. Results of the nitrogen conversions in the SHARON reactor

Parameter	Value
pH	7.8±0.5
influent NH_4-N (mg/L)	968±30
effluent NH_4-N (mg/L)	292±46
effluent NO_2-N (mg/L)	497±47
effluent NO_3-N (mg/L)	155±16
NH_4-N conversion to NO_X (%)	67.2
effluent NO_2-N/NH_4-N	1.5±0.9
NH_4-N loading rate (kg NH_4-N/m^3-day)	0.97±0.09
NH_4-N removal rate (kg NH_4-N/m^3-day)	0.68±0.06

3.2. The combined SHARON-ANAMMOX

The ANAMMOX reactor had been operated with the mixture of nitrite stock solution and piggery waste for about 300 days in feasibility test. At about 30 days after SHARON reactor was start-up, the effluent of SHARON reactor was fed as the influent of ANAMMOX reactor. During 20 days after the combined SHARON and ANAMMOX start-up, the effects of previous test used mixture substrate with piggery waste and nitrite stock solution was appeared in the effluent of ANAMMOX reactor.

As shown Fig. 3(a), in steady state of ANAMMOX reactor, the ratio between removed NO_2-N and NH_4-N was 2.41. Soluble nitrogen removal rate was 0.7 kg soluble N/$m^3{}_{reactor}$-day at average soluble nitrogen loading of 1.36 kg soluble N/$m^3{}_{reactor}$-day in stable state, Soluble nitrogen was defined as the sum total of NH_4-N, NO_2-N and NO_3-N. In this operation, specific soluble nitrogen removal rate was 0.11 kg soluble N/kgVSS-day (Fig. 3(b)). In this period, the conversion rate was higher than that in other period by denitrification because of higher content of organic matters compared to that after 50 days of operation. Van Dongen et al.(2001) reported that 0.91~0.96 kgN/m^3-day (0.18~0.33 kgN/kg DS-day) was converted and removal NO_2-N/NH_4-N ratio was 1.19~1.2 from the combined SHARON-ANAMMOX processes using sludge digester effluent.

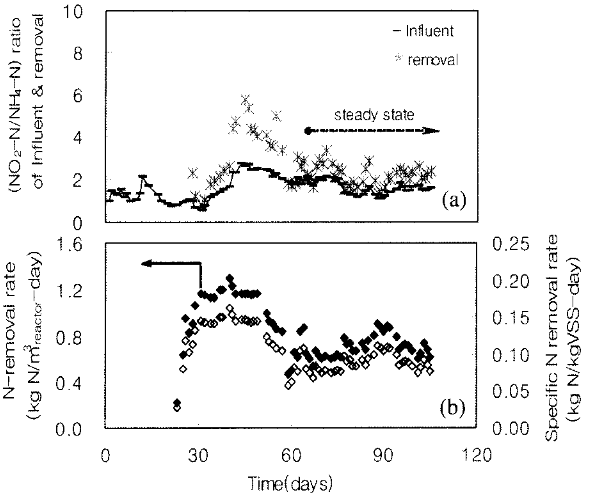

Fig. 3. Nitrogen removal in ANAMMOX reactor.

Table 4. Nitrogen removal in the ANAMMOX reactor of the combined SHARON-ANAMMOX system during steady state

Parameter	Value
Stable period (day)	76~99
HRT (day)	2.5
influent NH_4-N (mg/L)	242±47
influent NO_2-N (mg/L)	388±86
Soluble N loading rate (kg soluble N/$m^3{}_{reactor}$-day)	1.36±0.1
effluent NH_4-N (mg/L)	124±27
effluent NO_2-N (mg/L)	90±44
effluent NO_3-N (mg/L)	179±46
removal NO_2-N/NH_4-N ratio	2.41±0.43
Soluble N removal rate (kg soluble N/$m^3{}_{reactor}$-day)	0.7±0.07
Specific soluble N removal rate (kg soluble N/$m^3{}_{reactor}$-day)	0.11±0.04

Note) Soluble N means the sum total of NH_4-N, NO_2-N and NO_3-N

Summary of nitrogen removal in the ANAMMOX reactor of the combined SHARON-ANAMMOX system was shown in Table 4.

