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Study on the optimization of partial nitritation using air-lift granulation reactor for two stage partial nitritation/Anammox process

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Abstract. This study aimed to develop a compact partial nitritation step by forming granules with high Ammonia-Oxidizing Bacteria (AOB) fraction using the Air-lift Granulation Reactor (AGR) and to evaluate the feasibility of treating reject water with high ammonium content by combination with the Anammox process. The partial nitritation using AGR was achieved at high nitrogen loading rate (2.25±0.05 kg N m^{-3} d^{-1}). The important factors for successful partial nitritation at high nitrogen loading rate were relatively high pH (7.5~8), resulting in high free ammonia concentration (1~10 mg FA L^{-1}) and highly enriched AOB granules accounting for 25% of the total bacteria population in the reactor. After the establishment of stable partial nitritation, an effluent NO_2^--N/NH_4^+-N ratio of 1.2±0.05 was achieved, which was then fed into the Anammox reactor. A high nitrogen removal rate of 2.0 kg N m^{-3} d^{-1} was successfully achieved in the Anammox reactor. By controlling the nitrogen loading rate at the partial nitritation using AGR, the influent concentration ratio (NO_2^--N/NH_4^+-N = 1.2±0.05) required for the Anammox was controlled, thereby minimizing the inhibition effect of residual nitrite.

Keywords: reject water; AOB granules; partial nitritation; nitrogen removal; anammox

1. Introduction

The anaerobic ammonium oxidation (Anammox) process is an energy-efficient and economical nitrogen removal process, as it doesn't require organic carbon or aeration in a process, and produces much less sludge in comparison to a conventional process (Jetten *et al*. 2001, Sliekers *et al*. 2002). Recently, the combined partial nitritation and Anammox process has been regarded as one of the most efficient ways for achieving autotrophic nitrogen removal, and there are more than 100 full-scale Anammox based nitrogen removal plants around the world, and this number is rapidly increasing (Susanne *et al*. 2014, Muhammad and Satoshi 2015). The Anammox based nitrogen removal process represents a more sustainable alternative, due to the reduced aeration and external organic carbon requirements (Liang and Liu 2008).

In the partial nitritation (PN) process, ammonium is partially oxidized to nitrite under aerobic conditions by ammonium oxidizing bacteria (AOB) (Eq. (1)). Further, nitrification to nitrate (Eq. (2)) carried out by nitrite oxidizing bacteria (NOB) must be avoided in order to allow optimal nitrogen removal by the Anammox bacteria (Yamamoto *et al*. 2008). In addition, biodegradable organic matter should be removed to avoid its negative effects on the following Anammox process (Chamchoi *et al*. 2008, Rescalleda *et al*. 2008).

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$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad (1)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (2)$$

The nitrite produced in the nitritation process is then converted to nitrogen gas with the remaining ammonium as an electron donor by Anammox bacteria. Eq. (3) represents the stoichiometry of the Anammox process (Boran *et al*. 2012).

$$\begin{aligned} &1NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \\ &\rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \end{aligned} \quad (3)$$

For the successful application of the combined partial nitritation and Anammox process, the partial nitritation of ammonium to nitrite should be focused because it is often the rate-limiting step and is difficult to control under normal conditions (Cho *et al*. 2011). Nitrite is seldom accumulated in the nitrifying reactor since nitrite is oxidized rapidly to nitrate (Okabe *et al*. 1996, Satoh *et al*. 2003). Thus, nitrite oxidation must be restrained to accumulate AOB but NOB should be washed out or depress its activity.

According to the literature, accumulation of nitrite could be achieved successfully by oxygen limitation (Aslan *et al*. 2009), as well as high temperature coupled with the low sludge residence time (Fux *et al*. 2002, Hellinaga *et al*. 1998). It can also be accomplished by inhibiting NOB with free ammonia (FA) and/or free nitrous acid (FNA) (Lai *et al*. 2004).

Partial nitritation using various types of reactor combined with the Anammox process has been successfully studied in recent years (van Dongen *et al*. 2001, Fux *et al*. 2002). Sequential partial nitritation and Anammox process

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can be performed in two different reactors namely the SHARON-ANAMMOX process (Hellinga *et al*. 1998, van Dongen *et al*. 2001) or in a single reactor such as CANON (Completely Autotrophic Nitrogen removal Over Nitrite) process (Third *et al*. 2001, Fux *et al*. 2002, Cho *et al*. 2011).

However, the SHARON reactor for partial nitritation has a long hydraulic retention time (HRT) and requires a large reactor volume (WERF. 2015). In order to shorten the HRT, the mixed liquor suspended solids (MLSS) concentration should be maintained at a high level in the reactor, however, the poor settleability remains to be addressed. De Kreuk and De Bruin (2004) reported that increased MLSS concentration and improved settleability can be achieved by using aerobic granular sludge, which can shorten the residence time required for nitrification. Aerobic granular sludge is perceived as one of the most promising wastewater treatment technologies in this century. Aerobic granular sludge can be applied to wastewater treatment containing high strength organic matter and nitrogen in various ways. Oliver *et al.* (2017) reported that membrane bioreactor using aerobic granular sludge can contribute to membrane fouling reduction and enhanced performance compared to conventional MBR.

In order to stably remove the nitrogen of the reject water, a two stage partial nitritation/Anammox process was developed in which the partial nitritation using the Air-lift Granulation Reactor (AGR) was combined with Anammox. And to achieve stable nitrogen removal efficiency in Anammox reactor, it is important to optimize of AGR for forming granules with high AOB fractions and producing a stable nitrite under short HRT operation. In this study, the objectives of this paper were not only to evaluate factors for the cultural enrichment and granule formation of AOB but also to evaluate nitrogen loading rate for stable production of a suitable mixture of ammonium and nitrite.

2. Materials and methods

2.1 Partial nitritation using AGR and operation conditions

A pilot plant was built to evaluate AOB enrichment, granulation and partial nitritation using high ammonium concentration reject water in Daejeon sewage treatment plant in South Korea. The AGR was made of stainless steel with a height of 4 m, head diameter of 1.2 m, down comer part diameter of 0.86 m, riser part diameter of 0.6 m and the effective volume of 2 m^3. This partial nitritation pilot plant consisted of a blower for air supply, influent feed pumps and motorized valves for the discharge of treated water. Aeration was supplied through the nozzle (diameter 3mm) of the circular diffuser installed at the bottom of the riser part. A diffuser was installed inside the riser to allow air and the liquid flow from the riser to the down comer. A discharge valve was installed in the lower part for withdrawing sludge and granules in the reactor. The AGR was operated as a sequencing batch reactor manner, with one cycle adjusted from 4 hours to 12 hours depending on the ammonium concentration and the influent loading. The operation modes of the AGR consisted of the input (10 min), aeration (215 ~ 695 min), settling (5 min) and discharge (10 min).

