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Evaluation of Non-Biodegradable Organic Matter and Microbial Community's Effects on Achievement of Partial Nitrification Coupled with ANAMMOX for Treating Low-Carbon Livestock Wastewater

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Abstract: After the anaerobic digestion of livestock manure, high concentrations of nutrients still remain. Treatment of livestock wastewater through partial nitrification coupled with anaerobic ammonium oxidation (ANAMMOX) could be a useful technology depending on the investigation of microorganism enrichment and partial nitrification coupled with achievement of the ANAMMOX process. The results show 78.4% and 64.7% nitrite accumulation efficiency was successfully obtained in an intermittent aeration sequencing batch reactor and a continuous aeration sequencing batch reactor, respectively, at a loading rate of 0.93 kg ammonium/(m^3·d). The main reason for the high nitrite accumulation efficiency was the intermittent aeration strategy which generated a 20–30 min lag reaction for nitrite oxidation and promoted the growth of the dominant ammonium oxidation bacteria (*Nitrosomonas*). Non-biodegradable organic matter in the effluents of partial nitrification did not have obvious influence on ANAMMOX activity at low loading rates (118 ± 13 mg COD/L and 168 ± 9 mg COD/L), and up to 87.4% average nitrite removal rate was observed. However, with the influent COD concentration increasing to 242 ± 17 mg/L, the potential inhibition of ANAMMOX activity was exerted by non-biodegradable organic matter.

Keywords: livestock wastewater; partial nitrification; ANAMMOX; non-biodegradable organic matter; nitrogen removal; microbial community

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1. Introduction

Every year, a large amount of livestock manure containing high concentrations of nutrients and solids is produced from centralized livestock farms [1,2]. Anaerobic digestion is one of the sustainable technologies for livestock manure management. However, after anaerobic digestion treatment, most ammonium and non-biodegradable organic matter (mainly cellulose and hemicelluloses) in livestock manure still remains in the digestate wastewater [3,4]. The lack of a biodegradable carbon source in the livestock wastewater could lead to difficulties in the operation of the conventional nitrification–denitrification process. In this case, further studies of the livestock wastewater treatment biotechnology with low carbon cost and the effects of non-biodegradable organic matter on nitrogen removal are necessary.

Partial nitrification is an efficient technology for treating low-carbon wastewater by achieving biological nitrogen removal via nitrite rather than nitrate. This process has two advantages over the conventional nitrification–denitrification process: (I) reduction of the carbon demand of denitrifying bacteria by 40%; and (II) reduction of the aeration energy by 25% [5]. In the partial nitrification process, nitrite accumulation efficiency is related to nitrogen levels, pH, dissolved oxygen (DO) and loading rate, which are suggested to be the important factors for the washing out of nitrite-oxidizing bacteria (NOB) [6–8]. The high concentrations of ammonium and non-biodegradable organic matter in the livestock wastewater were unfavorable for partial nitrification. Thus, effective control strategies should be developed and investigated to achieve stable nitritation.

Anaerobic ammonium oxidation (ANAMMOX) has been considered as an effective technology for the reduction of accumulated nitrite by oxidation of ammonium rather than organic carbon [9]. In the ANAMMOX process, bacteria grow with bicarbonate as the sole carbon source. Thus, without the need for organic matter, this technology has been exploited to treat nitrogen-rich wastewater, such as landfill leachate, animal manure and supernatant of digested sludge [10,11]. ANAMMOX bacteria are considered to be difficult to cultivate because of their low specific growth rate (μ_{max} = 0.065/d) and sensitivity to organic matter, which could induce the inhibition of ANAMMOX enrichment [12]. Thus, the non-biodegradable organic matter withdrawn from partial nitrification reactors should be an important issue on the successful use of ANAMMOX for treating livestock wastewater.

In livestock wastewater, there is not enough biodegradable organic matter for the conventional biological nitrogen removal process. Thus, partial nitrification coupled with ANAMMOX was considered to be an efficient combined technology for nitrogen removal. In this research, in order to evaluate the influences of non-biodegradable organic matter and control strategies on livestock wastewater treatment efficiency, long-term partial nitrification coupled with ANAMMOX reactors were established via microbial community analysis to investigate the performance of nitrogen removal from the livestock wastewater.

2. Materials and Methods

2.1. Experimental Setup

Two identical laboratory-scale sequencing batch reactors (SBRs) and an ANAMMOX reactor were constructed (Figure 1). The reactors were made from transparent Plexiglas, each having an effective volume of 10 L.

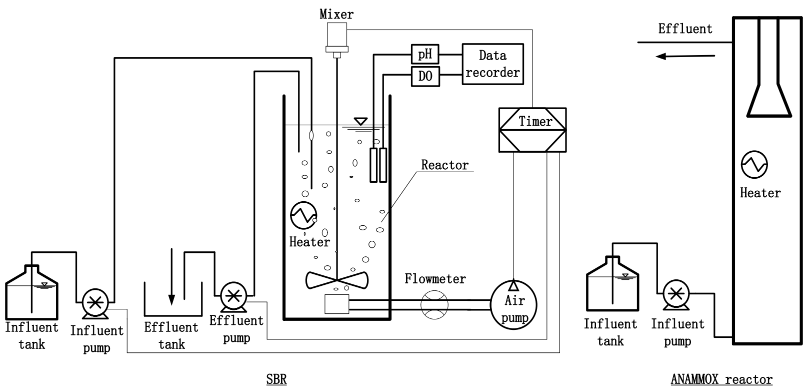

Figure 1. Schematic diagram of SBRs and ANAMMOX reactor systems.

