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Aerobic Granules for the Effective Oxidation of Ammonium Nitrogen

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Abstract

In this study, aerobic granules were applied to a lab-scale aerobic granule sludge airlift reactor (AGSAR) and the ammonium nitrogen oxidation performance was evaluated at different ammonium nitrogen loading rate (NLR). At least 99% of the initial ammonium nitrogen was oxidized at an NLR of 0.27 and 0.53 kg NH_4^+-N/m^3·day, for both aerobic granules (control), and nitrifying aerobic granules (NAGs). The ammonium nitrogen oxidation deteriorated, when the NLR was increased to 1.07 kg NH_4^+-N/m^3·day. The NAGs were characterized by complete nitrification, while partial nitrification was observed in the control.

Keywords: Aerobic granular sludge, Ammonium loading rate, Coating, Nitrification, Nitrifying bacteria

1. Introduction

It is generally recognized that excess nutrients, such as nitrogen (N), need to be removed to prevent oxygen depletion and eutrophication in bodies of water. For nitrogen removal from wastewater, biological treatment using nitrification-denitrification is carried out, because of its ease of operation and cost-effectiveness [1, 2]. Nitrification is the rate-limiting step in this process, which is performed by chemoautotrophic bacteria under aerobic conditions [3]. Because of the sensitivity of nitrifying bacteria to environmental factors as well as their extremely low growth rate, it is difficult to obtain and retain sufficient nitrifying bacteria in wastewater treatment plants [4, 5]. In order to solve this problem, advanced techniques for retaining high density of nitrifying bacteria in reactors have been developed and implemented for nitrogen removal in wastewater, e.g., immobilized cell, such as entrapment in a material, and attached cell (biofilms), such as growth on the surface of the carrier; but these techniques still have major drawbacks [6-8]. The cell-entrapping suffers from mass transfer resistance. It takes a long time to construct a nitrifying biofilm on the surface of a carrier in ammonium-rich wastewater containing few organic compounds. Moreover, it also has the problems of biofilm-associated clogging and sloughing [9]. For this reason, aerobic granule has recently become of particular interest to keep a large amount of nitrifying bacteria. Aerobic granules are formed through self-immobilization of microorganisms without any external support [10-12]. The self-immobilization that takes place during granule formation prevents slowly growing bacteria, e.g., nitrifying bacteria, from being washed out from the reactor [13]. However, information on nitrifying bacteria granulation with ammonium-rich wastewater is not well known [8, 14]. Previous research has shown that nitrifying granules could be formed in a two-phase (liquid-solid) fluidized bed reactor with an external aerator [15]. According to Tsuneda et al. [16], nitrifying bacteria could also self-immobilize in an aerobic upflow fluidized bed (AUFB) reactor. Shi et al. [17] proved that spherical and elliptical nitrifying granules were formed in a sequencing batch reactor (SBR). However, in these studies, the granulation speed of nitrifying bacteria was still low with inorganic wastewater rich in ammonium without organic compounds, and the formation of granules was unstable, due to lack of extracellular polymeric substances (EPS) [16-18]. If aerobic granules intentionally coated with nitrifying bacteria can be made, it is efficient to keep a large amount of nitrifying bacteria on the surface of aerobic granules, which are useful to oxidize ammonium nitrogen in wastewater. Meanwhile, there is no information about the characteristics of ammonium nitrogen oxidation using fresh aerobic granules, such as whether nitrifying bacteria start to grow on the surface of fresh aerobic granules and as a result whether ammonium nitrogen oxidation occurred or not. Therefore, in this study, we investigate the ammonium nitrogen oxidation efficiency, and the characteristics of both aerobic granules intentionally coated with nitrifiers (nitrifying aerobic granules [NAGs]) and non-coated with nitrifiers

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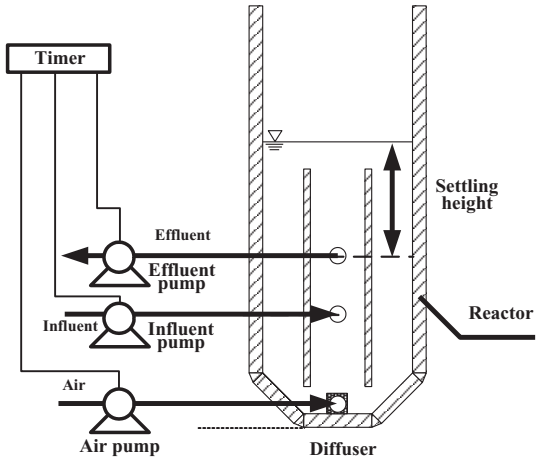

Fig. 1. Schematic experimental set-up of the aerobic granule sludge airlift reactor (AGSAR) used in this experiment.