The activated sludge in the aeration tank from the Daejeon sewage treatment plant was used as seed sludge for the AGR with an initial MLSS concentration was 10,000mg/L. The influent flow rate per cycle was 1 m^3, which is half of the effective volume of the total reactor. For the selective separation with the settling velocity of sludge, the discharge of the effluent was positioned in the middle of the reactor. To stabilize the seed sludge, the AGR was initially operated with a long HRT. In order to increase the ammonium loading after stabilization, HRT was shortened by increasing the duration of each cycle step by step. The air flow rate was adjusted between 0.8~1 m^3/min.

2.2 The combined of partial nitritation using AGR and Anammox process

After achieving stable partial nitritation using AGR, the effluent of PN using AGR was connected to the Anammox reactor. Fig. 1 shows a schematic diagram of the two-stage partial nitritation/Anammox process. Half of the ammonium in the influent was oxidized to nitrite in the PN using AGR, resulting in ammonium to nitrite ratio of about 1:1.2±0.05 in the effluent. The effluent of the AGR was then introduced into the Anammox reactor via a storage tank (volume 1 m^3), wherein the temperature was maintained at 35±2°C by a submerged heater. The HRT of storage tank was set to 2.4 hours so that to be introduced into the Anammox reactor until the next discharge of AGR without any change in water quality.

The Anammox reactor was made of stainless steel with a diameter of 1.2 m, the height of 1.8 m and an effective volume of 2 m^3. The reactor was equipped with thermal insulation on the outside, and the complete mixture was achieved by means of a mechanical mixer. The Anammox bacteria have a low biomass yield. Also the Anammox bacteria have the ability to aggregate into a granulated form or readily attach to surfaces with excessive formation of exocellular polymeric substances. Due to these fortuitous properties, the Anammox reactor can be readily achieved to the high SRT required to sustain and accumulate by various mode of operation (suspended growth, biofilm on support media, granular sludge) (WERF. 2015). In this study, the Anammox bacteria were attached to the moving bed carrier to achieve high SRT and allowed to flow by the mixer. The moving bed carrier had an active surface area of 800 m^2/m^3 and was charged at a 50% of reactor volume. The influent to the Anammox reactor was continuously supplied for 24 hours. After stabilization, HRT was shortened step by step to increase the nitrogen loading rate (NLR). In addition, a chemical facility was installed to control the rise of the reactor pH during operation.

2.3 Reject water

The reject water (i.e. dewatering water of anaerobic digestion sludge) treated in this study, which came from the Daejeon sewage treatment plant, varied in composition. More than 87% of total nitrogen was in the form of ammonium, and the ratio of alkalinity to ammonium was

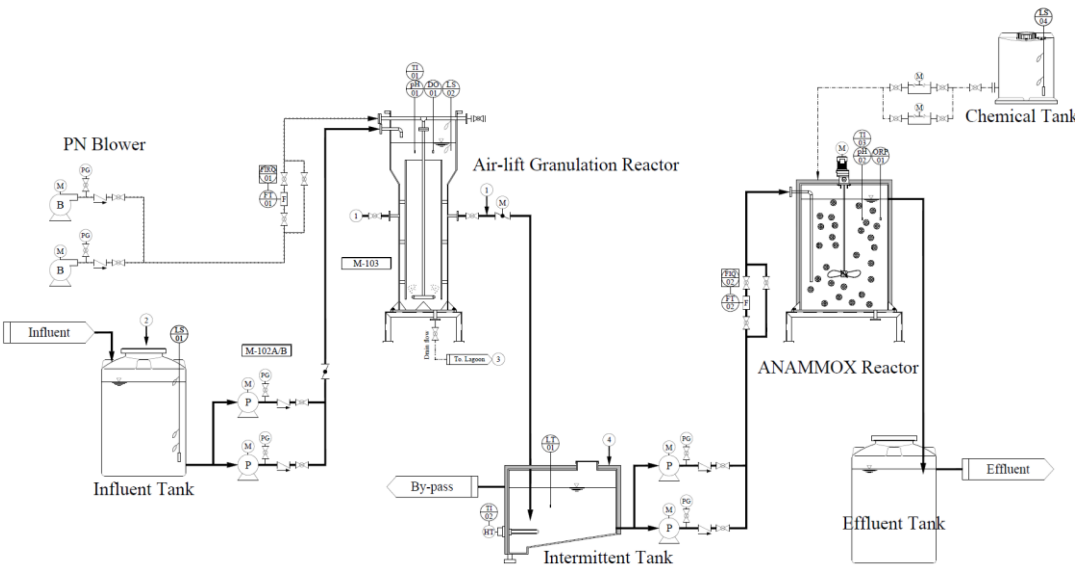

Fig. 1 Scheme of the two-stage partial nitritation and Anammox process

Table 1 The characteristics of reject water in the study

Compound	Unit	Range	Mean value
Total Nitrogen	mg N/L	551 ~ 785	596.4
Ammonium	mg NH_4^+-N/L	488 ~ 561	517.8
Nitrite	mg NO_2^--N/L	0.0 ~ 1.2	0.2
Nitrate	mg NO_3^--N/L	0.0 ~ 4.0	0.8
Alkalinity	mg $CaCO_3$/L	2,020 ~ 3,511	2,542
Total Chemical Oxygen Demand	mg O_2/L	510 ~ 850	679.5
pH	-	7.9 ~ 8.4	8.2
Temperature	°C	31 ~ 35	32.3
Ammonium/T-N ratio	-	0.7 ~ 0.9	0.9
Alkalinity/Ammonium ratio	-	4.1 ~ 6.3	4.9

4.91. The composition and mean values of the reject water, along with the alkalinity and pH, are summarized in Table 1.

2.4 Analytical methods

Total suspended solids (TSS), chemical oxygen demand (CODcr), total nitrogen (TN), ammonium, nitrite and nitrate were all measured according to Standard Methods (APHA. 2005). Concentrations of free ammonia (FA) were calculated as a function of pH, temperature and total ammonium as nitrogen (TAN) for FA, according to Anthonisen *et al*. (1976). The granule size analysis of the AGR was carried out using a standard sieve size (KS A5101-1~3) according to the sieve analysis test, and the sieve number was #30 (opening size 0.6 mm), #50 (opening size 0.3 mm) and #100 (opening size 0.1 mm). Granules filtered to each size sieve measured TSS based on the standard method and calculated the distribution ratio.

