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Efficient Nitrogen Removal of Reject Water Generated from Anaerobic Digester Treating Sewage Sludge and Livestock Manure by Combining Anammox and Autotrophic Sulfur Denitrification Processes

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Abstract: The reject water from anaerobic digestion with high (Total Nitrogen) TN concentration was treated by a demonstration plant combining the anammox process and SOD (SOD®; Sulfur Oxidation Denitrification) process. The anaerobic digestion was a co-digestion of livestock wastewater, food waste water, and sewage sludge so that the TN concentration and conductivity of the reject water were very high. This anammox plant was the first anammox demonstration plant in South Korea. The maximum TN removal efficiency of 80% was achieved for the anammox reactor under nitrogen loading rate (NLR) of 0.45 kg-N/m^3·d. As a result of decreasing the dilution of the reject water, the influent conductivity and NLR values were increased to 7.8 mS/cm and 0.7 kg/m^3·d, causing a rapid decrease in the TN removal efficiency. The sludge concentration from the hydro-cyclone overflow was about 40 mg-MLVSS/L in which small sized anammox granules were detected. It was proven that the increase in (Mixed Liquor Volatile Suspended Solids) MLVSS concentration in the anammox reactor was not easy under high influent conductivity and NLR. 97% of NO_2^--N+NO_3^--N generated from the anammox process could be treated successfully by the SOD reactor. A TN removal efficiency of 35% under poor annamox treatment could increase to 67% by applying the SOD reactor post treatment for the removal of NO_3^--N. The dominant anammox bacteria in the anammox reactor was identified as *Brocadia fulgida* and 9.3% (genus level) of the bacteria out of the total bacteria were anammox bacteria.

Keywords: nitrogen removal; anammox; reject water; co-digestion; sulfur denitrification; partial denitrification

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1. Introduction

The South Korean government prohibited all ocean dumping in accordance with the London Dumping Convention from 2016. Organic waste (food wastewater, sewage sludge, livestock manure) that was dumped in the ocean must be disposed of within land now. As a land treatment method for organic waste, anaerobic digestion treatment is receiving much attention [1].

Organic waste treatment using the anaerobic digestion process has a great advantage that it can obtain energy through biogas generation as well as waste treatment. Recently, efforts have been made to combine sewage sludge with livestock manure and food waste produced by municipalities to produce self-sufficient energy in sewage treatment plants of local governments [2]. However, the reject water from the anaerobic digestion contains a high concentration of nitrogen. Therefore, when a high concentration of nitrogen in the mainstream is treated using a conventional nitrogen removal process, the nitrogen removal capacity of the mainstream is insufficient and causes an increase in operational costs. As a result, a self-sufficient sewage treatment plant requires an economical nitrogen treatment process [3–5].

Compared to the conventional nitrogen removal technology based on nitrification and denitrification, anammox technology is regarded to be a very economical technology. [6,7]. Since the first full scale anammox plant was operated in 2000, about 100 anammox plants have been operating, mainly in Europe [8,9]. Under this situation, there is no case of full scale application of the anammox process in South Korea. Since the first paper was published in 2004, a variety of attempts have been made to establish a way of enriching anammox sludge by lab scale research [10]. The reasons for failing to establish a full scale anammox plant in South Korea is the difficulty in obtaining sufficient quantities of anammox sludge, and complicated operation of the anammox process compared with that for the conventional nitrogen removal process.

The anammox process has been successfully commercialized mainly in Europe. Representative technologies for each company in Europe are EssDe® (Switzerland, EssDe), ANITATMMOX (France, Veolia), ANAMMOX® (Netherland, PAQUES), and NAS® (Netherland, Colsen). Several Japanese companies (Hitachi, Ltd, Kurita Water Industries Ltd, Takuma Co. Ltd., and Meidensha Cooperation) succeeded in the commercialization of the anammox process. Recently, China is also spurring the development of anammox technology with intensive investments. In this trend, efforts are being made not only to develop South Korea's own anammox technology, but also to improve and apply foreign advanced anammox technology to suit the characteristics of South Korea wastewater.

In the case of the anaerobic co-digestion (food wastewater, sewage sludge, and livestock manure), the nitrogen concentration of reject water is about five to six times higher than that for usual anaerobic digestion. Lackner et al. [8] reported that the influent ammonium nitrogen (NH_4^+-N) concentration in the investigated facilities was below 1,000 mg/L. However, in South Korea, where most digestion is co-digestion, the concentration of NH_4^+-N of the reject water from anaerobic digestion is as high as 1,000~3,000 mg/L and the conductivity is about 20~30 mS/cm. Thus, a high concentration of NH_3 (FA, free ammonia) suppresses the anammox process [11–13]. Scaglione et al. [14] also reported that IC_{50} (concentration corresponding to the 50% activity reduction) was observed at 6.1 mS/cm. Therefore, there is a need for an anammox process applicable to high concentration wastewater characteristics.

Theoretically, 11% of NH_4^+-N removed by the anammox reaction remains as NO_3^--N [15]. Therefore, influent with a high NH_4^+-N concentration is introduced to the anammox reactor, and a high concentration of NO_3^--N remains in the effluent. As a result, post treatment is required to satisfy the effluent water quality standards. Studies have also reported the use of heterotrophic denitrification. However, the supply of external carbon sources and the generation of excess sludge are caused, which limits the advantages of the anammox process [16].

Sulfur denitrification has various advantages over heterotrophic denitrification. Sulfur denitrification is a technology for the removal of NO_2^--N and NO_3^--N using sulfur as an electron donor. The amount of excess sludge production for the sulfur oxidizing bacteria (SOB) process is one tenth of that for the heterotrophic denitrification process, thereby sludge disposal cost can be reduced [17]. Conventional sulfur denitrification has the disadvantage of a pH decrease caused by the alkalinity consumption. To compensate for this disadvantage, sulfur denitrification was combined with heterotrophic denitrification for supplementing alkali substances were applied [18–20]. In addition, a sulfur denitrification method using a special carrier, which can prevent the decrease in pH by mixing elemental sulfur and an alkaline substance, was developed. Therefore, combining this sulfur denitrification process with the anammox process makes it possible to remove NO_3^--N from the effluent of the anammox process.