(control), respectively, in different ammonium nitrogen loading rate (NLR). It is expected that this study could be useful for the development of technology for ammonium nitrogen oxidation using aerobic granules.

2. Materials and Methods

2.1. Enrichment of Nitrifying Bacteria and Manufacture of Aerobic Granules

Seed sludge obtained from the aeration tank of a Yangpyong (Korea) municipal wastewater treatment plant had been acclimated to P-medium (NH_4^+-N 500 mg/L), using the proposed method for cultivating ammonium oxidizing bacteria (AOB) [19, 20]. This cultivation was conducted for 7 months and resulted in obtaining AOB enriched sludge (data not shown). After 7 months cultivation, to identify AOB enriched sludge, sludge was taken and plated on a solid complex medium (NWRI agar) for heterotrophs and a minimal medium (HEPES solid) for AOB, respectively [21, 22]. Grown colonies were counted after 5 days of incubation for heterotrophs and after 14 days of incubation for AOB. The percentage of AOB in total colony counting showed about 85% of the entire colonies (data not shown). Even if polymerase chain reaction amplification and detection of *amoA* occur, fluorescent *in situ* hybridization (FISH) analysis and terminal restriction fragment length polymorphism (T-RFLP) analysis are needed to verify the existence of AOB. In our study, it was confirmed that AOB was successfully enriched by AOB—selective cultivation methods.

The aerobic granules used in this study were cultivated in an SBR with a working volume of 5 L. The SBR was operated in a time sequence of 5 min of feeding, 240 min of aeration, 5 min of settling, and 1 min of effluent withdrawal. The treated wastewater was discharged from the middle port of the SBR. Synthetic wastewater used in this study was mainly composed of sodium acetate as sole carbon source, while its detailed compositions can be found elsewhere [23]. Mature granules were nearly spherical in shape and had a compacted and integrated structure. The mean size of the granules was about 0.5 mm.

2.2. Reactor Set-up and Operation

The schematic diagram of the aerobic granule sludge airlift reactor (AGSAR) used in this study is shown in Fig. 1. The AGSAR is an SBR with a working volume of 4.5 L and height/diameter ratio of 7.75. One AGSAR was used for making NAG by coating the enriched AOB to the surface of aerobic granules. This reactor was also used to investigate the nitrification efficiency of NAGs. Another reactor of the same configuration was used to investigate the nitrification efficiency of aerobic granules. The initial aerobic granules concentration was 4,000 mg/L. In both experiments, air was pumped through a diffuser at the bottom of the reactor at a rate of 4–5 L/min. Influent was injected from the lowest port of the reactor, and effluent was drawn from the middle port of the reactor. The filling and withdrawal times were 6 and 18 min, respectively. The exchange ratio was 56%. The experiment was performed at room temperature and pH range of 7.4–8.8, respectively.

2.3. Manufacture of NAGs

The aerobic granules (4,000 mg/L) and AOB enriched activated sludge (400 mg/L) mixing ratio was 10:1 based on mixed liquor suspended solids (MLSS) for making NAGs, by coating AOB onto the surface of aerobic granules. After the aerobic granules and AOB enriched activated sludge were mixed, aeration was maintained during 43 hr for mixing together. When the SBR operation was started, the aeration time was fixed at 22 hr for an ammonium loading rate of 0.09 kg NH_4^+-N/m^3·day and 4 hr for that of 0.53 kg NH_4^+-N/m^3·day in one cycle of SBR operation mode, respectively. The filling and withdrawal times were 6 and 18 min, respectively. The settling time was changed during the different operation periods. The settling time was gradually decreased from 30 to 6 min to prevent discharge of AOB in the reactor.

