B−13 Development of an aerobic granule sequencing batch airlift reactor for partial nitrification

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1. Introdction

Anaerobic ammonium oxidation (anammox), in which dinitrogen gas (N_2) is produced from ammonium (NH_4^+) and nitrite (NO_2^-) by anammox bacteria, is a new cost-effective technology to remove ammonium from wastewater. Nitrogen compounds in most wastewater are in the form of NH_4^+; therefore, half of the NH_4^+ in wastewater has to be converted to NO_2^- (partial nitrification; PN) by ammonia oxidizing bacteria (AOB) prior to anammox process. However, since favorable growth conditions for AOB and nitrite oxidizing bacteria (NOB) are different, operation conditions for the PN reactor have to be optimized to enhance the growth of AOB and inhibit the growth of NOB by controling parameters such as temperature, pH, and DO. One problem low nitritation efficiency that has to be addressed in the pretreatment often limit the overall maximum rate of nitrogen removal.

Granular sludge technology has been attracted due to its fast sludge settling property, long sludge retention time, tolerance to shock loading, and no sludge bulking. Most aerobic granules have been successfully developed by using sequence batch reactor (SBR)[1]. Several researchers have developed PN aerobic granule reactors[2-5]. But there are still a paradox between partial nitrification and granule formation under low DO concentration, furthermore, details about microbial structure and in situ activity of the PN aerobic granules are still limited.

The objectives of this study were to: (1) develop PN aerobic granules in SBAR; (2) evaluate the performance of the PN aerobic granule reactor; (3) clarify the identity and the localization of AOB and other microbes in the PN aerobic granule.

2. Materials and methods

(1) Reactor set-up and operation

The internal-circulate sequencing batch reactor with working volume of 5 L was designed as shown in Fig. 1. Composition of the synthetic wastewater was 200-600 mg-N L^{-1} $(NH_4)_2SO_4$, 200 mg-TOC L^{-1} sodium acetate, 24 to 72 mM $KHCO_3$ (varied to maintain the influent pH at 7.5-8), 0.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.2 mM $CaCl_2$, and 1 mL of trace element solutions I and II. NH_4^+-N was stepwise increased from 200 mg L^{-1} to 600 mg L^{-1}.

The reactor was inoculated with 1.5 L of fresh activated sludge taken from the secondary settling tank of the municipal wastewater treatment plant located in Sosegawa, Sapporo. The reactor was operated in successive cycle conditions composed of substrate feeding, aeration, sludge settling, and effluent withdrawal. At the effluent withdrawal, only upper half of the liquid was removed; thereby, settled sludge was retained in the reactor.

For the first 4 days, one cycle composed of 2 min for substrate feeding, 360 min aeration, 60 min settling, and 2 min effluent withdrawal. Thereafter, the reactor was operated in one cycles of 4-h each, which

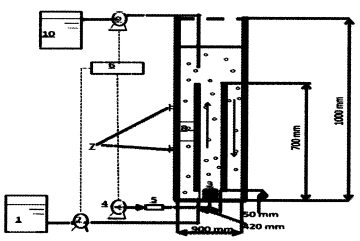

Fig. 1. Schematics of the SBAR expirement set-up.

was composed of 2 min for substrate feeding, 3-30 min for sludge settling, 2 min for effluent withdrawal, and the rest of time for aeration. After development of PN granules (after 83 days), sludge-settling time of 3 min was fixed. The reactor was operated of room temperature (15-25°C).

(2) Analytical procedure

Suspended solids (SS), volatile suspended solids (VSS), sludge volume index (SVI), concentrations of NH_4^+, NO_2^-, NO_3^- and Total organic carbon (TOC) were measured regularly according the standard mothed[6].

(3) Clone library analysis

Genomic DNA was extracted from individual mature granules (n=3) by using Fast DNA spin kit (Bio 101, Qbiogene, CA., USA). After confirming that the microbial community structures were similar among the three granules by denaturing gradient gel electrophoresis (DGGE) analysis, DNA were pooled and used for clone library analysis as described by Ishii et al. (2009)[7].