Recent studies have shown that partial denitrification converts NO_3^--N into NO_2^--N and then NO_2^--N and NH_4^+-N was removed by the anammox reaction [21–23]. Many reports showed an increase in total nitrogen removal efficiency by the combination of sulfur denitrification as the post treatment of anammox process [24–26].

In this paper, the results of first full scale operation of the anammox process in South Korea are described. In particular, the anammox process was firstly applied to treat reject water from an anaerobic digester where sewage sludge, livestock manure, and food wastes water are co-digested. Sulfur oxidizing denitrification (SOD) was applied to remove NO_3^--N in the effluent of the anammox process. Influent conductivity affecting the nitrification and anammox reaction was investigated. In addition, changes of the microbial community were monitored and confirmed at different operating conditions during long term operating duration.

2. Materials and Methods

2.1. Site Status

The site where the full scale anammox plant is installed is the local wastewater treatment plant (WWTP), treating 30,000 m^3/day of sewage by the A_2O method. The anaerobic digester accepts 150 m^3/day of sewage sludge, 100 m^3/day of livestock manure, and 1 m^3/day of food waste water. The reject water generated from the anaerobic digester is treated by long-term aeration and chemical treatment, and the final produced reject water is returned to the primary settling tank of WWTP. Due to the shortage of treatment capacity of the WWTP, it is difficult to treat the reject water properly, especially in the winter season. Therefore, it is necessary to increase the facility size or reduce the nitrogen concentration of the reject water.

As a result, the anammox process, which can remove high concentration nitrogen, can be regarded as the most suitable process to reduce the nitrogen load to the mainstream. In this study, the anammox process was applied to remove high concentration nitrogen from the reject water. The anammox process was designed and installed to treat about 40% of the total reject water volume. A one stage anammox process (EssDe®) was designed and operated in the SBR (sequencing batch reactor) mode. The nitrogen load was more than 100 kg-N/day on average.

2.2. Reactor Setup and Operating Methods

Figure 1 shows the process schematics. The remaining pre-installed tank, which was previously used as a sludge thickening tank, was modified to the anammox reaction tank. The anammox reactor volume is 330 m^3 and its effective volume is 230 m^3. This anammox tank was operated under a hydraulic retention time (HRT) of 1.0–1.5 days.

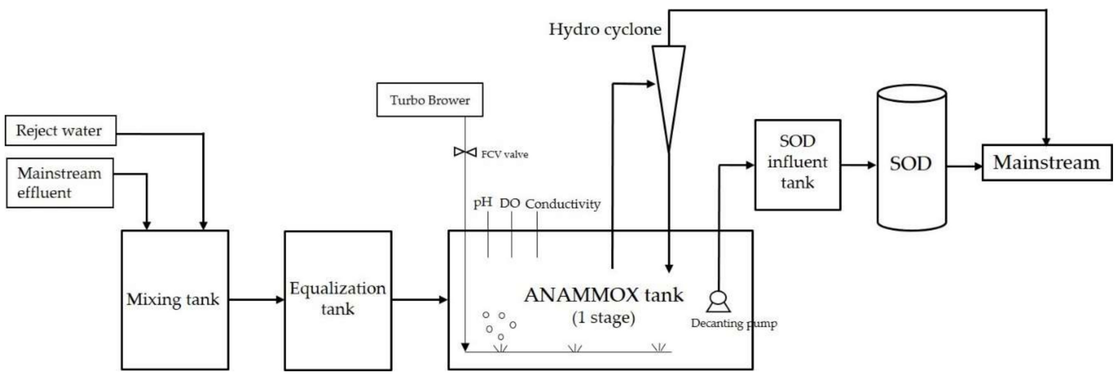

Figure 1. Process schematics.

The reject water is stored in the reject water reservoir and then flows into the anammox equalization tank. The reject water was diluted with mainstream effluent and then introduced to the anammox reactor. The reason for this dilution is to minimize the influence of conductivity, which adversely affects the ammonium oxidizing bacteria (AOB) and anammox bacteria. In the equalization tank, mainstream effluent was allowed to flow into the anammox reaction tank according to the dilution ratio. Wastewater inflow can be introduced into the anammox tank after mixing the reject water and mainstream effluent, which is automatically controlled through the inflow control of the (Human Machine Interaction)HMI program.

In the anammox tank, a submerged mixer was installed for mixing with the reactor. To control the DO concentration, a turbo blower and a flow control valve (FCV) were installed so that the DO could be controlled minutely. Using hydro-cyclone, anammox sludge was returned (underflow) to the reaction tank and nitrate oxidation bacteria (NOB) and heterotrophic bacteria were discharged (overflow) from the reaction tank.

The anaerobic digester is mid-temperature digestion and the temperature of the recirculating water is about 30–35 °C. Therefore, if the reject water is introduced into the anammox reaction tank without dilution, the heating facility is unnecessary. However, in this study, the reject water was diluted with the mainstream effluent. During the winter season, the temperature of the mainstream effluent was decreased to about 10 °C, so that the heating system is needed. The heating of the mainstream effluent was carried out by the steam of the biogas boiler. After introducing the heating system, the anammox reactor temperature could be maintained at about 30 °C. Experiments confirming the effect of conductivity on treatment performance were conducted.

The operational conditions of the reactor are shown in Table 1. Operational conditions were changed in eight steps. The flow rate, NLR, and nitrogen concentrations of each step are shown as an average value. Run 1, which was the stabilization step after inoculation, was operated under the minimum inflow of reject water. After the system was stabilized, in Run 3, NRLs were increased carefully, but the nitrogen removal efficiencies were not improved. Therefore, the nitrogen removal rate (NRR) was reduced again. In Run 4, the dilution ratio was increased to restore the decreased nitrogen removal efficiency. In Run 5 and 6, stable operation was achieved. In Run 7, influent nitrogen concentration was the highest under low dilution. In Run 8, the operation was performed to recover the nitrogen removal efficiency again.

Table 1. Operational conditions.

