

Research Article

Start-Up and Aeration Strategies for a Completely Autotrophic Nitrogen Removal Process in an SBR

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The start-up and performance of the completely autotrophic nitrogen removal via nitrite (CANON) process were examined in a sequencing batch reactor (SBR) with intermittent aeration. Initially, partial nitrification was established, and then the DO concentration was lowered further, surplus water in the SBR with high nitrite was replaced with tap water, and continuous aeration mode was turned into intermittent aeration mode, while the removal of total nitrogen was still weak. However, the total nitrogen (TN) removal efficiency and nitrogen removal loading reached 83.07% and 0.422 kgN/(m³·d), respectively, 14 days after inoculating 0.15 g of CANON biofilm biomass into the SBR. The aggregates formed in SBR were the mixture of activated sludge and granular sludge; the volume ratio of floc and granular sludge was 7 : 3. DNA analysis showed that Planctomycetes-like anammox bacteria and *Nitrosomonas*-like aerobic ammonium oxidization bacteria were dominant bacteria in the reactor. The influence of aeration strategies on CANON process was investigated using batch tests. The result showed that the strategy of alternating aeration (1 h) and nonaeration (1 h) was optimum, which can obtain almost the same TN removal efficiency as continuous aeration while reducing the energy consumption, inhibiting the activity of NOB, and enhancing the activity of AAOB.

1. Introduction

Biological nitrogen removal processes are generally used for the elimination of nitrogen from wastewater. However, wastewater with low carbon to nitrogen ratios (C/N), such as supernatants of anaerobic sludge digesters, landfill leachate, and special industrial wastewater, makes the conventional nitrification and heterotrophic denitrification processes more difficult [1, 2]. The discovery of the anaerobic ammonium oxidation (anammox) process has revolutionized the removal of nitrogen from wastewater that contains high amounts of nitrogen and has low C/N [3]. The anammox process involves the oxidation of ammonia into nitrogen gas, with nitrite serving as an electron acceptor in the absence of oxygen and organic carbon compounds [4]. Anammox is a promising process in which the costs related to aeration and external carbon addition are lowered by 60% and 100%, respectively, and which results in 90% less sludge compared to conventional nitrification-denitrification processes [5–7]. Several

autotrophic nitrogen removal processes have been developed based on anammox, such as the two-stage SHARON-ANAMMOX process [8] and the one-stage CANON (completely autotrophic nitrogen removal via nitrite) [9, 10] and OLAND (oxygen-limited autotrophic nitrification-denitrification) [11] processes. One-stage processes combine two reactions that are catalyzed by two different microbial groups, aerobic ammonium oxidizing bacteria (AerAOB) and anaerobic ammonium oxidizing bacteria (AnAOB). Initially, AerAOB oxidizes NH₄⁺ to NO₂⁻ under aerobic conditions (i.e., partial nitrification), which is followed by AnAOB converting NH₄⁺ and NO₂⁻ into N₂ [12]. CANON is one of the autotrophic nitrogen removal processes that maintain AerAOB and AnAOB in a single reactor by controlling low DO levels [13].

The long start-up period of the CANON process is the bottleneck for its application due to the very slow growth rate (doubling time is 11 d) and the low yield coefficient (0.13 g dry weight/g NH₄-N oxidized) of AnAOB [4, 14, 15]. There are two strategies used to initiate the CANON process [16]. The

first strategy is to inoculate an anammox reactor with nitrifying biomass and subsequently maintain the oxygen-limited conditions [13, 17]. The second is to operate a nitrifying reactor under oxygen-limited conditions in order to wash out NOB and later inoculate the AnAOB biomass [18, 19]. For the application of the CANON process, the strategy of initially inoculating universally nitrifying activated sludge, and then inoculating small amounts of AAOB in order to partially nitrify the reactor, was the most practical [20].