For scanning electron microscopy (SEM) observation, the granule samples were placed for 2 hours in a 1% glutaraldehyde solution (pH 7.4). The samples were washed with 0.12 M phosphate buffer solution two times for 15 min at 4°C, fixed in 2% osmium tetroxide for 2 hours at 4°C. The samples were shaken with a mixed solution of ethanol: acetone (2:1, 1:1) and then dehydrated for 30 min by 100% acetone. Thereafter, the samples were dehydrated with a mixed solution of acetone: isoamylacetate (1:1) for 1 hour. The dehydrated samples were dried using a critical point dryer(HCP-2, Hitachi Koki, Japan) before they were coated with platinum and view under a SEM (LEO SUPRA55, Carl Zeiss, Germany).

2.5 Distribution analysis of AOB and NOB

To analyze the distribution of AOB and NOB, sludge or granules were sampled from the partial nitritation reactor at regular intervals. Sampled sludge and granules were sent to Chunlab Service (Chunlab Inc., Seoul Korea, www.chunlab.com). Microbial community analysis through nucleotide sequence analysis of 16s rRNA using next-generation sequencing (NGS) was carried out to quantify the total microorganism species of sludge and granules. Among the identified microorganisms from the analysis, the amount of AOB related species (*Nitrosomonas, Nitrosococcus, Nitrosospira*) and NOB related species (*Nitrobacter, Nitrospina, Nitrococcus, Nitrospira*) was calculated, respectively. The portion of each AOB and NOB in the total microbial community was estimated and compared.

3. Results and discussion

3.1 Nitrogen removal performance of partial nitritation using AGR and Anammox process

3.1.1 Performance of the partial nitritation using AGR

The AGR pilot was operated for 1~150 days to form AOB granules. During the initial period (phase 1), the

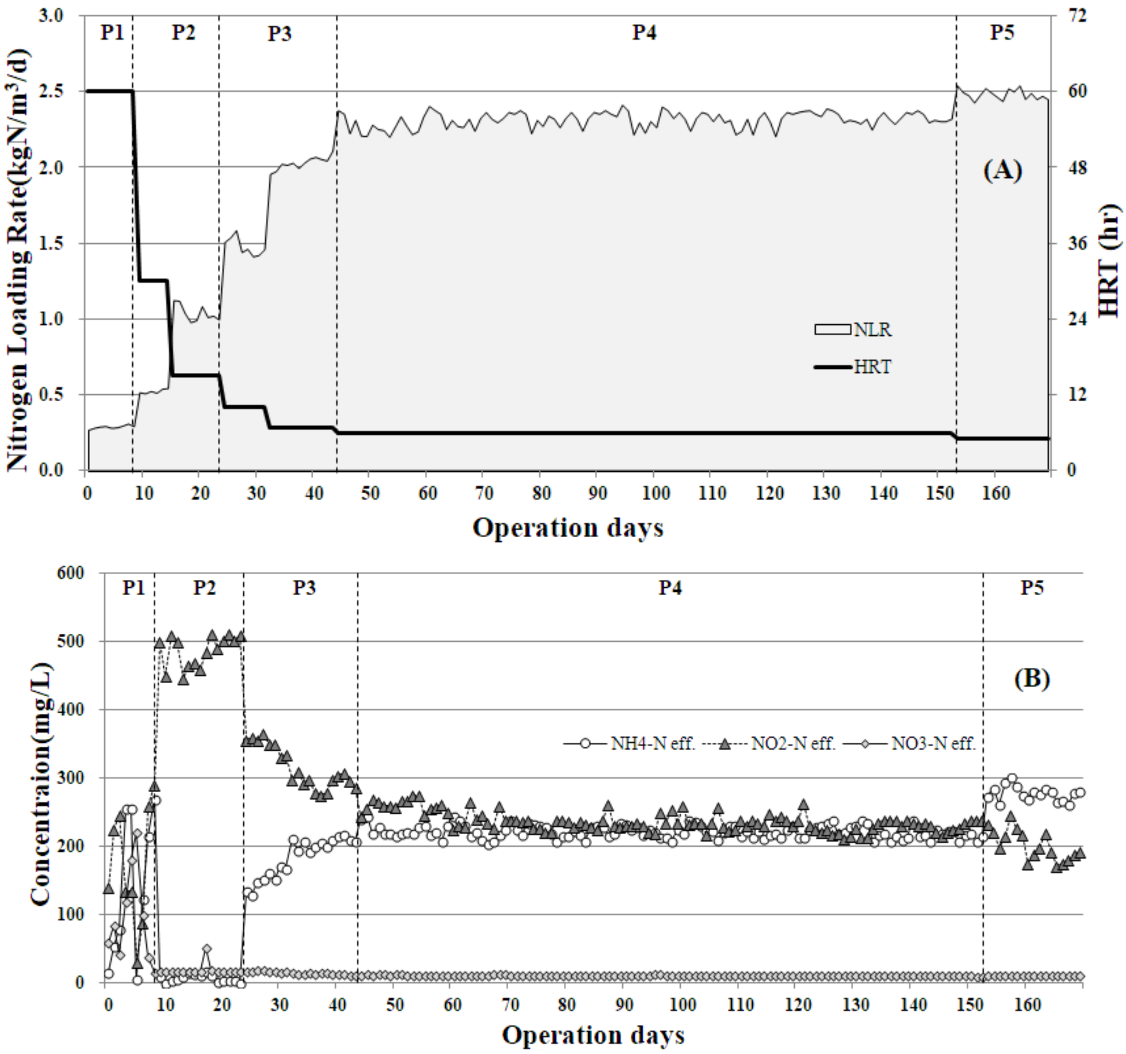

Fig. 2 Performance of the partial nitritation using AGR over the course of the study (phases 1, 2, 3, 4 and 5) showing (A) the nitrogen loading rate and HRT of the reactor, and (B) the concentration of ammonium, nitrite and nitrate in the effluent

settling time of the AGR pilot was periodically decreased (from 60 minutes to 5 minutes) to wash-out unwanted sludge having poor settleability. After the stabilization periods, the AGR pilot was operated at a fixed settling time of 5 minutes for the phase 2 to 5. The number of operation cycles of the reactor was increased for increasing NLR. The performance of the AGR is summarized in Fig. 2.

During phase 1, nitrate concentration was increased because high SRT (over 50 days) and low NLR (0.3 kg $N/m^3/d$) had been maintained. These operating conditions had kept NOB in the AGR and resulted in full nitrification. During phase 2, the HRT was reduced from 60 hours to 15 hours to increase the NLR. The NLR was increased from 0.5 kg $N/m^3/d$ to 1.1 kg $N/m^3/d$. The settling time in this period was fixed at 5 minutes. Because of short HRT and SRT, NLR of 1.7 to 3.7 times higher than phase 1, the oxidation of ammonium to nitrate was inhibited. Nitrate in the effluent ranged from 2 to 3% of the influent ammonia.