Run	Elapsed Time (Day)	Reuse Water Flow Rate (m^3/d)	Reject Water Flow Rate (m^3/d)	Dilution Rate	Influent TN Concentration (mg/L)	Nitrogen Load (kg/d)
1	0–90	95 ± 33	32 ± 14	4.0	411 ± 58	51 ± 27
2	91–105	121 ± 9	40 ± 3	4.0	411 ± 65	71 ± 7
3	106–151	136 ± 16	44 ± 8	4.1	436 ± 63	79 ± 12
4	152–236	127 ± 17	39 ± 7	4.3	482 ± 64	83 ± 16
5	237–326	141 ± 24	47 ± 8	4.0	549 ± 22	105 ± 16
6	327–390	154 ± 23	52 ± 10	4.0	542 ± 59	111 ± 17
7	391–411	112 ± 19	58 ± 9	2.9	661 ± 44	111 ± 21
8	412–440	160 ± 16	55 ± 5	3.9	463 ± 37	100 ± 6

The SOD reactor was applied to treat effluent from the anammox reactor. The SOD reactor was filled with JSC-Pellet® (JEON TECH. CO., LTD. Suwon, South Korea), which contains sulfur and alkali substances, and operated under up flow mode. The working volume of the SOD reactor was 0.5 m^3 and the reactor was filled with 330 kg of JSC pellet. The flow rate was adjusted to approximately 1.3 m^3/d and the internal recirculation rate from the upper to the lower part was approximately 1.8 m^3/hr.

2.3. Inoculation

Initial seed sludge for the anammox process was supplied by EssDe. Concentrated anammox sludge had a TS value of about 30,000 mg/L (VS 71%) and an MLVSS value of about 1,900 mg/L after seeding of anammox sludge into the reaction tank. By keeping the reactor temperature above 27 °C after seeding, the influent NH_4^+-N concentration was maintained at about 400 mg/L. NLRs were increased by increasing the influent flow rate.

Nitritation did not occur well just after inoculation. It was concluded that the activity of ammonia oxidizing bacteria (AOB) was reduced during long-term (60 day) transportation. For the purpose of supplementing AOB, activated sludge from the aeration tank of the existing reject water treatment facility was inoculated into the anammox reaction tank. Activated sludge from the existing reject water treatment facility was additionally inoculated with 5 m^3/d for 10 days. The existing aeration tank consists of four tanks in total. It is a facility operated by the point gauging technique, which operates the aeration tanks from 1 to 4 with a DO of 2.0 mg/L, 1.5 mg/L, 1.0 mg/L, 0.5 mg/L, or less, respectively. Approximately 50% of the influent NH_4^+-N was converted to the NO_2^--N form at the first stage of the aeration tank by introducing the reject water without dilution (NH_4^+-N concentration of reject water about 2000 mg/L).

Activated sludge of the aeration tank of the existing reject water treatment facility was also seeded as an inoculation source in the SOD reactor. About 4 kg MLVSS of activated sludge was seeded as noculum. An internal circulation was conducted for 2 days after seeding. Then, influent was fed to the SOD reactor.

2.4. Analytical Methods for Liquid Samples

Daily sampling and water quality analysis were conducted to evaluate the treatment efficiency for each reactor. Sampling was carried out for the reject water, mainstream effluent, equalization tank, anammox tank, and the influent and effluent of the SOD reactor. CODcr, TN, TKN, NH_4^+-N, NO_2^--N, NO_3^-N, and SO_4^{2-} were measured by the colorimetric method (APHA, 2005). MLSS and MLVSS were measured by the Standard Method (APHA, 2005). pH, temperature, electrical conductivity, inflow air volume, and flow rate were monitored in real time through HMI.

2.5. Analytical Methods for Microbial Community Samples

The samples were taken 6 times for monitoring any microbial community change. The microorganisms were analyzed as follows, at the Molecular Microbial Ecology Laboratory at the Chun Lab in the Republic of Korea. For the bacterial 16S rRNA amplification, the barcoded primers, 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGYTACCTTGTTACGACTT-3′), were used. The PCR reactant (50 μL) consisted of 1X solution (100 mM Tris-HCl, 1.5 mM $MgCl_2$, 500 μg/mL BSA, pH 8.3), 160 μM dNTPs, 0.3 μM primer, cleaned DNA (10-15 ng/μL), and 1 unit Taq polymerase (HanTaq, Genenmed). The PCR conditions were an initial cycle at 95 °C for 3 min followed by 30 cycles at 95 °C for 3 s, 49 °C for 30 sec and 72 °C for 1 min, and a final step at 72 °C for 10 min.

The PCR product was purified with a PureLinkTM Quick Gel Extraction Kit (Life Technology, USA) after electrophoresis in 1% agarose gel. The 16S rRNA clone library was constructed using pGEM-T vector (Promega, USA). The direct amplified PCR was carried out using the prGTf ('5-TACGACTCACTATAGGGCGA-3′) of T-vector and the 1492R primers of 16S rRNA. The amplification was done under the following conditions: Initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final elongation at 72 °C for 5 min. The PCR product was confirmed by using 2% agarose gel electrophoresis and visualized under a Gel Doc system (BioRad, Hercules, California, USA).

2.6. Particle Size and Morphological Analysis

Particle size distribution of total biomass (granular biomass and floc type biomass) was measured. The laser light scattering technique was used to characterize the particle size distribution of biomass in the reactors. A laser particle size analysis system (HELOS, Sympatec GmbH, Germany) was used. Every sample was measured in triplicate.

3. Results and Discussion

3.1. Nitrogen Removal Performance of Anammox Process

COD_{cr}, NH_4^+-N, and TP concentrations of the reject water were 2,670 ± 271, 1,762 ± 144 (1,732 ± 131), and 22 ± 6.2 mg/L, respectively. The major nitrogenous components of reject water, NH_4^+-N. NO_2^--N and NO_3^-N, were not detected in the reject water. The average conductivity of reject water was 17,822 ± 860 µS/cm. The maximum and minimum conductivity of reject water were 20,200 µS/cm and 15,200 µS/cm, respectively. The mean TSS concentration, pH temperature, and alkalinity of reject water were 678 mg/L, 8.25 ± 0.1, 31.5 ± 1 °C, and 10,000 mg/L, respectively.