The CANON process can be developed in both biofilm and suspended systems. For suspended-growth systems, there are two challenges: biomass retention and the equilibration of different microbial group activities [21]. Granular sludge not only guarantees high biomass retention efficiency but also protects inner AAOB from dissolved oxygen [22]. Recent studies state that small and large aggregates play different functional roles in the nitrite-anammox process [21, 22]. It was reported that large type aggregates ($>500 \mu\text{m}$) accounted for 68% of the AnAOB, whereas 65% of the nitrification potential was observed in the smaller aggregates ($<500 \mu\text{m}$) in a granular reactor [21]. The aggregates formed in granular sludge reactors contain a mixture of flocs, small granules, and large granules [23–26]. Granular sludge can be defined as compact and dense aggregates with an approximately spherical external appearance that did not coagulate under decreased hydrodynamic shear conditions and that settle significantly faster than floc [27]. In comparison, floc is characterized by loose, permeable aggregates that are composed of microcolonies enmeshed in extracellular polymers [28]. In granular aggregates, AerAOB lies in the outer layer and AnAOB in the inner layer, while in floc, AerAOB were the dominant bacteria [22]. To achieve high nitrogen removal efficiency, a good balance of AerAOB and AnAOB activity is needed, which is related to the aggregate characteristics in the reactor.

In the CANON process, the continuous aeration mode was applied more often than the intermittent aeration mode. Lackner stated that continuous aeration is preferred under normal operating conditions, while intermittent aeration is primarily used during start-up or periods of low sludge activities [1]. It was also reported that the intermittent aeration strategy can obtain similar total nitrogen removal efficiency as the continuous aeration strategy, thereby saving energy consumption due to shorter aeration times [29], but the proper ratio of aeration to nonaeration time should be well considered.

In this study, partial nitrification was established in a sequencing batch reactor by carefully controlling the aeration, initially using activated sludge as seed sludge and then inoculating CANON biofilm in the SBR. This method achieved both partial nitrification and anammox in one single-stage reactor by reducing the aeration amount and altering the continuous and intermittent aeration amounts. This process was examined, the sludge characteristics were studied with SEM, and the polymerase chain reaction (PCR) technique was used to analyze the microbial community in the reactor. Additionally, the influence of aeration strategy on the CANON process was studied in batch tests.

2. Materials and Methods

2.1. Reactor Description. This study used a SBR with a working volume of 13 L. Dimensions of the SBR were the following: height of 545 mm, inner diameter of 174 mm, and height to diameter ratio of 3.13:1. Two or three cycles were performed each day during the whole experiment. One cycle consisted of a 20 min filling period, an aerating period (continuous or intermittent) of 11 h in 12 h cycle or 7 h in 8 h cycle, a 30 min settling period, a 5 min drawing period, and a 5 min idling period. The exchange volume was fixed at 50%, and a mass-flow controller was used to keep aeration constant. Two different aeration strategies were applied in this study, namely, continuous aeration and intermittent aeration, both characterized by the ratio (R) between the aerated and nonaerated duration times. The reactor was stirred at a rate of 700 rpm and maintained at a temperature of $30 \pm 2^\circ\text{C}$.

2.2. Biomass and Synthetic Wastewater. In this study, the conventional nitrification sludge originated from the oxidation ditch of a municipal wastewater plant, was inoculated and developed to partial nitrification in the SBR, and was later inoculated with a small CANON biofilm biomass (0.15 g) to partial nitrification in the SBR.

The synthetic wastewater used in this study mainly contained NH_4^+ from NH_4HCO_3 (N source, C source, and buffer), NaHCO_3 (supplementary C source and buffer), and phosphate from KH_2PO_4 . The influent concentrations were sampled from the filling tank. The influent ammonium concentration was in the range of 100–300 mg/L and the pH was between 7.4 and 8.2.

2.3. Chemical Analysis. The concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, MLSS, and MLVSS were measured according to standard methods (APHA, 1995). Total nitrogen (TN) was defined as the sum of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$. Dissolved oxygen (DO) concentrations and pH values in the reactor were determined using a portable analyzer (HQ30d, Hach, USA) and a pH meter (PHS-10, Fangzhou, China). Nitrite accumulation ratio (NAR) and free ammonia (FA) were calculated as follows [30]:

$$\text{NAR}\% = \frac{[\text{NO}_2^-\text{-N}]_{\text{Eff}}}{[\text{NO}_2^-\text{-N}]_{\text{Eff}} + [\text{NO}_3^-\text{-N}]_{\text{Eff}}} \times 100\% \quad (1)$$

$$\text{FA} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{17}{14} \times \frac{([\text{NH}_4^+\text{-N}] + [\text{NH}_3\text{-N}])(\text{mg/L}) \times 10^{\text{pH}}}{e^{(6334/(273+T))+10^{\text{pH}}}} \quad (2)$$