Partial nitritation began to take place in phase 3 when the HRT was shortened from 15 hours to 7 hours. Overall, 55 ~ 70% of the ammonium was oxidized to nitrite. During this period, the NLR loading rate was around 1.1 ~ 2 kg $N/m^3/d$. As NLR increased (Fig. 2A), the amount oxidized to nitrite in the effluent decreased (Fig. 2B). The average ratio of ammonium and nitrite in the effluent was 1:1.42 at the maximum NLR of 2 kg $N/m^3/d$, and the nitrite was higher than the optimal influent conditions (ammonium to nitrite ratio of about 1:1.2±0.05) for Anammox.

Half of the ammonium began to convert to nitrite in phase 4 that was 6 hours of HRT and 2.2 ~ 2.4 kg $N/m^3/d$ of NLR. The ratio of ammonium and nitrite in the effluent was 1:1.11±0.05 and a similar ratio as in the influent conditions required for Anammox reactor were obtained in phase 4. This value was higher than those reported in biofilm systems (at 1.76 kg $N/m^3/d$) (Satoshi Okabe *et al*. 2011) and suspended systems (at 1.2 kg $N/m^3/d$) (Moomen Sloliman and Ahmed Eldyasti 2011). However, in phase 5, which had an HRT of 5 hours and a NLR of 2.5 $kgN/m^3/d$, only 40% of the ammonium was converted to nitrite. The average effluent ammonium to nitrite ratio was at 1:0.7 due to the insufficient reaction time of partial nitritation, and the ammonium was higher than the optimal influent conditions for Anammox.

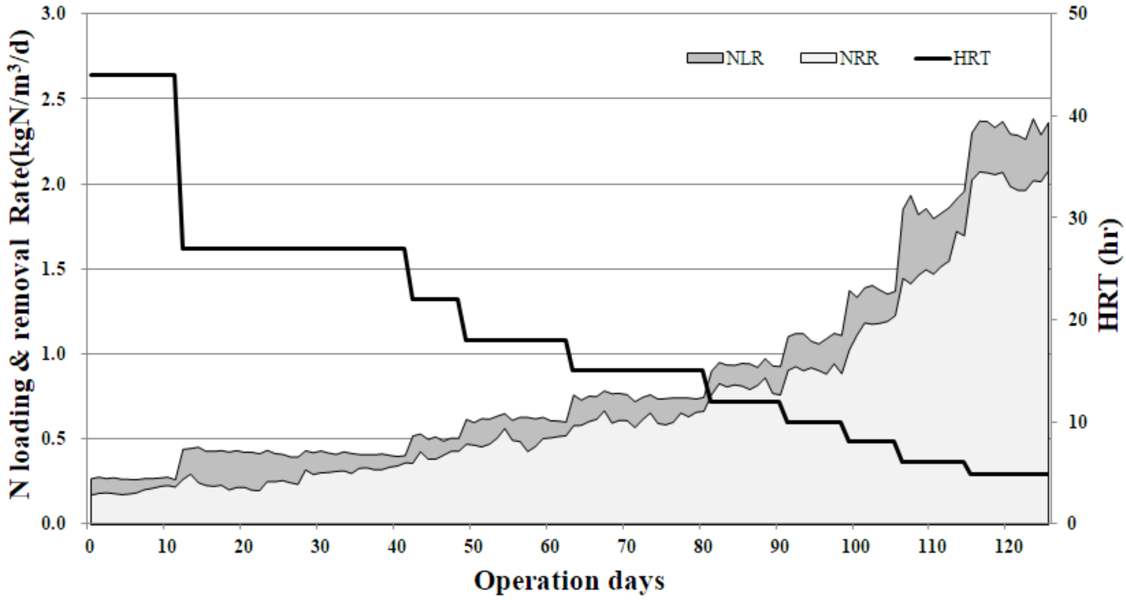

Fig. 3 The performance of the Anammox reactor as a function of the nitrogen loading rate (NLR) and nitrogen removal rate (NRR)

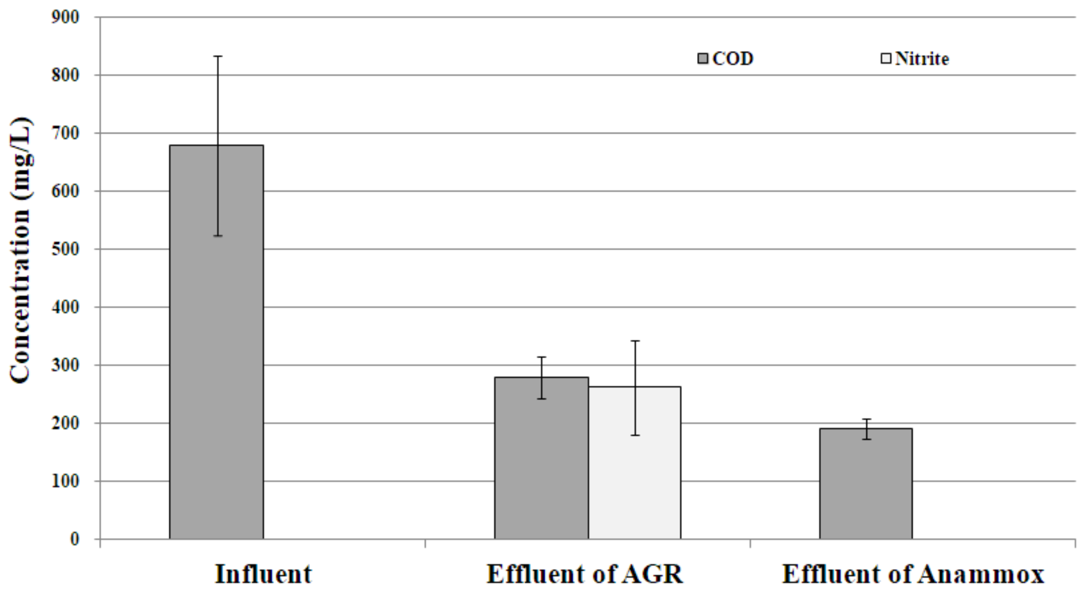

Fig. 4 The change of COD at influent, AGR and Anammox

3.1.2 Nitrogen removal performance of the Anammox reactor

After establishing stable partial nitritation, the PN reactor was connected to an Anammox reactor. The ammonium and nitrite concentrations after PN in the AGR were in the range of 204 ~ 245 mg/L and 224 ~ 275 mg/L, respectively. The ratio of ammonium to nitrite are around 1:1.15, which was an acceptable ratio for the subsequent Anammox activity. Fig. 3 shows that the NLR was increased as step by step manner as a periodic reduction of HRT in the Anammox process.