Figure 2 (A) shows the changes in nitrogen concentrations, TN removal efficiency, and reactor temperature for the anammox tank. Figure 2 (B) shows the daily changes in NLR, NRR, SNR (specific nitrogen removal), and the ratio of NO_3^--N production to NH_4^+-N removal.

Run 1 showed the results of operation during the stabilization period after seeding. After inoculation, NLR was kept at 0.06 kg-N/m^3·d and 0.44 kg/m^3·d of NRR was obtained after 60 days of operation. At this period, the NRR was increased from 0.05 kg/m^3·d up to 0.32 kg/m^3·d and the TN removal efficiencies were maintained at about 80%. However, effluent NO_3^--N concentrations were increased from around day 90 of operation. Then, the TN removal efficiencies began to decrease, and this phenomenon lasted until Run 2.

The TN removal efficiencies were gradually recovered on day 120 in Run 3. NLR was increased to 0.4 kg-N/m^3·d. However, seasonal changes in influent temperature caused a decrease in the reactor temperature. As the mainstream effluent was used for the dilution of the reject water, the temperature of the anammox reactor also decreased. The temperature dropped to 23 °C on day 150. Then, the biogas boiler was applied for heating the anammox tank.

Foaming occurred in the reaction tank under the poor nitrogen removal condition. There are various reasons for this foaming phenomenon. One reason was the inflow of antibiotics used for foot-and-mouth disease. At the same time, lime was used to prevent such a disease in the pig keeping farm. Therefore, it is considered that the inflow of antibiotics and lime might affect the TN removal efficiency and thus caused the foaming in the anammox reactor.

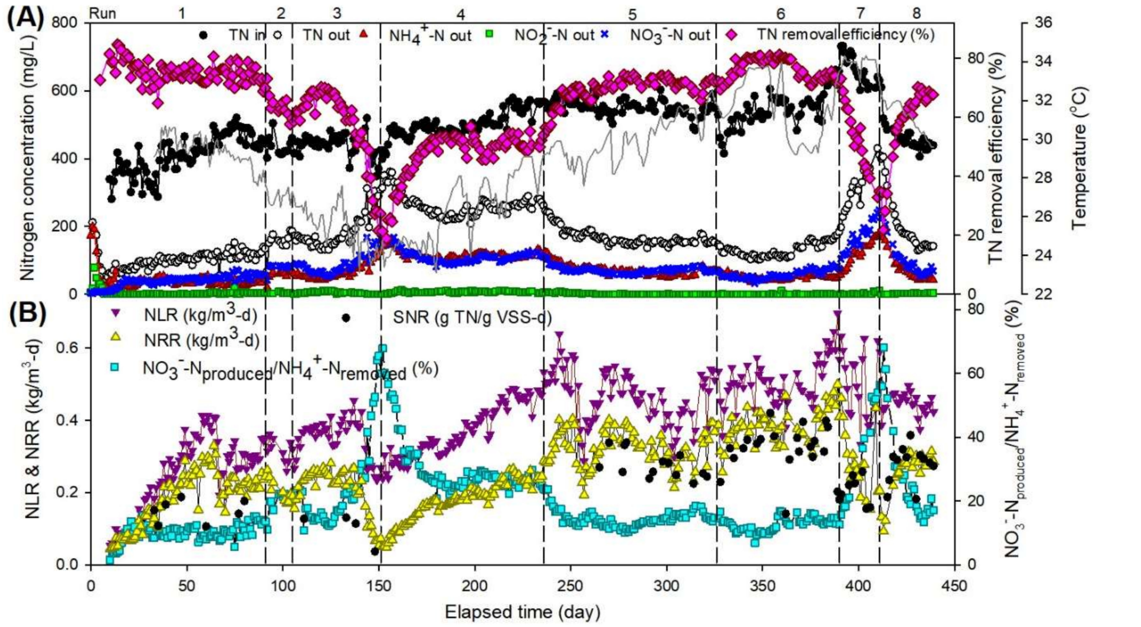

Figure 2. Treatment performance of the anammox process during long-term operation: (**A**) Changes in TN, NH_4^+-N, NO_2^--N, and NO_3^--N concentrations, TN removal efficiency, and temperature (**B**) Changes in NLR, NRR, SNR, and the ratio of produced NO_3^--N concentration to removed NH_4^+-N concentration.

Fernandez et al. [27] reported the effects of antibiotics on anammox bacteria. Tetracycline hydrochloride and chloramphenicol were studied in the range of 100–1000 mg/L and 250–1000 mg/L, respectively. 20 mg/L of chloramphenicol was reported to cause a 25% reduction in the nitrogen removal efficiency. Specific anammox activity (SAA) was also decreased from 0.25 to 0.05 g-N/g-MLVSS·d. A similar phenomenon was reported for tetracycline hydrochloride.

To improve the TN removal efficiency, the washing out of toxic substances from the reactor was tried. At this time, 100% throughput through the hydro-cyclone was started. By operating the hydro-cyclone separator, granular anammox sludge was returned to the reactor and the floc type sludge was discharged from the reactor. Accompanying this operation, AOB sludge was also washed out from the reactor, so that supplementation of AOB was needed. For this purpose, activated sludge from the aeration tank of WWTP was added again to the anammox reactor by 5 m^3/d (dry weight approximately 20 kg/d). This supplementation was continued from day 152 to 199.

To increase the AOB activity and nitration rate, DO was also increased to approximately 1 mg/L. Increasing the reactor DO could enable an increase of the influent flow rate. The reduction in SRT was proven to be effective for NOB washout. These applied measures resulted in an increase of NLR. In our system, the influent flow rate was determined by the pH high/low setting. At the start of the inflow time, the pH in the reaction tank was increased due to the high influent alkalinity, and the influent stops at the 'high' value. As nitrification occurs by aeration, the pH was reached by getting a 'low' value. Then, the inflow started again and the reactor pH reached a pH high value. Therefore, the faster the nitrification rate, the faster the pH decrease rate. Inflows occurred frequently. Additionally, the NLR was increased. As a result of this operational condition, the removal efficiency of Run 4 recovered to about 55%.