2.4. Batch Tests. Tests to determine the effect of different aeration strategies (including continuous and intermittent aeration) on process efficiency were performed in batch reactors inoculated with the mixed liquor collected from the SBR prior to the end of the reaction period during the quasi-steady state. Tested sludge was washed to remove nitrogen compounds and was divided into four parts. Each part of the sludge was put into a 1.5 L batch reactor. Four batch reactors were supplied with synthetic wastewater. The initial

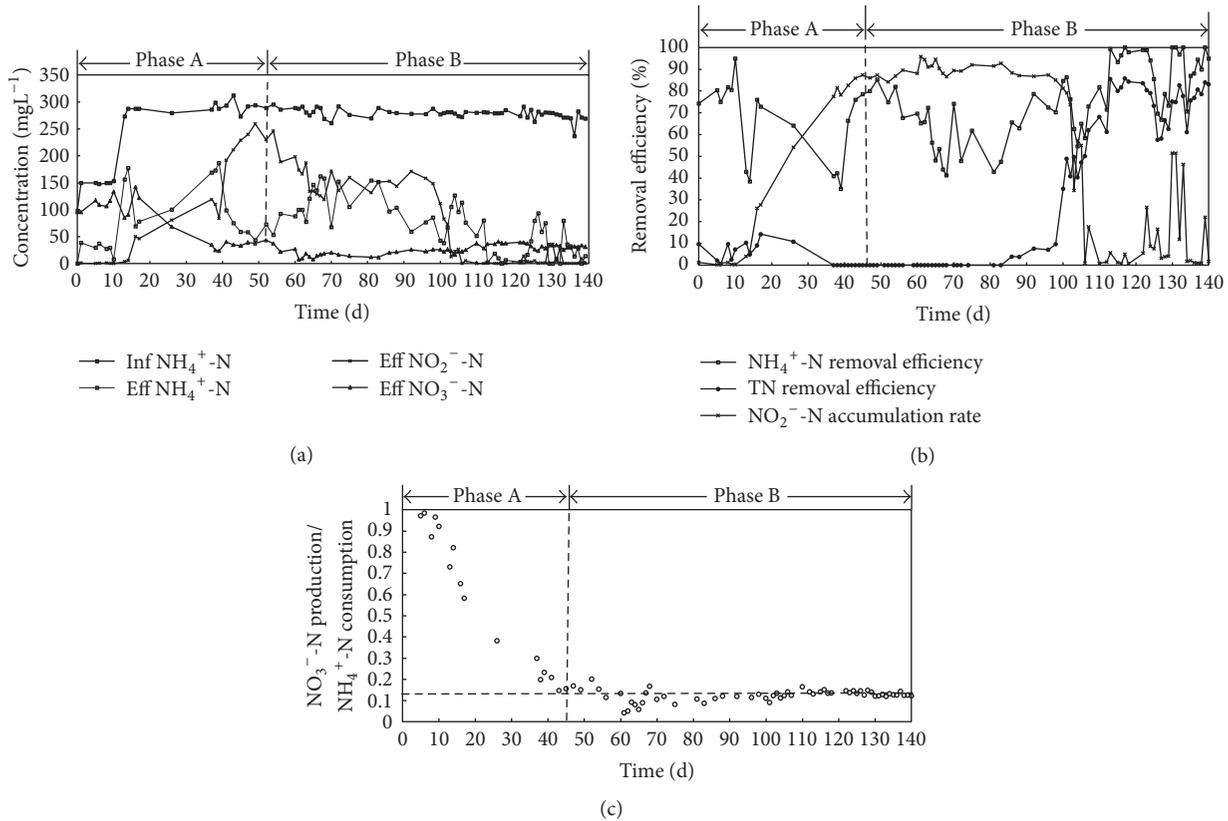


FIGURE 2: Performance of the CANON reactor: (a) influent and effluent concentrations of nitrogen species; (b) ammonium, TN removal efficiency, and nitrite accumulation rate (NAR); (c) ratio of nitrate production to ammonium consumption.