3.3. Mass balance

Nitrogen balance in the combined SHARON-ANAMMOX reactor were shown in Fig. 4. The reduction of about 21.8% of soluble nitrogen with COD consumption itself occurred at between SHARON effluent tank and ANAMMOX influent tank for oxygen removal. It is noted that a significant ammonium conversion occurred in the effluent reservoir of SHARON reactor because of the nitrification with residual DO while nitrite and nitrate also were probably removed in the reservoir via anoxic denitrification for ANAMMOX influent, which is considered as a significant contribution.

Without an external carbon energy, about 49% of ammonium nitrogen and about 71% of nitrite nitrogen from influent nitrogen were removed by ANAMMOX while nitrite nitrogen of 0.0047 g/day was removed through partial denitrification. In addition, nitrate nitrogen of 0.005 g/day was produced. In the ANAMMOX reactor, removed nitrogen to nitrogen gas by ANAMMOX and denitrification were about 96% and 3.3% of influent nitrogen, respectively. The remaining was removed by cell synthesis. In overall, the combined SHARON-ANAMMOX system could remove 65.5% of soluble nitrogen (NH_4-N + NO_2-N + NO_3-N) without an aid of external carbon energy.

3.4. FISH for the microorganisms in the ANAMMOX reactor

The fractions of microorganisms were estimated with FISH using the 16S rRNA gene probes using Pla46, Kst1275, NSO190 and NIT3 in the biopellets of ANAMMOX reactor (Fig. 4). They specificities *Planctomy-*

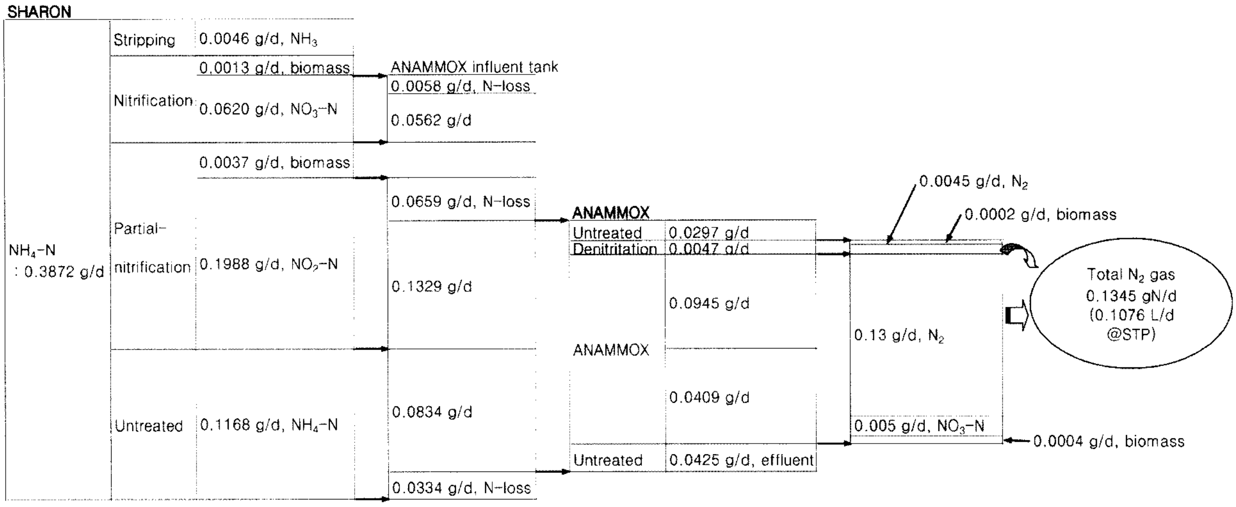

Fig. 4. Nitrogen mass balance in steady state.