Although the temperature was about 24 °C until 160 days, the removal efficiency was increased. If it had not been diluted with mainstream effluent, heating would have been unnecessary. The temperature of the digester effluent was about 30 °C. In general, the optimum temperature range for the anammox reaction is 30-40 °C [13,28,29]. However, there have also been successful cases of anammox operation at lower temperatures of 18 °C and 20 °C [30,31]. It was reported that the activity of anammox bacteria is reduced at temperatures below 30 °C. However, in this study, it is considered that the influence of temperature is not obvious even in operations under 24 °C.

Energy loss by heating is great in winter when using mainstream effluent as dilution water. However, it was difficult to treat reject water containing high concentrations of NH_4^+-N and conductivity by the anammox process without dilution. Therefore, when dilution of reject water is required, the energy cost for heating the influent must be minimized by checking the optimum dilution factor.

In Run 5, DO was reduced to less than about 0.5 mg/L. Under the operating reactor DO concentration of 1 mg/L, the TN removal efficiency could not increase. The TN removal efficiency was increased to about 70% after decreasing the DO concentration to less than 0.5 mg/L. Effluent NH_4^+-N and NO_3^--N concentrations were decreased simultaneously. The ratio of NO_3^--N production/NH_4^+-N removal also decreased gradually. It was proven that anammox activity was increased and NOB activity was decreased with a decrease in the reactor DO concentration. It is considered that the effect of adjusting DO to 0.5 mg/L was more influential because a large amount of NOB was washed out during the period of 100% discharge by hydro-cyclone.

However, the decrease in the reactor DO concentration resulted in a decrease of the nitritation rate, which caused a reduction of NLR to 0.29 kg-N/m^3·d. Then, an increase in the nitrification rate by securing AOB activity in the anammox tank was attempted by reducing the volume of hydro-cyclone discharge (=reducing floc type discharge).

About 80% of the TN removal efficiency was obtained in Run 6. At this time, the ratio of NO_3^--N production/NH_4^+-N removal remained stable at about 10%.

In Run 6, the dilution ratio was decreased to investigate the change of the removal efficiency by the conductivity. At the same time, influent NH_4^+-N concentrations and NLR were also increased.

From day 353, the dilution rate was reduced from 4.5 to 4 times. After that, the dilution ratio was decreased to 3 times in a stepwise manner until day 390. The influent conductivity was about 5.8 mS/cm under a 4.5 time dilution. Under a decreasing dilution ratio to 3 times, the influent conductivity was increased to about 7.0 mS/cm. During this period, the NLR was increased from 0.4 kg-N/m^3·d to 0.7 kg-N/m^3·d. During days 373-390, the TN removal efficiency decreased by about 5%, but it was not decreased more. However, the TN removal efficiency began to decrease sharply after changing the dilution ratio to 2.8 on day 391. The influent conductivity was increased to about 7.8 mS/cm at under 2.8 times dilution. Unlike the decrease in the TN removal in Run 3, effluent NO_3^--N was increased obviously. This is probably due to the decrease in the anammox activity. NO_2^--N and NH_4^+-N must be removed simultaneously by the anammox reaction, but the NH_4^+-N concentration was increased under the condition of high NO_3^--N production by NOB. Thus, NOB washout was needed. In order to recover the TN removal efficiency, the volume of hydro-cyclone overflow discharge was increased. Additionally, the DO concentration was increased to enhance the nitritation activity during days 397–411. However, the TN removal efficiency could not be recovered. However, TN removal efficiency was gradually improved after changing the dilution rate to 4.0. In Run 8, the TN removal efficiency was restored to about 70% after 20 days of operation.

Salinity is well known as one of the important inhibition factors of anammox bacteria [32]. There are many studies evaluating specific anammox activity (SAA) with increasing salinity levels in the anammox reactor. Most of these studies used single salt (usually NaCl) for their experiments. Scaglione et al. [14] conducted an anammox activity batch test on supernatants from four full scale anaerobic digestion plants. Short term test results reported IC_{50} (corresponding to the 50% activity reduction) values was 6.1 mS/cm. This is 1.9-3.8 times lower than the IC_{50} value of 11.4 mS/cm [33] and 22.9 mS/cm [31] in single salt experiments. In the activity test using inoculum stored at 11 mS/cm for approximately 4 months, the inhibition response decreased by 12-14% in synthetic saline medium and real wastewater. Therefore, it was reported that the inhibition of conductivity can be reduced through adaptation.

In this study, the influent conductivity and the reactor conductivity for more than 350 days before reducing the dilution ratio was 5.8 mS/cm and 3.2 mS/cm, respectively. Although our anammox reactor was operated for a long time, anammox bacteria failed to adapt to high concentrations of 11.4 mS/cm or more. The dilution factor was reduced by about 2 weeks of the adaptation period for each step of reducing the dilution ratio. However, the removal efficiency was decreased sharply.

3.2. Anammox Process Problems and its Improvement

Figure 3(A) shows the changes in MLVSS concentrations in the reactor, hydro-cyclone overflow, and underflow. During the process operation, the MLVSS concentration in the reactor remained at an average of 1,150 mg/L. The MLVSS concentration of the underflow was maintained at 2,900 mg/L by the day 280, but gradually decreased. About 60-70% of the overflow sludge was composed of granules type sludge.

Figure 3(B) shows the changes in the specific nitrogen removal rate in the anammox tank. After 250 days in Run 6, the specific TN removal rate was increased up to 0.39 kg-N/kg-MLVSS·d, but decreased again to 0.15 kg-N/kg-MLVSS·d at Run 7. As the influent conductivity and NH_4^+-N concentration increased, the specific nitrogen removal rate also decreased. In this study, it would be necessary to increase the biomass concentration in the reaction tank for more stable anammox operation.