As a result, the NLR of the Anammox reactor increased from 0.27 to 2.33 kg N/m^3/d. When nitrite concentration in the effluent of Anammox reached 10 mg/L, the nitrogen loading rate was increased. After about 116 days, NLR was reached around 2.33 kg N/m^3/d and more than 87% of nitrogen was removed about 2.0 kg N/m^3/d of nitrogen removal rate (NRR).

Even though an oxygen controlled simple single stage partial nitritation/Anammox process such as the CANON process (Third *et al*. 2001, Fux *et al*. 2002, Cho *et al*. 2011) is available in the market, however, two stage partial nitritation/Anammox process is more effective for nitrogen removal than single reactor types. When partial nitritation and Anammox reactors are separated, the inhibitory effect of oxygen on the Anammox reactor is relieved and thus the performance of each reactor could be optimized more efficiently.

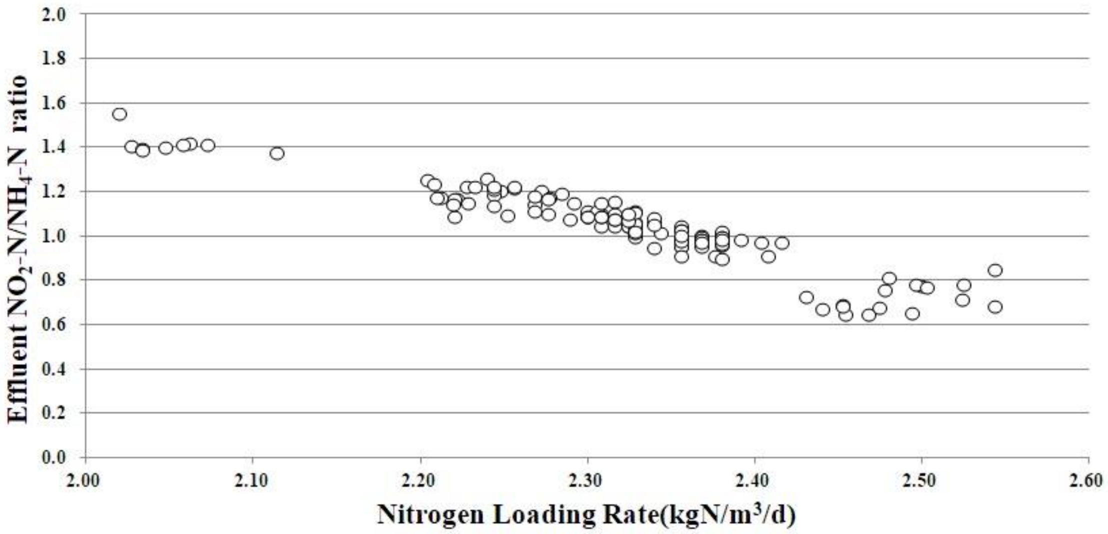

Fig. 5 The ratio of ammonium to nitrite according to the nitrogen loading rate of AGR at phase 4

The heterotrophic bacteria compete with the autotrophic Anammox bacteria that also exist in Anammox systems. Because the heterotrophic bacteria grow faster than the autotrophic Anammox bacteria under high concentrations of organic matter, the heterotrophic bacteria eliminate the Anammox bacteria and thereby reduce the Anammox nitrogen removal capability. This type of inhibition could be considered as a phenomenon of "out-competition" (Ren-Cun Jin *et al*. 2012). Mumtazah Ibrahim *et al*. (2016) reported that nitrogen removal in the Anammox reactor was achieved with simultaneous Anammox and denitrification at an influent COD to nitrite ratio of 2. Therefore, in order to minimize inhibition of Anammox by denitrification of heterotrophic bacteria, it is important to lower COD to nitrite ratio of 1.5 or less.

Fig. 4 shows the change of COD at influent, AGR and Anammox. 59% of organic matter was removed at AGR, thereby reducing the COD and nitrite ratio of Anammox reactor influent to 1.07, It was possible to minimize inhibition of Anammox activity by out-competition.

3.2 Production rate of nitrite at AGR according to nitrogen loading rate (NLR)

For better nitrogen removal in the Anammox process, optimizing the ratio of ammonium and nitrite, at 1:1.2±0.05, in the effluent of AGR was important. The nitrite conversion rate of ammonium as a function of NLR was evaluated in phase 4 of AGR as shown in Fig. 5.

During this period, the average ammonium to nitrite ratio in the effluent of AGR was maintained at 1:1.1 with 2.2 ~ 2.4 kg N/m^3/d of NLR. Especially, 2.25 ± 0.05 kg N/m^3/d of NLR with 1:1.2 of ammonium to nitrite ratio showed the best NRR performance. At 2.4 kg N/m^3/d of NLR, the ratio of nitrite was decreased such as 0.6 ~ 0.8 compared to 1 of ammonia. With 2.0 ~ 2.1 kg N/m^3/d of NLR, the ratio of nitrite was increased as 1.4 compared to 1 of ammonia. Therefore, based on the above results, it is possible to control the ammonium to nitrite ratio as a function of the AGR nitrogen loading rate.

The ratio of ammonium and nitrite in the Anammox influent affects the nitrogen removal efficiency and residual nitrite in the Anammox reactor after reaction because they might inhibit the activity of the Anammox process. Accordingly, it was necessary to check the specific anammox activity (SAA) and the removal efficiency of nitrogen with residual nitrite concentration for confirming the anammox activity.

The NLR of the AGR was controlled to change the nitrite content in the effluent. The influent ratio of ammonium to nitrite to the Anammox reactor were in the range of 1:1.2 ~ 1.7. When the concentration of nitrite in the reactor was below 10 mg/L, 90% of nitrogen was removed, but the removal efficiency decreased as residual nitrite concentration in the Anammox process increased. For example, when the nitrite concentration continuously exceeded 50 mg/L, the nitrogen removal efficiency decreased to 60% or less. A plausible explanation for this observation is the inhibitory effect of nitrite concentration since SAA value was maintained, 130 g N/kg VSS/d, until 40 mg/L of nitrite concentration, but the value of SAA was decreased when the nitrite concentration was maintained over 40 mg/L.

A previous study reported that over 100 mg/L of nitrite can decrease nitrogen removal efficiency in the Anammox process (Elena Bettazzi *et al*. 2010, Ren-Cun Jin *et al*. 2012). In this study, it was confirmed that the partial nitritation using AGR can easily control the influent ammonium and nitrite and result in a stable Anammox operation in the following process.