2.4. Medium

For evaluating the performance efficiency of AOB coated aerobic granules and fresh aerobic granules, synthetic wastewater was used as influent, which consisted of NH_4Cl (nitrogen source), $NaHCO_3$ (inorganic carbon source and pH buffer), 30 mg $CaCl_2$/L, and 4.5 mg K_2HPO_4/L, 20 mg $FeSO_4 \cdot 7H_2O$/L, 25 mg $MgSO_4 \cdot 7H_2O$/L, respectively. During the experiment, the concentrations of NH_4Cl and $NaHCO_3$ in the influent were adjusted by the respective ammonium nitrogen loading rate. The theoretical amount of $NaHCO_3$ for nitrification (7.14 g as $CaCO_3$/g NH_4^+-N) was added to the synthetic wastewater following the ammonium nitrogen loading rate. The N (NH_4^+-N)/COD ratio was changed following the ammonium nitrogen loading rate. The 30 mg/L of sodium acetate was added as a carbon source, because it promotes the growth of heterotrophic microorganisms in the granules and supports the formation of granules. Microelements, including trace metals, were also supplemented in the influent [23].

2.5. Analytical Methods

During the whole of the experimental periods, samples were taken at the beginning of the aeration time and effluent time. All samples were passed through a 0.45 μm glass fiber syringe filter (Whatman, Kent, UK) and used in water measurement. NH_4^+-N,

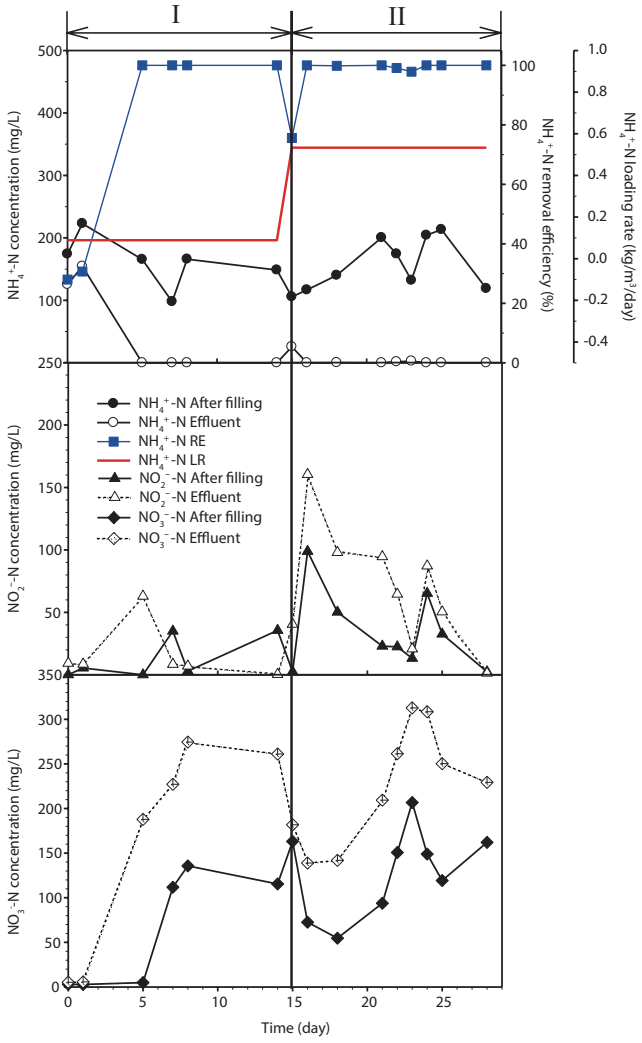

Fig. 2. Nitrogen profiles during the development of nitrifying aerobic granules by coating fresh aerobic granules with ammonium oxidizing bacteria. NH_4^+-N loading rate was 0.09 (period I) and 0.53 (period II) kg N/m^3·day.