TABLE 1: Operating parameters of each phase in SBR.

Parameters	Phase A	Phase B
Time range (d)	0~50	51~140
Hydraulic retention time (h)	16	24 (51~123 days), 16 (124~140 days)
Aeration mode	Continuous	Intermittent (2 h aeration : 2 h no aeration : 2 h aeration : 1.5 h no aeration)
DO concentration during aeration phase	1~2 mg/L (1~11 days), 0.16~0.18 mg/L (12~51 days)	0.04~0.15 mg/L (aeration time), 0 mg/L (no aeration time)
Intermittent aeration (R)	—	1/1 (except the last nonaeration duration)

During phase B, two cycles were performed each day and intermittent aeration was applied from day 51 to day 123, after which three cycles were applied again to increase the nitrogen load of the SBR. The DO concentration in the aerated portion of the SBR cycle was maintained in the range of 0.04~0.15 mg/L. The removal of TN was minimal and was increased slowly between days 51 and 98. On the 99th day, 0.15 g of CANON biofilm biomass was taken into the SBR, after which

the TN removal efficiency increased obviously, exceeding 80% on day 113 (14 days after CANON biofilm inoculation). The ratio of nitrate production to ammonium consumption was approximately 0.13, which one would expect for the CANON process [9] in the end of period B. This finding demonstrates the successful start-up of the CANON process in the SBR and indicates that the inoculation of AAOB biomass can significantly shorten the start-up time of the

CANON process. Low DO concentrations and intermittent DO modes also played important roles in the realization of autotrophic nitrogen removal via nitrite.

3.2. Aeration Strategies. Changes of nitrogen compounds under different aeration strategies are depicted in Figures 3(a), 3(b), 3(c), and 3(d). It is demonstrated in Figure 3(a) that ammonia concentrations decreased almost linearly under continuous aeration. Nitrite concentration increased in the first 60 min, later decreased, and subsequently remained steady between 1.5 and 2.0 mg/L. Nitrate concentration increased from 28.33 mg/L to 57.48 mg/L. The removal efficiency of ammonia was 73.72%, the total nitrogen removal efficiency was 65.56%, and the ratio of nitrate production to ammonium consumption was 0.154, which was much higher than what one would expect for the CANON system [9]. The second aeration strategy included 5 aeration times and 5 nonaeration times (Figure 1), where each aeration time lasted 60 min and each nonaeration time lasted 30 min. The nitrogen compounds of the second aeration strategy are shown in Figure 3(b). It can be seen that ammonia decreased in each aeration time, while the variation of ammonia in each nonaeration time differed. Ammonia concentration decreased in the first two nonaeration times but remained unchanged in the following three nonaeration times. Additionally, it was observed that nitrite concentrations were very low in the subsequent three nonaeration times, which limited the activity of AAOB and resulted in unchanged ammonia concentrations. The total nitrogen removal efficiency of the second aeration strategy was 67.02%, and the ratio of nitrate production to ammonia consumption was 0.137, which was still higher than what one would expect for the CANON system. There were 4 aeration times and 4 nonaeration times in the third aeration strategy. All aeration times were 1 h, and nonaeration times were 1 h, 1 h, 1 h, and 0.5 h. Variation of nitrogen compounds used in the third aeration strategy was similar to those used in the second aeration strategy. The ammonia and total nitrogen removal efficiencies of the third aeration strategy were 62.93% and 69.21%, respectively. The ratio of nitrate production to ammonium consumption was 0.128, which was closest to what one would expect for the CANON system in the four batch tests. The fourth aeration strategy also included 5 aeration times and 5 nonaeration times, and the ratio of aeration time to nonaeration time was 0.5 h : 1 h. The ammonia and total nitrogen removal efficiencies of the fourth strategy were only 49.83% and 54.22%, respectively, due to the aeration times being too short to convert enough ammonia to nitrite. The activity of AAOB was also limited by the lack of nitrite.