3.3 Formation of granular sludge in AGR

The sludge volume index (SVI) and the MLSS concentration during the operation of the AGR pilot are presented in Fig. 7. Initially, MLSS concentration was about 10,000 mg/L and the SVI was higher than 200. The SVI decreased rapidly after 10 days of operation when the

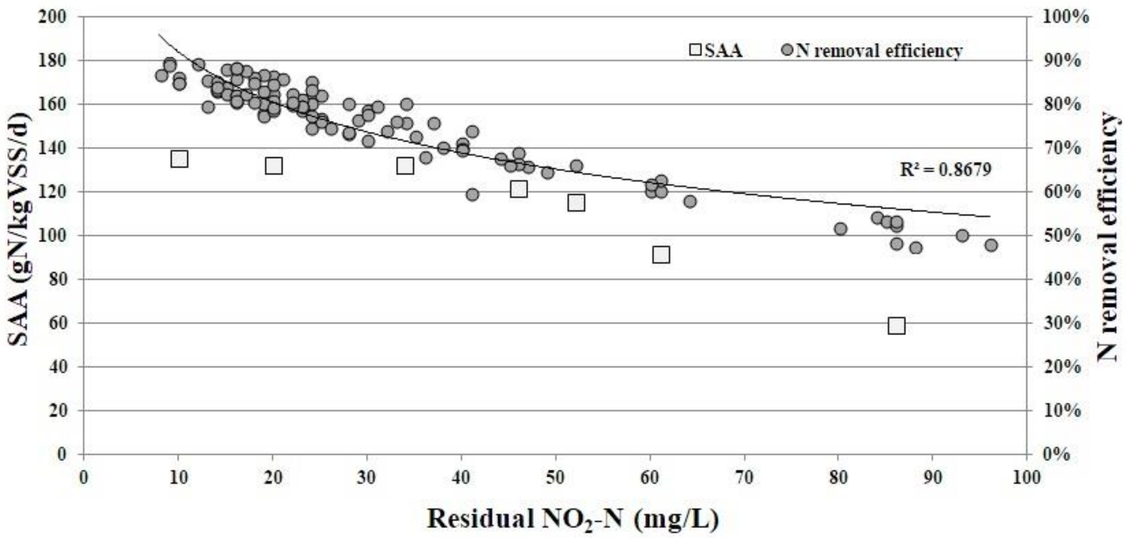

Fig. 6 The specific anammox activity (SAA) and the removal efficiency of nitrogen as a function of the concentration of the residual nitrite in the Anammox reactor

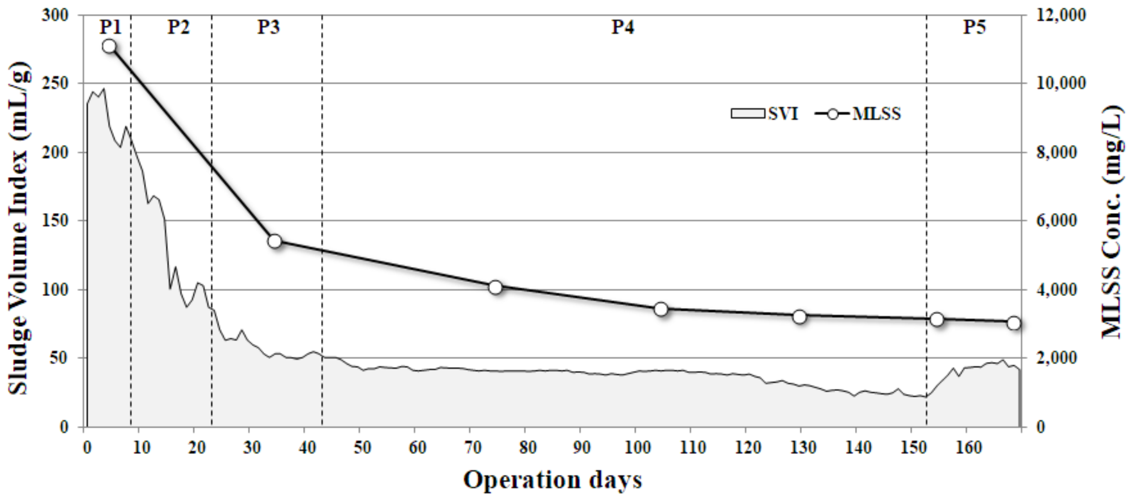

Fig. 7 The sludge volume index (SVI) and the MLSS concentration measured during operation of the AGR pilot reactor

nitritation activity progressed. About one month later, SVI was lower than 50.

After 130 days of operation, the AGR process showed excellent biomass settleability since the MLSS concentration and SVI were maintained about 3,200 mg/L and 30, respectively. The SVI started to increase to 50 as NLR increased in phase 5. During phase 5, the free ammonia (FA) concentration also increased from 10.1 ± 3.95 mg/L (phase 4) to 20.4 ± 1.82 mg/L because of residual ammonium and high pH. Yang *et al*. (2004) reported that when the concentration of FA in aerobic granulation was 23.5mg/L or higher, it can inhibit granulation. The high FA concentration could induce the suppression of polymer secretion which can essential microbial product for agglomeration of microorganisms.

The granulated sludge in the AGR process was observed with a microscope (Fig. 8). In phase 1, the size of the sludge was under 0.1 mm. After 35 days of operation (phase 3), small sized granules were observed. After 50 days (phase 4), granule sizes increased to more than 0.3 mm. The granules obtained after 75 days were observed to be larger than 0.6 mm size.

In Fig. 9, granule size of sludge and MLSS concentrations were compared in each phase. The average size of attained granules was 0.1 ~ 0.3 mm after 35 days. After 75 days, the distribution of granules over 0.6 mm, 0.3 ~ 0.6 mm, and 0.1 ~ 0.3 mm were at 52%, 28% and 18%, respectively. It was hard to find less than 0.1 mm granule during this working period. After 130 days, the distribution of the 0.1 ~ 0.3 mm size granules decreased to 8% and the granule size of 0.6 mm or larger increased to 80% among attained total granules. Although the increase of the granule size was mainly investigated by visual observation (Fig. 8), the increase of average granule size can be numerically estimated because the portion of bigger granules had been increased as operation days passed (Fig. 9)

This could be explained by the operating conditions with short settling time and increased internal hydraulic shear-force in AGR process. This resulted in the wash-out of sludge with poor settleability and selected granular sludge in the AGR process.