Lackner et al. [8] compared eight operating cases of the DEMON® (Gommiswald, Switzerland) process, which are the same as our anammox process. According to this report, TS in the reaction tank varied from 1,000 mg/L to 4,500 mg/L. The operation was carried out under higher NLR than that of our reactor. Attainable NLR is varied depending on the operating conditions and the concentration and characteristic of target wastewater.

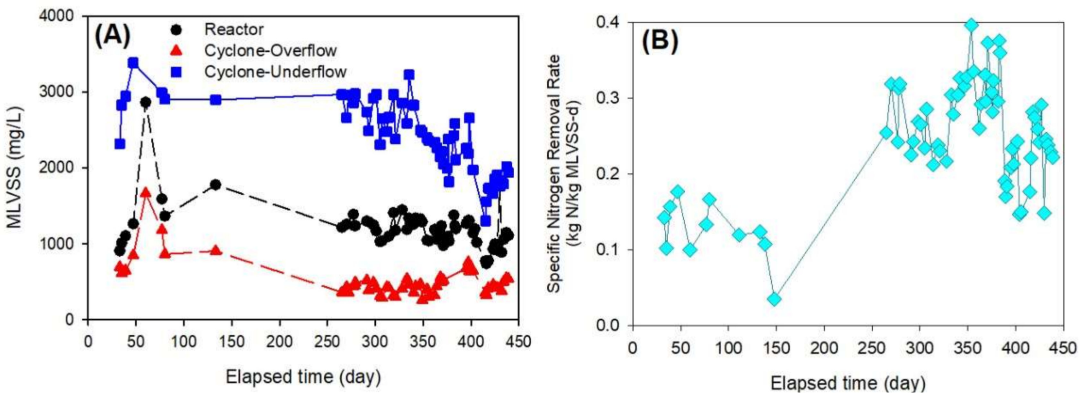

Figure 3. Daily changes in the MLVSS concentration (**A**) and the specific nitrogen removal rate (**B**).

The collection of anammox granules by hydro-cyclone was very effective. However, about 40 mg MLVSS/L of anammox granule was washout in the overflow. Also, about 20 mg MLVSS/L of the anammox granule was washout in the supernatant at the decanting time. Approximately 7 kg/d of anammox biomass should increase based on the anammox yield of 0.1 kg-biomass/kg-N and the removal amount of 70 kg-N/d. Approximately 6 kg/d of biomass was washout under 200 m^3/d of the inflow rate with 100 m^3/d of hydro-cyclone discharge and 100 m^3/d of decanting discharge. Therefore, it was very difficult to maintain the high granular anammox concentration in the reactor.

A method for increasing the anammox granular concentration in the reactor was successfully confirmed in this study. It was not easy to confirm the increase in anammox granular concentration because the anammox sludge in the reactor was a mixture of flocs type anammox sludge and granule type anammox sludge. Figure 4(A) shows the size distribution of granular anammox sludge. Our obtained anammox granules showed a wide distribution in particle size with a mean size of 320 µm. In addition, it was found that the anammox granule whose size was 200 µm or more occupied about 50% or more of the whole anammox granules. Floc type anammox sludge was sieved by a 150 µm sieve, but the sieve clogged quickly (data not shown). Finally, more than 90% of our anammox granule could be successfully recovered by the simple sieving with a 200 µm sieve alone.

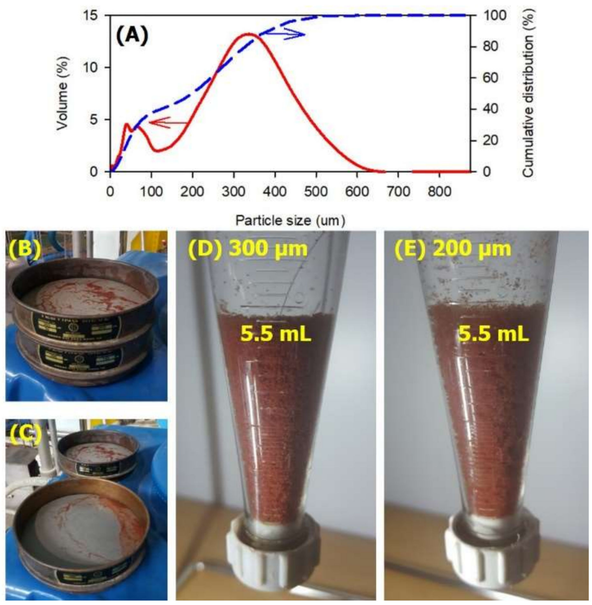

Figure 4. Size distribution of anammox sludge (**A**) and the sieve screening result (**B**)-(**E**).

Anammox granules were sieved by a 200 μm and 300 μm size in a stepwise manner as shown in Figure 4(B),(C). As a result, the same volume of anammox granules was recovered from this two-step sieving as shown (Figure 4 (D),(E)). The floc type anammox sludge passed through this sieve.

3.3. Feasibility and Application of Combined Anammox and the SOD Process

From day 245, operation of the SOD reactor was started. The aim of the SOD reactor is the removal of NO_3^-N, which is produced during the preceding anammox reaction. A high concentration of effluent NO_3^--N is produced during the anammox treatment for high NH_4^+-N containing wastewater. Hence, post treatment is required to satisfy the effluent TN standard.

In this study, residual NO_3^--N was removed by applying the SOD reactor, in which elemental sulfur is used as an electron donor. Sulfur denitrification has a disadvantage of consuming alkalinity and its corresponding decrease in reactor pH. In this study, a special sulfur pellet was used. The special pellet was a JSC Pellet, which was a mixture of elemental sulfur (S) and calcium carbonate ($CaCO_3$). The JSC pellet can keep a neutral pH (7.0–8.0) by preventing a pH decrease through constant supplementation of alkalinity from the pellet. By using this pellet, stable operation of the SOD reactor under neutral conditions without pH adjustment is feasible. The SOD reactor was filled with JSC pellets and operated under the up-flow method.