The total aeration times in the four aeration strategies were 450 min (continuous aeration), 300 min (aeration time : nonaeration time = 1 h : 0.5 h), 240 min (aeration time : nonaeration time = 1 h : 1 h), and 150 min (aeration time : nonaeration time = 0.5 h : 1 h). The total nitrogen removal efficiencies of the first, second, third, and fourth strategies were 65.56%, 62.93%, 65.33%, and 54.22%, respectively. The ratios of nitrate production to ammonia consumption for the first, second, third, and fourth strategies were 0.154, 0.142, 0.128, and 0.103, respectively. This finding suggests

that the ammonia conversion rate and the nitrate production rate decreased with decreasing aeration times, which were also related to the activities of AOB, NOB, and AAOB. The CANON process relied on the incorporation of AOB and AAOB, while NOB was not expected in the reactor. The ratio of nitrate production to ammonia consumption was 0.13 when the NOB had washed out completely [9]. A ratio higher than 0.13 indicated that NOB was not washed out completely from the SBR. The activity of AAOB was inhibited by oxygen when oxygen penetrated into the inner of floc in the aeration duration, while the nonaeration duration can not only inhibit the activity of NOB but also help the AAOB to restore its activity, which was inhibited by oxygen in the aeration duration. The strategy of 0.5 h aeration and 1 h nonaeration is not recommended because the short aeration time limited the production of nitrite, resulting in ammonia and total nitrogen removal efficiencies being very low. The very low ratio of nitrate production to ammonia consumption further verified that the short aeration time limited the conversion of ammonia. The strategy of 1 h aeration and 1 h nonaeration was the optimal one, which resulted in similar total nitrogen removal and continuous aeration efficiency and shortened the duration of aeration. The ratio of nitrate production to ammonia consumption under this strategy was also close to the one expected for the CANON system [9].

3.3. Biomass Morphology. At the start of this experiment, 1 liter of activated sludge from a municipal WWTP was taken into the SBR, and a small CANON biofilm was inoculated in the SBR after the partial nitrification was set up. Over time, the granular sludge started to grow in the SBR. At the end of the experiment, the sludge in the SBR was a mixture of floc and granules, with the granules occupying 31% (w/w) and the flocs occupying 69% (w/w). Three scanning electron microscopy (SEM) images of the granules are shown in Figure 4: (a) the outside of a granule, (b) one slice of a granule (a rectangle), and (c) another slice of a granule (an ellipse). Arrows in Figure 4(b) indicated holes inside the granule. Granules had distinct boundary and had spherical or ellipsoidal appearance, while flocs were more loose and amorphous. The average diameter of the granules was 900 μm , while the size of floc was smaller than 300 μm .

3.4. DNA Analysis. PCR-DGGE was used to examine the microbial community in the SBR, and parts of the results are shown in Table 2. Results show that six groups had high identity to *Candidatus Brocadia* belonging to the order Planctomycetales. *Candidatus Brocadia* was identified as the dominant species of AnAOB in the CANON process. This finding indicates that only one genus of anammox bacteria is dominant under the applied growth conditions [32]. Three groups of AerAOB belonging to β -proteobacteria were found in this reactor, all of which were closely related to *Nitrosomonas*, which have been found as dominant populations in other CANON systems [33, 34]. NOB belonging to *Nitrospira* was also present in the reactor, which further supports the results of batch tests. The NOB was present in floc when the DO was sufficient, producing nitrate and resulting in unstable performance of the CANON system.