NO_2^--N, and NO_3^--N were analyzed based on the standard methods using Optizen POP QX spectrophotometer [24]. SCODcr was also analyzed (HACH, Loveland, CO, USA). The pH and dissolved oxygen were monitored using ThermoProbes. The free ammonia (FA) concentration was calculated using the following equation [25].

$$\text{FA (mg/L)} = 17/14 \times \frac{TAN(\frac{mg}{L})\times 10^{pH}}{(\frac{K_b}{K_W})+10^{pH}}, \qquad \frac{K_b}{K_W} = e^{(6{,}344/(273+^{\circ}C))}$$

Table 1. Experimental conditions of the aerobic granule sludge airlift reactor (AGSAR)

	Experiment A		Experiment B		
	I	II	III	IV	V
Operation (day)	1–15	16–29	1–6	7–22	23–50
NH_4^+-N (mg/L)	200	200	200	200	200
NH_4^+-N load (kg N/m^3·day)	0.09	0.53	0.27	0.53	1.07

3. Results and Discussion

The operational condition of the AGSAR throughout the study is shown in Table 1. In parts I and II (experiment A), NAGs were developed, by coating with AOB. In parts III, IV, and V (experiment B), the nitrification efficiency was monitored, according to the change of influent NLR with both aerobic granules and NAGs.

3.1. Development of NAGs

NAGs in an AGSAR were investigated (experiment A). NAGs were developed by coating aerobic granules with enriched AOB from the activated sludge. The nitrification profiles during the development of NAGs are shown in Fig. 2. The settling time required for keeping aerobic granules in the reactor would not be longer than 5 min to prevent the growth of suspended activated sludge [23]. However, if the settling time is too short during the start-up period, a lot of AOB could not be efficiently maintained in the reactor due to their low growth rate, which resulted in wash

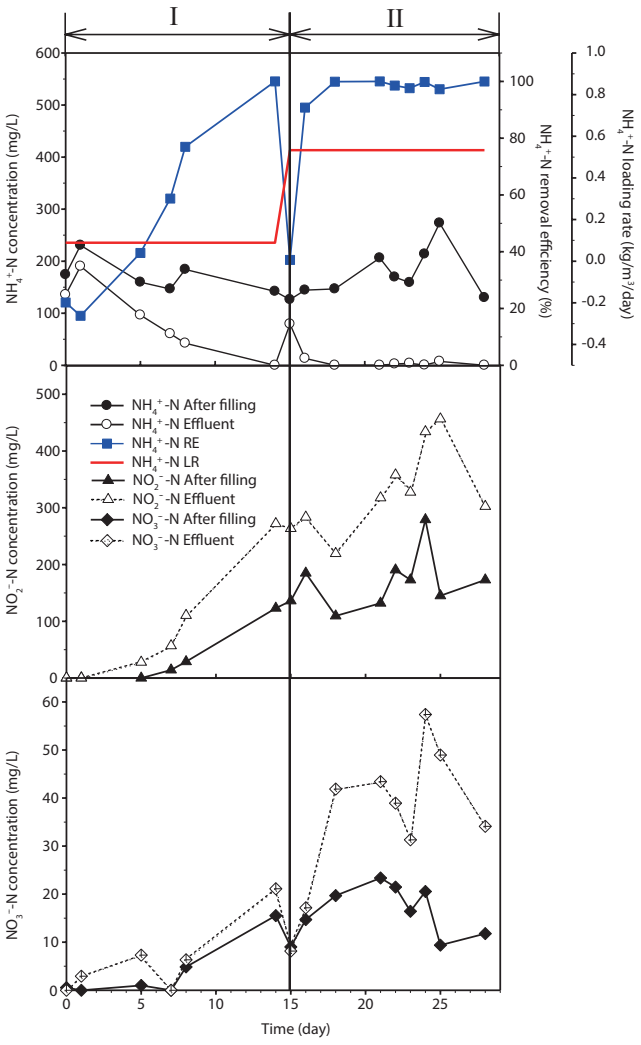

Fig. 3. Nitrification profiles using fresh aerobic granules non-coated with ammonium oxidizing bacteria (control group). NH_4^+-N loading rate was 0.09 (period I) and 0.53 (period II) kg N/m^3·day.

out [8, 17]. For coating AOB enriched sludge to the surface of aerobic granules and preventing wash out of AOB in the reactor in this study, a long settling time was retained during the start-up period. The settling time was gradually decreased from 30 to 6 min within 5 days of operation, as the effluent SS concentration measured in each cycle was decreased from 130 to 20 mg/L, respectively. After 5 days of operation, the settling time was set to 6 min. The MLSS concentration was maintained at 4,200–4,300 mg/L. The color of aerobic granules gradually changed from yellow to red within 5 days of operation. This result shows that AOB were well coated on the surface of aerobic granules within short periods of operation.