(4) Fixation and cryosection of granules and Fluorescence in situ hybridization (FISH)

Fixation and cryosection of granules and fluorescence in situ hybridization (FISH) was performed as described by Okabe et al. (1999)[8].

3. Results and discussion

(1) Development of PN aerobic granules

Figure 2 shows the nitrogen removal performance in the PN aerobic granule reactor during the start-up period. Air-flow rate was adjusted to keep DO blow 2 mg L^{-1}. After 2 months of operation, half of the NH_4^+ was stably oxidized to NO_2^- in one cycle, suggesting that partial nitrification was achieved. After PN achieved (83 days), the settling time was shorten from 5 min to 3 min . As a result, PN aerobic granules were successfully formed. Mature granules reached 1.5-4 mm (Fig. 3) with average settling velocity of 103 m h^{-1} and SVI of 60 ml g^{-1}. Kim et al. (2006)[2] first reported PN aerobic granules developed by using SBR. They first developed aerobic granules, then enrich AOB by controlling DO and ammonia loading rate. Similarly, Xu et al. (2011)[3] enriched AOB fromed aerobic granules by adding chlorate, an NOB inhibitor. In this study, we first enriched AOB, and then form PN aerobic granules, without addition of NOB inhibitors. We fixed volume exchange ratio as 50%, and changed the settling time from 60 min to 3 min. Free NH_3 and NH_2OH concentrations are known to inhibit the growth of NOB. These concentrations are function of NH_4^+ and NO_2^- concentrations, pH, and temperature, therefore, can be controlled by changing NH_4^+ loading rate. We added bicarbonate to keep pH between 7 and 8.5.

Granule size in our reactor (1.5-4 mm) was much larger than those previously reported (0.3–0.5 mm by Kim et al. (2006)[2], and similar to Xu et al. (2011)[3]. This difference may be due to the load of organic materials. High organic loading rate could stimulate the formation of large-size granules. Denitrifiers found in our granules may also contribute to the formation of porous structures by producing gas inside the granules. This results in the formation of large granules. Large granular size results in faster settling velocity (103 m h^{-1} average) than the activated sludge (e.g., 7-10 m h^{-1}). Similar to our study, large PN granules reported by Vazquez-Padin et al. (2010)[4]. showed fast settling velocity (150 m h^{-1}).

We used synthetic wastewater supplemented with acetate to mimic real wastewater. Since anammox bacteria are sensitive to organic materials, we need to remove TOC prior to Anammox process. The TOC removal efficiency was almost reached 95% during the operation. Heterotrophic bacteria or denitrifiers may have used acetate.

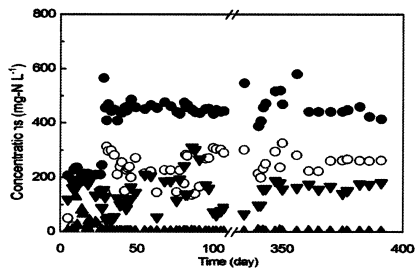

Fig. 2. The performance of the partial nitrification aerobic granule reactor. Ammonium (○), nitrite (▼) and nitrate (▲) concentrations in effluent and the ammonium (●) concentration in influent .

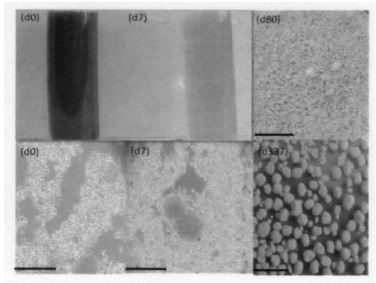

Fig. 3. Photographs of aerobic granules development in SBAR.