Figure 5 shows changes in TN, NH_4^+-N, NO_2^--N, and NO_3^--N concentrations and the removal efficiency for the SOD reactor. Stable operation of the SOD reactor was attained within 7 days after inoculation and NO_2^--N and NO_3^--N from the anammox reactor were stably removed. The average treatment efficiency of NO_2^--N+NO_3^--N was 90% and the maximum removal efficiency of NO_2^--N+NO_3^--N was 97%. The average TN removal efficiency of the SOD reactor was 44%. This is because NH_4^+-N was not removed by the SOD reactor. As shown in Figure 5, the effluent quality of SOD was very stable even under fluctuation of influent TN concentrations. The average SS of the anammox was about 200 mg/L, but the clogging of the SOD reactor was not significant. The treatment efficiency of the SOD reactor did not decrease too much even under changing influent TN concentrations for about 200 days of operation. Backwashing of the SOD reactor was not performed for 200 days of continuous operation.

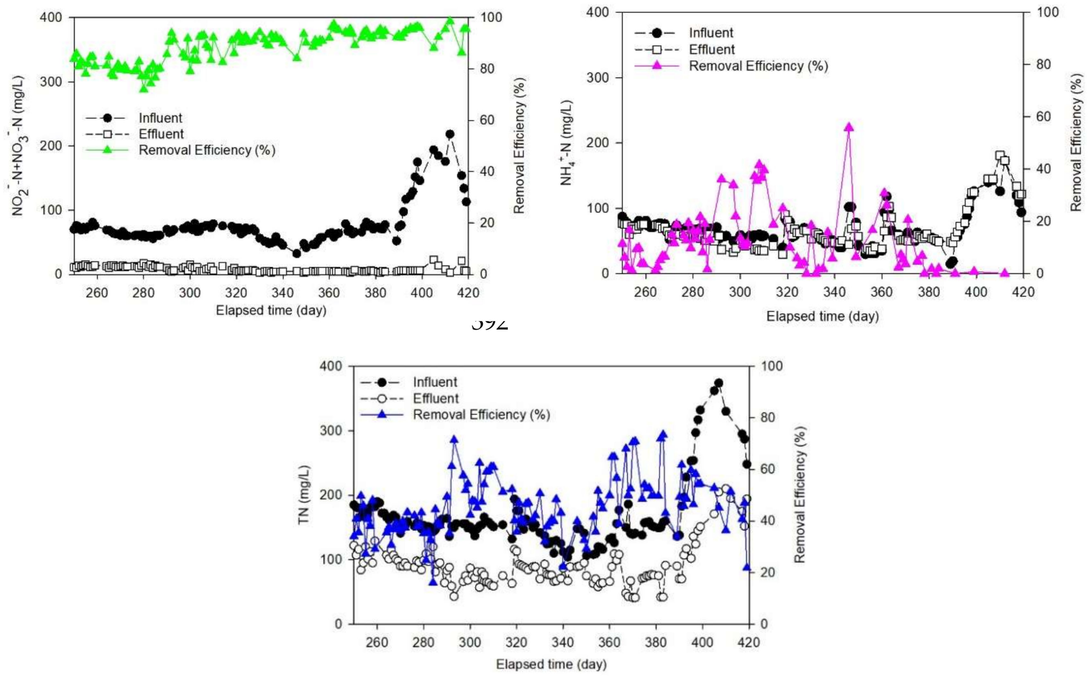

Figure 5. Treatment performance of the SOD process during long-term operation: Variations of TN, NH4+-N, NO2–N, and NO3–N concentrations and removal efficiency.

SOD was proven to be effective for maintaining the stable final effluent TN concentrations even under unstable operation of the anammox reactor. When the TN removal efficiency of the anammox process was decreased, the effluent NH_4^+-N to NO_3^--N ratio was always increased to about 1:1. NO_3^--N entering the anammox reactor can reduce by SOD reaction, so that about 50% of the effluent TN concentration from the anammox reactor can be removed. When the anammox process is operated properly, the remaining NO_3^--N can be removed to ensure a lower final TN concentration. On the other hand, the NO_3^--N concentration increases under unstable operation of the anammox process, so that the role of the SOD process becomes important. On day 412, influent and effluent TN concentrations of the anammox reactor were 610 and 400 mg/L (NH_4^+-N 200 mg/L, NO_3^--N 200 mg/L), respectively. Therefore, the TN treatment efficiency of the anammox reactor was only 35%. The remaining 200 mg/L of NO_3^--N was removed through SOD treatment and the effluent TN concentration from the SOD reactor reduced to 200 mg/L. Then, the TN removal efficiency for the whole process (anammox+SOD) was increased to 67%.

3.4. Nitrogen Removal Profile for Whole System

Figure 6 shows the overall nitrogen removal performances for the whole process (anammox + SOD). Average nitrogen concentrations obtained from operation during days 250-380 are shown in Figure 6. Inflow TN was mostly composed of NH_4^+-N, and TN and NH_4^+-N concentrations had almost similar values. 560 mg/L of TN was introduced to the anammox reactor. This TN was removed to 157 mg/L by anammox treatment, so the average TN removal efficiency reached 72%. At this point, NH_4^+-N and NO_3^--N were 59 and 63 mg/L, respectively, with a concentration ratio of 1:1.2. NO_3^--N from the anammox reactor was removed to an average of 8 mg/L by passing through the subsequent SOD reactor. As a result, the average TN treatment efficiency for the whole system increased to 85%.

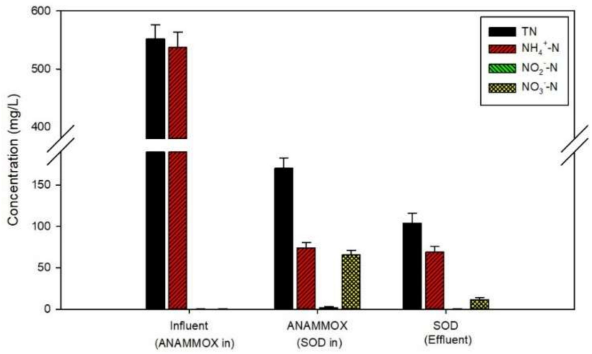

Figure 6. Nitrogen removal profile for the whole (Anammox+SOD) process.