It has been well known that cell hydrophobicity and EPS play a crucial role in the self-immobilization and attachment of cells to a surface [26, 27]. Generally, cell hydrophobicity increased and EPS decreased, as the substrate N/COD ratio increased [28]. Increasing the cell hydrophobicity leads to a decrease in the excess Gibbs energy of the surface at once, which results in promotion of cell-to-cell interaction, leading to the bacteria being aggregated tightly together [29]. Therefore, in this study, it is expected that cell hydrophobicity can be the driving force for the attachment of nitrifying bacteria on the surface of aerobic granules, despite the low production of EPS at high substrate N/COD ratio.

In the initial stage of operation, NH_4^+-N removal efficiency was about 30%, but reached more than 90% after 5 day. From then, NH_4^+-N removal efficiency was maintained to at least 97% on the average at an NLR of 0.09 (period I) and 0.53 (period II) kg NH_4^+-N/m^3·day, and NH_4^+-N was almost completely nitrified during the operation time. Nitrification in the reactor was expected to be terminated as accumulation of NO_2^--N, because AOB enriched activated sludge cultivated by P-medium was coated onto the surface of aerobic granules. However, complete nitrification occurred in the reactor. This could be due to the AOB enriched activated sludge also having a lot of nitrite oxidizing bacteria (NOB).

3.2. Nitrification Characteristics of Aerobic Granules (Control)

For the control reactor, in which the aerobic granules were put, one cycle of SBR mode was operated with the same conditions of manufacturing NAGs. The MLSS concentration was