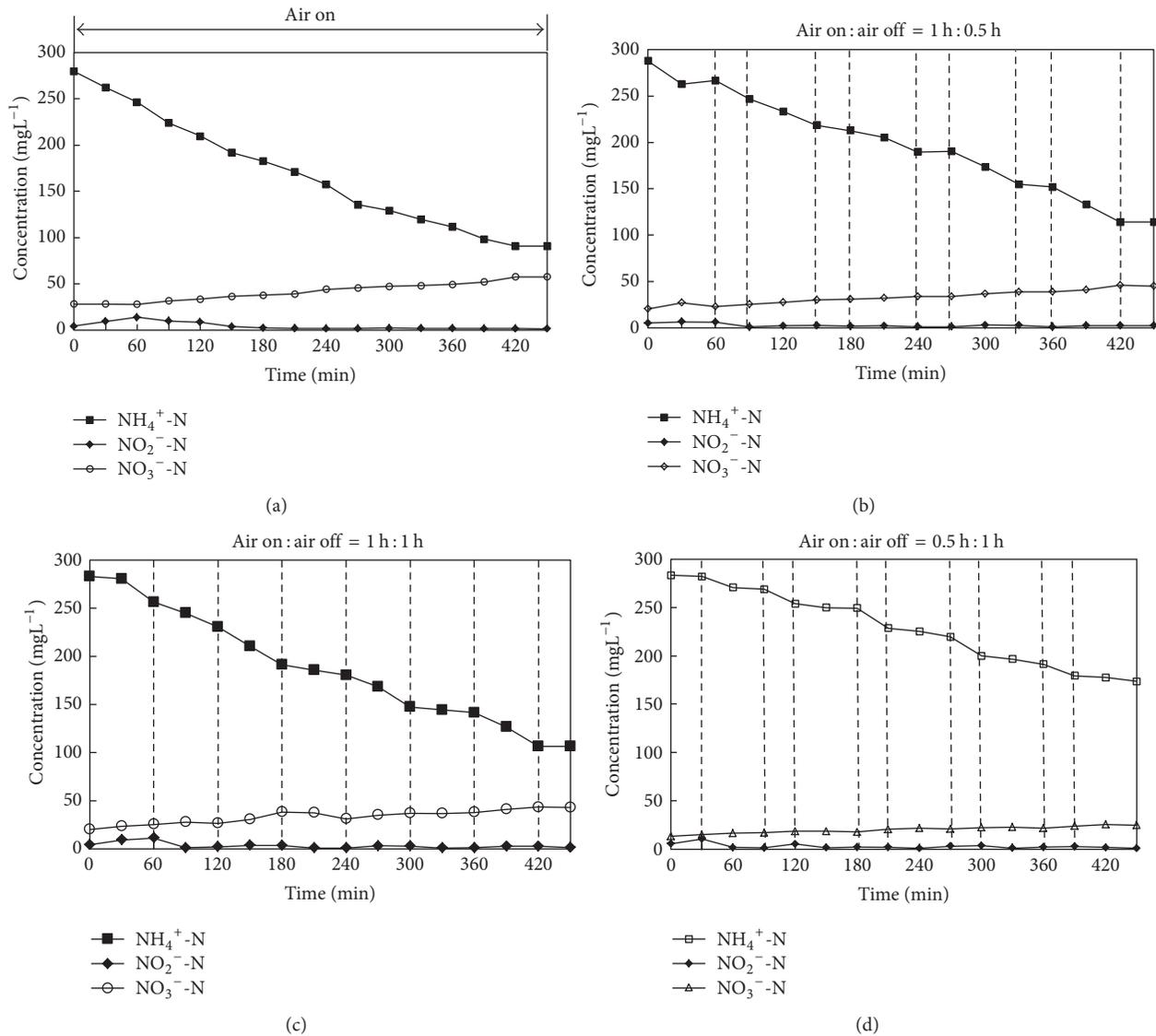


FIGURE 3: Profiles of nitrogen compounds in bulk solutions for a typical reaction time (450 min) of the SBR with four aeration strategies: (a) continuous aeration; (b) aeration time:nonaeration time = 1 h:0.5 h; (c) aeration time:nonaeration time = 1 h:1 h; (d) aeration time:nonaeration time = 0.5 h:1 h.

TABLE 2: Phylogenetic sequence similarities to the closest relative from DGGE bands.