3.5. Changes in Microbial Communities

Microbial community analysis was carried out for the reactor sludge, hydro-cyclone overflow, and hydro-cyclone underflow on day 86. The population ratio was confirmed at the phylum level and is shown in Figure 7. The ratio of Planctomycetes for each sludge were 14.7% (underflow), 2% (overflow), and 7.2% (reactor), showing the highest ratio of anammox microorganisms in underflow. These results are the same as those of genus level analysis.

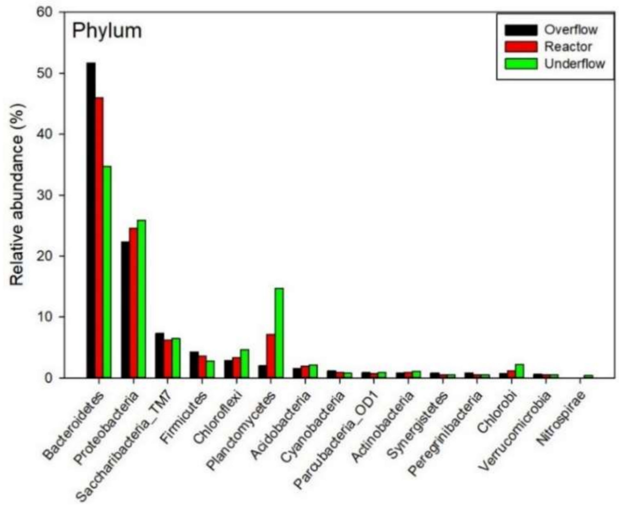

Figure 7. Taxonomic classification of the bacterial communities at each sampling point at the phylum level.

Table 2 shows the changes in the microbial community at the genus level over time. Only the data for microorganisms detected at more than 1% are presented. *Brocadia* was identified as a representative anammox bacteria in seed sludge, which was identified as *Brocadia fulgida* at the species level.

At the genus level, anammox began to be identified from day 86. In particular, the highest percentage of anammox bacteria was observed in the sludge recovered from the hydro-cyclone and less than 1% was found in the overflow. This indicates that the recovery of anammox bacteria by the hydro-cyclone separator was an effective way. In the anammox reactor, the sum of AOB and the anammox bacteria ratio were increased with operational time. The ratio of anammox bacteria on day 254 was increased by about 1.7 times as compared with the ratio on day 86. The ratio mixed with AOB was increased to 1.5 times.

Table 2. Changes in the microbial community at the genus level.

Elapsed Time (Day)	Sample Name	AOB *Nitrosomonas* (%)	NOB *Nitrospira* (%)	ANAMMOX *Brocadia* (%)	AOB+ ANAMMOX (%)
0	Seeding sludge	6.5	-	-	-
6	IN	2.6	-	-	-
86	Over (Out)	1.8	-	-	1.8
	Reactor	1.7	-	5.4	7.1
	Under (Recycle)	1.2	-	12.4	13.6
109	Over (Out)	6.5	1.3	-	6.5
	Reactor	7.3	-	6.5	9.8
	Under (Recycle)	12.4	-	11.4	23.8
215	Over (Out)	4.3	2.4	-	4.3
	Reactor	2.9	2.2	7.5	10.4
	Under (Recycle)	3	-	3	6
254	Over (Out)	1.8	-	-	1.8
	Reactor	1.7	-	9.3	11.0
	Under (Recycle)	1.2	-	6.5	7.7

The ratio of *Nitrosomonas* and *Nitrospira* as representative microorganism species of AOB and NOB was also compared. NOB must be discharged from the anammox reactor. Therefore, it should be discharged from the reaction tank and must be maintained at a low level. The proportion of *Nitrospira* was less than 1% at all time points except on day 215 (Run 4). Nitrogen removal efficiencies were low until day 150. In Run 4, activated sludge from the aeration tank of WWTP was inoculated to increase the AOB population and the hydro-cyclone discharge was increased. To increase AOB activity, the reactor DO concentration was also increased to about 1.0 mg/L. *Nitrospira* was found to be 2.2% in the anammox reactor. This result was closely related to the introduction of NOB from seed activated sludge and the rather high DO concentration of 1.0 mg/L. On day 254 (Run 5), *Nitrospira* was reduced to less than 1% again. After stabilization of TN removal efficiencies, the reactor DO concentration was reduced again to 0.5 mg/L or less to suppress NOB activity. NOB activity could be reduced, accompanying the gradual washout from the reactor by the hydro-cyclone overflow.

4. Conclusions

In this study, reject water from anaerobic digestion with a high TN concentration was treated by a demonstration plant combining the anammox process and SOD (SOD®; Sulfur Oxidation Denitrification) process. The anaerobic digestion was a co-digestion of livestock wastewater, food waste water, and sewage sludge so that the TN concentration and conductivity of the reject water were very high. This anammox plant was the first anammox demonstration plant in Korea. Problems encountered and methods to improve these problems during 450 days of operation were described in this paper. The major findings are summarized as follows:

1. The reject water was diluted with mainstream effluent and was used as the influent to the anammox reactor. The maximum TN removal efficiency of 80% was achieved for the anammox reactor under NLR of 0.45 kg-N/m^3·d.
2. As a result of decreasing dilution, influent conductivity and NLR values were increased to 7.8 mS/cm and 0.7 kg/m^3·d, causing a rapid decrease in TN removal efficiency.
3. The sludge concentration from the hydro-cyclone overflow was about 40 mg-MLVSS/L in which a small size of the anammox granules was detected. It was proven that an increase in MLVSS concentration in the anammox reactor was not easy under high influent conductivity and NLR.
4. 97% of NO_2^--N+NO_3^--N generated from the anammox process could be removed successfully by the SOD reactor. A TN removal efficiency of 35% under poor annamox treatment could increase to 67% by applying the SOD reactor as a post treatment for the removal of NO_3^--N.
5. The dominant anammox bacteria in the anammox reactor was identified as *Brocadia fulgida* and 9.3% of the bacteria out of the total bacteria were anammox bacteria. Accompanied with the continuing operation, the population of anammox bacteria was increased by about 1.7 times compared to the initial inoculation stage.

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