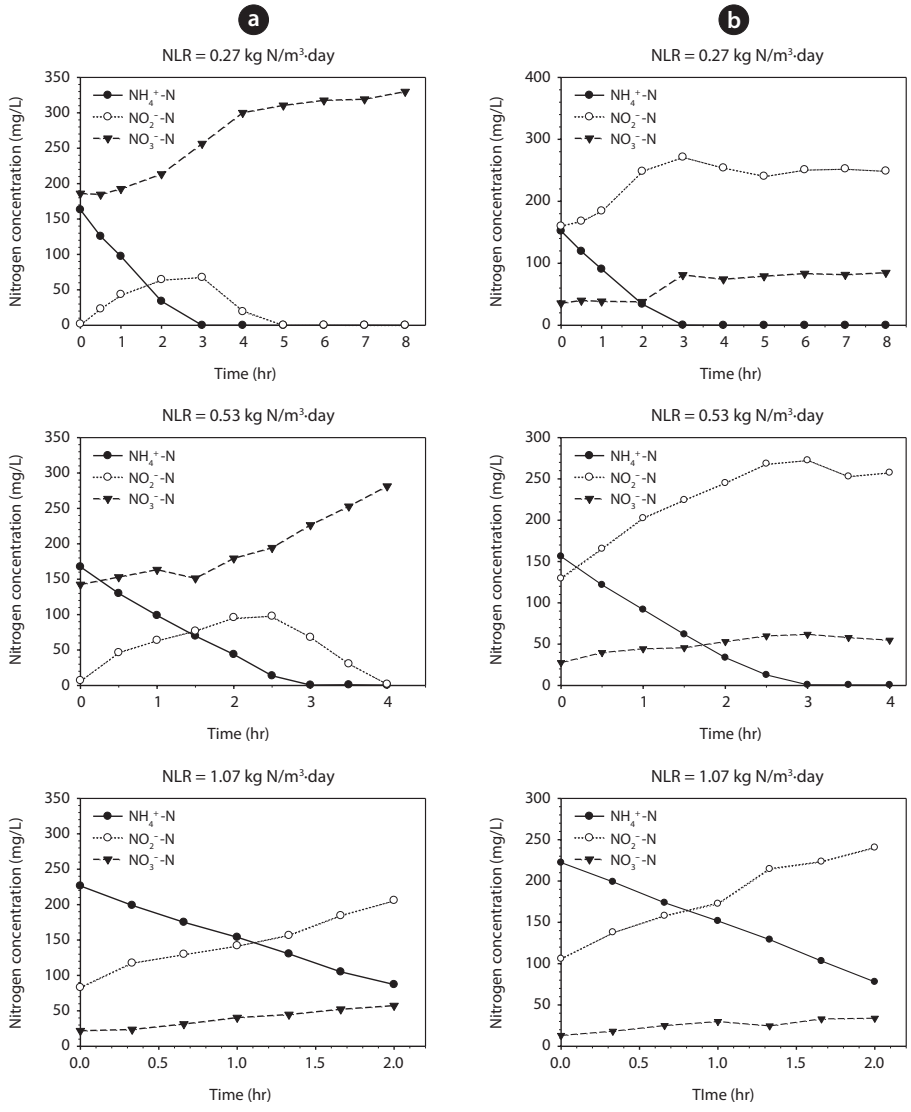

Fig. 4. Nitrogen profiles under different ammonium nitrogen loading rate (NLR): (a) nitrifying aerobic granules and (b) fresh aerobic granular. NH_4^+-N loading rate was maintained at 0.27 (1–6 days), 0.53 (7–22 days), and 1.07 (23–50 days) kg N/m^3·day.

maintained at 4,100–4,200 mg/L after 5 days of operation. The nitrification profiles of fresh aerobic granules (control) are shown in Fig. 3. In the initial stage of operation, NH_4^+-N removal efficiency was too low (about 20%). It gradually increased, as aerobic granules adapted to the high NH_4^+-N concentration in wastewater. This achieved more than 90% after 14 days, and then maintained more than 95% on average during the operation time (NLR 0.53 kg NH_4^+-N/m^3·day).

However, in this study, partial nitrification was observed in aerobic granules, while complete nitrification occurred by NAGs. This result shows that the NOB population in aerobic granules is lower than in AOB, and as a result, the nitrite oxidation is relatively low [30]. In nitrifying microbial aggregates, NOB is mainly distributed in the deeper area of the AOB in the aerobic granule [31, 32]. The substrate affinity for oxygen (K_s) with NOB (0.13 mg O_2/L) is also too low compared with that of AOB (1.98 mg O_2/L), and this is unfavorable to the NOB in terms of competition for dissolved oxygen under the same condition [33, 34]. Meanwhile, complete nitrification was evident in the NAGs. This is because the enriched nitrifying bacteria on the surface of the NAGs can efficiently access the available dissolved oxygen surrounding them.

Based on these results, it is obvious that the nitrifying bacteria were successfully coated on the surface of the NAGs in the reactor.

3.3. Nitrification Efficiency according to the Ammonium Nitrogen Loading Rate

3.3.1. NAGs

Fig. 4(a) illustrates the oxidation efficiency evaluated under different NLR using NAGs (experiment B), which were mentioned in Section 3.1 to show the trends of each nitrogen compound during the aeration time within one cycle of SBR mode operation. At an NLR of 0.27 and 0.53 kg NH_4^+-N/m^3·day, NH_4^+-N was oxidized by more than 99% in about 4 hr and was converted