Band	Taxon	Identity	Accession	Phylum (classifier)
4	<i>Candidatus Brocadia</i> sp. RAS-Ina-1 16S rRNA gene	97%	HM769652	Planctomycetia
5	<i>Candidatus Brocadia</i> sp. S08-01 16S rRNA gene	99%	JN205347	Planctomycetia
7	<i>Candidatus Brocadia fulgida</i> isolate R1 16S rRNA gene	97%	JQ864321	Planctomycetia
15	<i>Candidatus Brocadia</i> sp. ODS-1 16S rRNA gene	98%	HM769653	Planctomycetia
19	Uncultured <i>Candidatus Brocadia</i> sp. 16S rRNA gene	98%	KF606756	Planctomycetia
23	<i>Candidatus Brocadia caroliniensis</i> 16S rRNA gene	98%	KF810110	Planctomycetia
1	Uncultured <i>Nitrosomonas</i> sp. gene for 16S rRNA gene	100%	AB500059	β -proteobacteria
26	<i>Nitrosomonas</i> sp. HP8 partial 16S rRNA gene	99%	HF678378	β -proteobacteria
28	Uncultured <i>Nitrosomonas</i> sp. DGGE 16S rRNA gene	100%	KF452288	β -proteobacteria
2	<i>Nitrospira</i>	98%	KF472236	β -proteobacteria

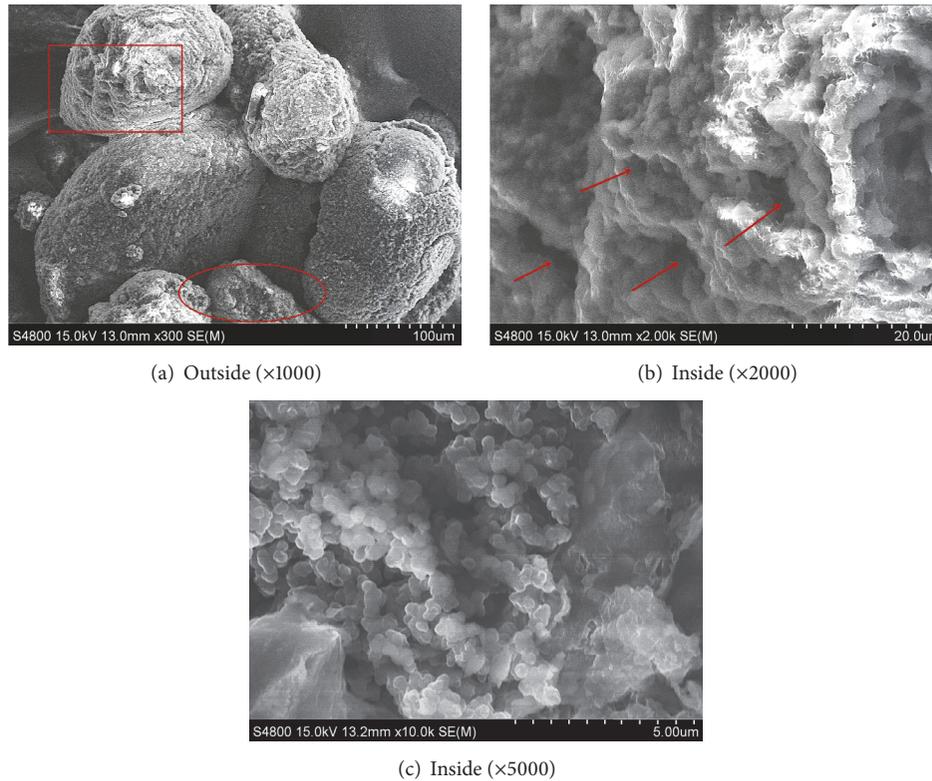


FIGURE 4: Scanning electron microscopy (SEM) images of granules.

4. Conclusions

Inoculating AAOB biomass in a partial nitrification system can start the CANON process rapidly, resulting in total nitrogen removal efficiencies of up to 80% after inoculation. The presence of complete granular sludge was not necessary. The mixture of granular sludge and floc present in the SBR and the NOB present in the floc and sludge influenced the total nitrogen removal efficiency. Granules had distinct boundary and were spherical or ellipsoidal, while floc was more loose and amorphous. The average diameter of the granules was $900\ \mu\text{m}$, while the size of floc was smaller than $300\ \mu\text{m}$. The intermittent aeration strategy was important not only for ammonia and total nitrogen efficiencies but also for the inhibition of NOB and the protection of AAOB from oxygen. The optimal ratio of aeration time to nonaeration time for the intermittent aeration strategy was 1 h : 1 h.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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