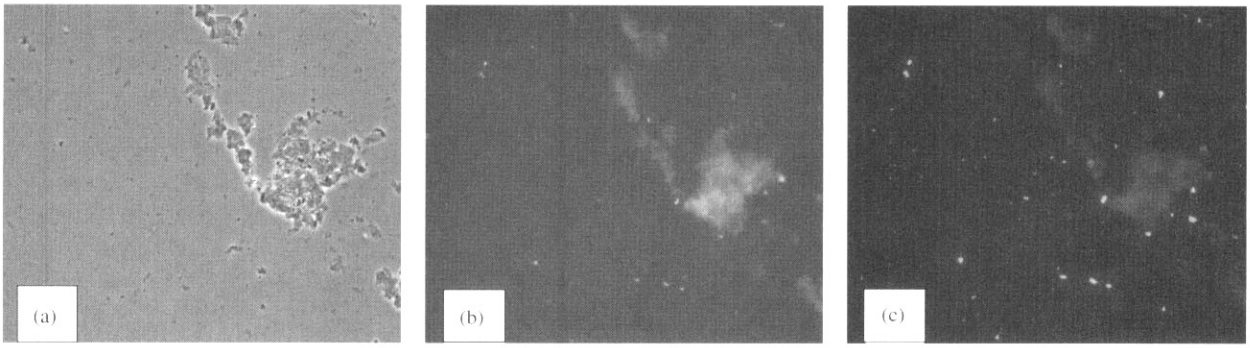

Fig. 5. Micrographs of ANAMMOX in granules. (a) Phase contrast-micrograph of biopellets, (b) *In situ* hybridization with FITC-labeled probe Pla46. Cells of planctomycetales are shown in green, (c) *In situ* hybridization with CY3-labeled probe Kst1275. Cells of *Candidatus* Kuenenia stuttgartiensis are shown in red.

cetales, *Candidatus* Kuenenia stuttgartiensis, ammonia-oxidizing β-subclass proteobacteria and *Nitrobacter* spp., respectively. Fractions of very little ammonia-oxidizing β-subclass proteobacteria and *Nitrobacter* spp. 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 played great role of nitrogen conversion in ANAMMOX reactor.

4. CONCLUSIONS

The nitrogen removal from anaerobic treatment effluent of piggery waste with high nitrogen content, especially ammonium nitrogen and very low BDSCOD/NH$_4$-N ratio as lower 0.6 using the combined SHARON and ANAMMOX process could be performed. The NO$_2$-N/NH$_4$-N ratio of SHARON effluent was about 1.5 in condition of 35°C and 1 day HRT. In ANAMMOX reactor at 1.36 kg soluble N/m$^3_{\text{reactor}}$-day of loading rate, nitrogen removal rate and specific nitrogen removal rate were 0.7 kg soluble N/m$^3_{\text{reactor}}$-day and 0.44 kg soluble N/kgVSS-day respectively. In addition, the ratio converted NO$_2$-N/NH$_4$-N was 2.41. In overall, the combined SHARON-ANAMMOX system could remove 65.5% of soluble nitrogen (NH$_4$-N + NO$_2$-N + NO$_3$-N) without an aid of external carbon energy.

FISH analysis verified that ANAMMOX bacteria especially *Candidatus* Kuenenia stuttgartiensis, was played great role of nitrogen removal in ANAMMOX reactor.

국문요약

SHARON (아질산성 질소로의 고효율 암모늄 전환)-ANAMMOX (혐기성 암모늄 산화) 결합공정을 이용하여 돈사폐수를 처리하는 매우 낮은 C/N비를 가진 ADEPT (고효율 혐기성 소화)공정의 유출수로부터 질소를 제거하는 연구를 수행하였다. 전단의 SHARON 반응조에서 암모니아성 질소의 부하 및 제거율은 각각 0.97 kg NH$_4$-N/m$^3_{\text{reactor}}$/day 및 0.68 kg NH$_4$-N/m$^3_{\text{reactor}}$/day였다. 안정상태에서 NO$_2$-N 또는 NO$_3$-N로 전환된 암모니아성 질소에 대한 중탄산알칼리도의 소모량은 전환된 암모니아성 질소 그램당 8.4 그램이었다. 후단의 ANAMMOX 반응조는 SHARON 반응조의 유출수가 공급되었다. NH$_4$-N, NO$_2$-N 그리고 NO$_3$-N의 합

으로 정의되는 ANAMMOX 반응조의 용존성 질소 부하와 제거율은 각각 1.36 kg soluble N/m$^3_{\mathrm{reactor}}$/day 및 0.7 kg soluble N/m$^3_{\mathrm{reactor}}$/day였다. ANAMMOX에 의한 평균 NO_2-N/NH_4-N 비율은 2.41이었다. FISH 기법을 이용한 염기서열 분석결과 ANAMMOX 반응조에서 *Candidatus* Kuenenia stuttgartiensis가 우점하였으며, 질소제거에 중요한 역할을 한 것으로 나타났다.

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