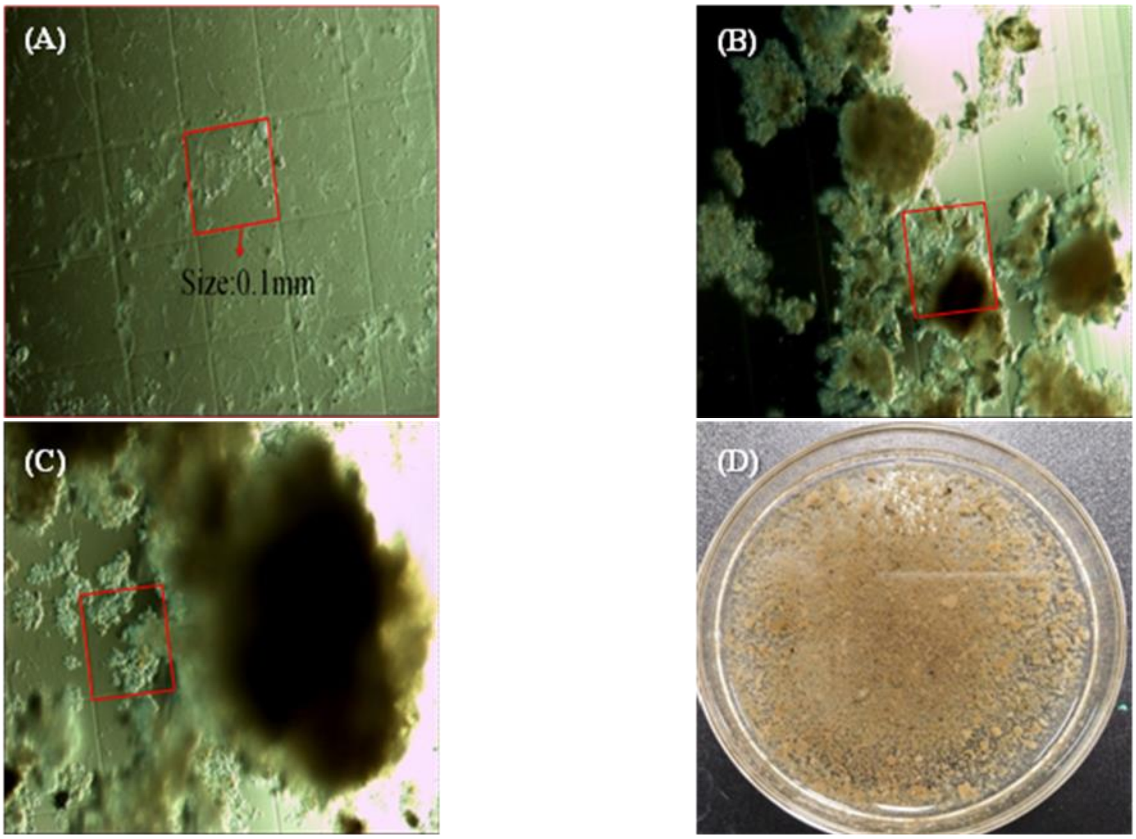

Fig. 8 Changes in granule size during operation period: (A) seeding sludge, (B) after 35 days, (C) after 50 days and (D) after 75 days

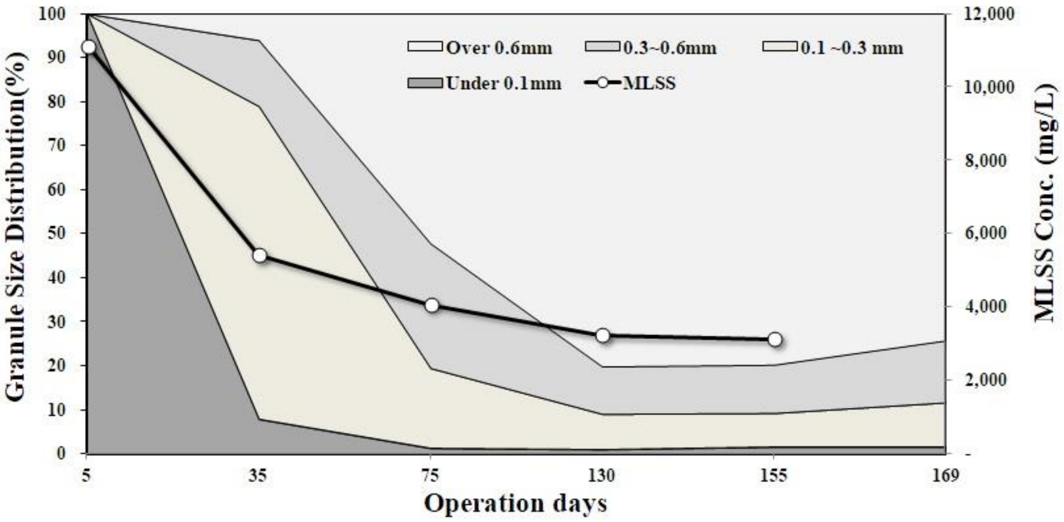

Fig. 9 Distribution of granule size and MLSS concentration during the operation period

After 130 days of the AGR pilot operation, the sampled granules in the reactor were observed with a scanning electron microscope (SEM) shown in Fig. 10. The average size of the granules was 1,000 μm and morphologically spherical or elliptical. Bacteria were distributed in the granules, which might be associated with nitrification.

3.4 Enrichment culture of AOB in AGR

FA was calculated based on the ammonium concentration and pH at the beginning and end of the operation cycle (data not shown). At the beginning of the cycle, the pH was 8.2 and FA concentration was calculated to have an average value of 76.1 ± 2.1 mg/L. At the end of the cycle, the average pH and FA concentration decreased to 7.6 and 9.2 ± 0.4 mg/L, respectively. Fig. 10 shows the distribution of AOB and NOB in the granules. Based on the results of microbial community analysis through nucleotide sequence analysis of 16S rRNA using NGS, the AOBs detected were from the genera *Nitrosomonas* and