(2) Performance of the PN aerobic granule reactor

Figure 4 shows typical concentration profiles of nitrogen, TOC and pH in one cycle (4 h) in the PN reactor at steady state operation with ammonium loading rate of 1.8 kg-N m^{-3} day^{-1}. As NH_4^+ concentration decreased, NO_2^- concentrations increased, supporting the occurrence of PN reaction. Decrease in NO_2^-, along with the decrease in TOC, was observed in the denitrification and aerobic respiration by heterotrophs. Since accumulation of NO_3^- was not observed, NO_2^- was most likely reduced to gaseous forms (e.g., N_2O or N_2). Based on the mass calculation, 35.8%, 0.1%, and 32.1% of NH_4^+ were converted to NO_2^-, NO_3^-, and gaseous forms, respectively. At this condition, NH_4^+ conversion rate was 1.22 kg-N m^{-3} day^{-1}; whereas nitrite production rate was 0.64 kg-N m^{-3} day^{-1}. Effluent NO_2^-/NH_4^+ ratio was around one. These results indicated that this effluent is suitable for anammox process. NO_2^- production rate in our PN reactor was greater than that reported by Shi et al. (2011)[5] (0.38 kg-N m^{-3} day^{-1}), but smaller than that reported by Kim et al. (2006)[2] (2.4 kg-N m^{-3} day^{-1}). Shi et al. (2011)[5] added C source in their influent; while Kim et al. (2006)[2] did not, suggesting that addition of C source may influence NO_2^- production rate. Since there is a room to improve NO_2^- production efficiency in our PN aerobic granule reactor, the reactor operation conditions should be further optimized in the future.

(3) Identification and localization of the AOB

In the present study, we identified *Nitrosomonas* sequences that were >96.7% similar to *Nitrosomonas europaea* in our PN aerobic granule. Since *N. europaea* is known to favor higher strength of NH_4^+ and have faster growth rates than other AOB, high concentration of NH_4^+ in our reactor may have selected AOB closely related to *N. europaea*. Similar to our study, *Nitrosomonas* AOB were detected in the PN aerobic granules[2-4]. In our PN aerobic granules, AOB occupied only 7% of the clones, and clones related to *Rhodocyclales* bacteria (e.g. *Thauera* spp.) and *Pseudomonadales* bacteria (*Pseudomonas* spp.) were detected more frequently. Although clone library results can underestimate the abundance of *Alphaproteobacteria* due to their small number of rRNA operons[7], our result indicate that majority of the microbes in the PN aerobic granules were composed of non-AOB bacteria. FISH results show *Nitrosomonas* AOB were present near the surface of the PN aerobic granules. In this area, AOB were tightly packed and surrounded by other bacteria probably aerobic acetate-oxidizing bacteria.

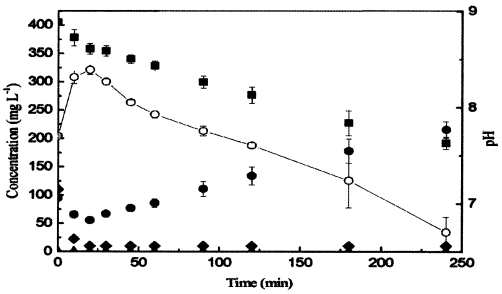

Fig.4 Typical pattern during a steady state of SBAR. Ammonium (■), nitrite (●), nitrate(▲), and TOC (◆) concentrations and pH (○) are shown.

4. conclutions

In this study, we successfully developed PN aerobic granule reactors that had the greater NH_4^+ removal efficiency than the PN reactors previously reported. The efficiency can be further enhanced by controlling airflow rate and reaction cycle time. The NO_2^-/NH_4^+ ratio in the effluent is around one, which is suitable for anammox process. *Nitrosomonas* AOB witch present near the surface was responsible in anmonia oxidization. In addition to ammonia oxidization, we also observed the occurrence of nitrite reduction (i.e., denitrification) in the granules.

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