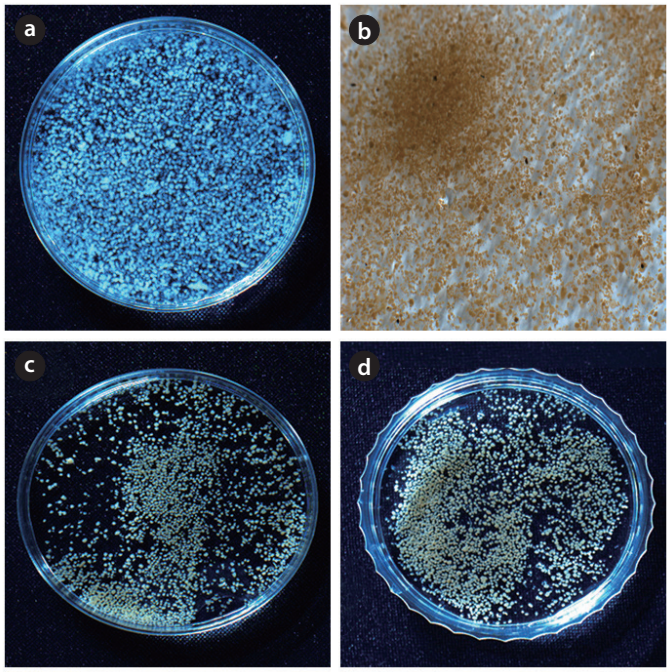

Fig. 5. Photographs of aerobic granules: (a) fresh aerobic granules, (b) nitrifying aerobic granules (NAGs) after 5 days of enrichment, (c) NAGs after 78 days of operation, and (d) fresh aerobic granules after 78 days of operation. For (c) and (d), NH_4^+-N loading rate was maintained at 0.27 (1–6 days), 0.53 (7–22 days), and 1.07 (23–50 days) kg N/m^3·day and the photographs were obtained at the end of 50 days of operation under 1.07 kg N/m^3·day loading rate.

to NO_3^--N. This result shows that complete nitrification could be achieved within a short aeration time, if NAGs were used. Because a lot of NOB already existed on that, the NOB was enough to oxidize NO_2^--N. FA concentration in the range of 1–150 mg/L does not cause any or partial inhibition effect to *Nitrosomonas* (AOB), so the conversion from NH_4^+-N to NO_2^--N is not inhibited. But this FA range is adequate to cause complete inhibition to *Nitrobacter* (NOB), so conversion from NO_2^--N to NO_3^--N nearly does not happen [20, 35]. At NLR of 0.27 and 0.53 kg NH_4^+-N/m_3·day, the initial FA concentration in this study was 28.7 and 18.4 mg/L, respectively. Therefore, most of the NH_4^+-N was converted to NO_2^--N without inhibition of AOB, but conversion from NO_2^--N to NO_3^--N almost did not happen. After NH_4^+-N was depleted after 2 hr of 4- and 8-hr cycle, FA concentration was less than 1 mg/L. Most of the NO_2^--N was converted into NO_3^--N after 2 hr. At 1.07 kg NH_4^+-N/m^3·day of ammonium nitrogen loading rate (aeration time was 2 hr), 54.3% of NH_4^+-N was converted to NO_2^--N and 38.5% of NH_4^+-N of influent remained. In this period, NO_2^--N was also accumulated during aeration time. As a result, it is considered that an appropriate aeration time and exchange ratio, depending on the NH_4^+-N concentration of the influent, are required for stable nitrification.

3.3.2. Aerobic granules

Fig. 4(b) illustrates the ammonium oxidation efficiency evaluated under different NLR, using aerobic granules (experiment B) to show the trends of each nitrogen compound during the aeration time within one cycle of SBR mode operation.

At an NLR of 0.27 and 0.53 kg NH_4^+-N/m^3·day, NH_4^+-N was removed by more than 99% in about 3 hr and most of the removed NH_4^+-N was converted to NO_2^--N, contrary to the NAGs. Low activity of the NOB of the aerobic granules could be due to few NOB present in the aerobic granules.

At 1.07 kg NH_4^+-N/m^3·day, 60.5% of NH_4^+-N was converted to NO_2^--N and 35.1% of NH_4^+-N of influent was not oxidized. As a result, it is considered that an appropriate aeration time and exchange ratio, depending on the NH_4^+-N concentration in influent, are required for stable nitrification.

In this study, the color of granules after operation is shown in Fig. 5. The color of aerobic granules show light yellow and NAGs after 5 days of enrichment show strong reddish color, respectively. Even if the NAGs and aerobic granules after 78 days of operation showed reddish color, the intensity of red color of the NAGs was weakened.

4. Conclusions

In this study, it was verified that complete nitrification occurred at different ammonium NLR by NAGs, while partial nitrification occurred, when aerobic granules were used. It is presumed that the NOB population of aerobic granules is relatively lower than that of the NAGs. If partial nitrification occurs during long-term operation, it is of more benefit to save energy and cost for denitrification. Consequently, aerobic granules, which are not necessary to enrich the nitrifying bacteria beforehand, could be an excellent and alternative biomass for nitrogen removal.

Acknowledgments

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