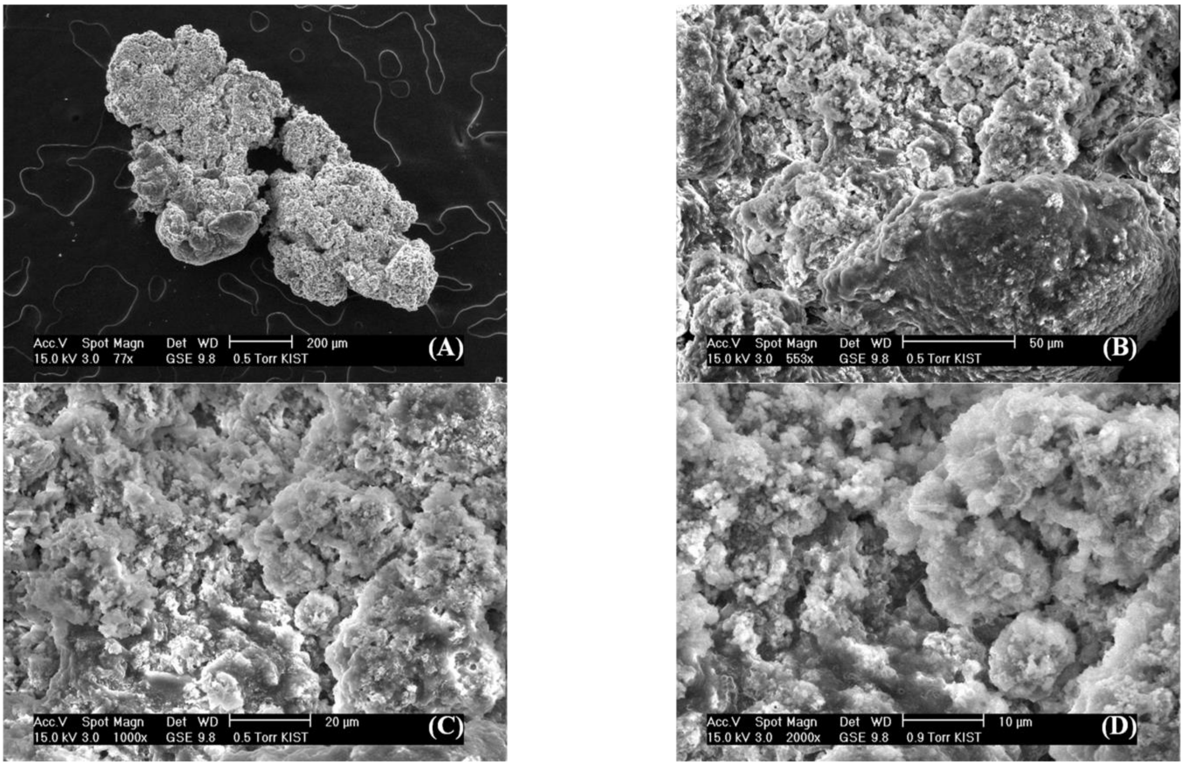

Fig. 10 Scanning electron micrographs (SEM) of the granules sampled after 130 days

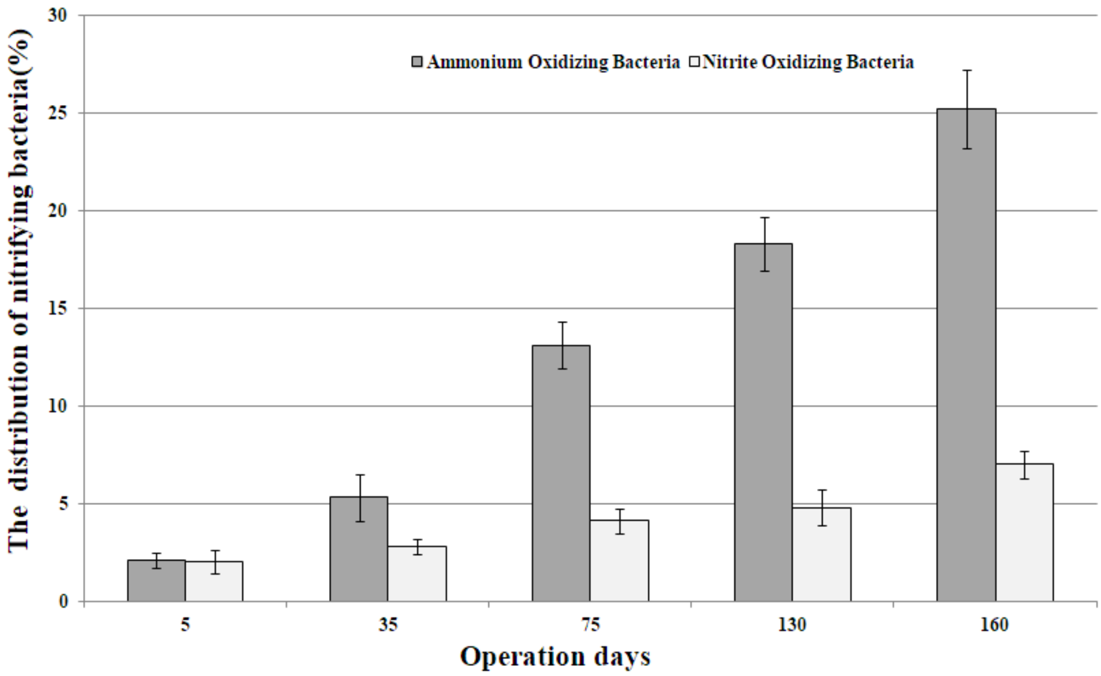

Fig. 11 Change in the distribution of AOB and NOB in the granules

Nitrosospira while for NOBs were *Nitrospira* and *Nitrobacter* in this study. After 35 days, the fraction of AOB began to increase. After 130 days, the distribution of AOB was 18%, and after 160 days, it increased to 25%, which was more than 12 times higher than the initial value. After 160 days, 7% NOB was observed, but the nitrate in the effluent was 2 ~ 3% of the input ammonium. High FA concentration ranges (9.2 ~ 76.1 mg/L) in this study could have inhibited the NOB activity (Anthonisen *et al*. 1976). Therefore, better acclimation of AOB rather than NOB is plausible.

4. Conclusions

The objectives of this paper were to develop a compact partial nitritation step by forming granules with high AOB fractions using AGR and to evaluate the feasibility of treating reject water with high ammonium by combination with Anammox process. This study not only demonstrated successful culture enrichment and granule formation of AOB, but also achieved stable production of a suitable mixture of ammonium and nitrite by controlling the nitrogen loading rate. Granules were formed after 35 days of AGR operation, and after 130 days, 80% of the granules were 0.6 mm or bigger in size. These granules were successfully formed due to the internal hydraulic shear-force of the AGR, and the short settling time, which select only biomass with good settleability. The percentage of AOB in granules was increased to 25% but not that of NOB. This could be the effect of FA concentration in the process. Stable Anammox influent ammonium to nitrite ratio (1:1.2±0.5) were also achieved at high nitrogen loading rate (2.25±0.05kgN/m^3/d). This was possible because granules with the AOB composition of 25% were formed. The partial nitritation using AGR was connected to the Anammox reactor, which successfully attained a high nitrogen removal rate of 2.0 kg N/m^3/d in the Anammox reactor. By controlling the nitrogen loading rate during the partial nitritation in the AGR, the influent ammonium to nitrite ratio required for the stable Anammox operation can be achieved as minimizing residual nitrite inhibition effect.

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