One SBR was operated with an intermittent aeration (IASBR) strategy and the other was operated with continuous aeration (CASBR) in an 8 h cycle. For the IASBR, after a 60 min non-aeration period, the reactor was intermittently aerated with successive 50 min aeration/30 min non-aeration periods. For the CASBR, 250 min continuous aeration was adopted after a 180 min non-aeration period. Then, the settle phase lasted 40 min before the effluent was withdrawn in the last 10 min. Each SBR was stirred with a rectangular mixing paddle. The air was supplied using aquarium air pumps at an air flow rate of 0.8–0.9 L air/min.

The characteristics of the livestock wastewater taken from a mesophilic manure digester were: 10,260 ± 780 mg/L of COD; 3112 ± 94 mg/L of NH_4^+-N; 3478 ± 75 mg/L of total nitrogen (TN); 1.25 ± 0.18 g/L of suspended solids (SS); and pH of 8.26 ± 0.05. The wastewater had a COD to TN ratio of 2.96, and the BOD_5 to COD ratio was only 0.38, indicating a low proportion of biodegradable organic matter in the wastewater. The livestock wastewater was fed to SBRs with peristaltic pumps at loading rates of 3.08 kg COD/(m^3·d) and 0.93 kg NH_4^+-N/(m^3·d). The temperature of two SBRs was controlled at 26 ± 1 °C, simulating the digestate liquid temperature after anaerobic digestion.

An upflow anaerobic sludge blanket (UASB) reactor was built for cultivating ANAMMOX biomass (Figure 1). The effluents of IASBR were collected and adjusted with NH_4Cl to obtain a desirable nitrogen ratio (NH_4^+-N: NO_2^--N = 1: 1.32) for the influent of the ANAMMOX reactor [9]. The seed sludge fed to the UASB was a mixture of anaerobic denitrification sludge, anaerobic composting sludge, river sediment sludge, local soil and partial nitrification activated sludge. Before treating the raw effluents of IASBR, influent synthetic wastewater (30 mg/L NH_4^+-N and 40 mg/L NO_2^--N from Day 1 to Day 100; 60 mg/L NH_4^+-N and 80 mg/L NO_2^--N from Day 101 to Day 223; and 90 mg/L NH_4^+-N and 120 mg/L NO_2^--N from Day 223 to Day 289) was continuously fed into the UASB at a hydraulic retention time (HRT) of 8 h for ANAMMOX biomass cultivation. The temperature of the UASB was controlled at 35 ± 2 °C, and an optimum pH of 8.0 ± 0.4 was controlled through adding $NaHCO_3$ into the influent.

2.2. Analytical Methods

COD, BOD_5 and SS were tested in accordance with the standard APHA methods [13]. NH_4^+-N, NO_2^--N and NO_3^--N were measured with Hach kits (Hach, Ames, IA, USA). A pH probe (pH 320, WTW, Weilheim, Germany) and a DO sensor (Unisense, Aarhus, Denmark) were used for pH and DO measurement, respectively.

The nitrite accumulation efficiency (η) is a term used to describe the performance of partial nitrification as per Equation (1):

$$\eta = \frac{S_{NO_2^- - N}}{S_{NO_2^- - N} + S_{NO_3^- - N}} \times 100\% \quad (1)$$

where $S_{NO_2^- - N}$ and $S_{NO_3^- - N}$ are the concentrations of NO_2^--N and NO_3^--N in the effluent, respectively.

2.3. Batch Experiments

All batch experiments were carried out in 1 L beakers. Air diffusers were placed at the bottom of the beakers. Washed activated sludge (Day 120) from two SBRs was added to the beakers, and the sludge concentrations in the beakers were adjusted to close to those inside the SBRs. The pH was controlled at 8.20 ± 0.05.

Specific oxygen uptake rate (SOUR) measurements: (I) NH_4Cl or $NaNO_2$ was fed into the beaker to obtain specified 30 mg/L NH_4^+-N or NO_2^--N, respectively; (II) after feeding, the aeration commenced until DO became saturated in the beakers; (III) aeration was turned off, and DO concentrations consumed by the oxidation of NH_4^+-N or NO_2^--N were recorded.

Influence of intermittent aeration on partial nitrification: (I) 0, 10, 20, 30 and 40 min non-aeration periods were set in 5 beakers, where argon gas was striped at the bottom of the beakers in order to continuously remove DO; (II) specified 30 mg/L NH_4^+-N and NO_2^--N were added into the beakers through adjusting NH_4Cl and $NaNO_2$ dosages, (III) aeration commenced, and liquid samples were taken at intervals for the measurement of NO_2^--N and NO_3^--N concentrations.

2.4. Microbial Population Analysis by 16S rRNA Gene Sequencing

In order to assess potential population changes of the partial nitrification biomass in IASBR and CASBR, 2 mL of sludge was sampled from each reactor. DNA was extracted using the repeated bead beating and column purification extraction process [14]. Modified 16S Illumina adapter fusion primers were used to generate amplicon libraries. The primers were CaporasoNexF and CaporasoNexR [15,16]. PCR was conducted by using 20 ng of DNA from the sludge samples as a template and Kapa HiFi Hotstart ReadyMix (Kapa Biosystems, London, UK) according to the manufacturer's instructions. The thermocycling conditions were: one cycle of 95 °C for 3 min, then 26 cycles of 95 °C for 30 s, 55 °C for

30 s, 72 °C for 30 s, followed by one cycle of 72 °C for 5 min. QIAquick PCR Purification Kits (Qiagen, UK) were used to purify libraries. Two unique 8 bp indices were then added (one index at the 5′ end of the amplicon and the other at the 3′ end) to each amplicon in a second round of PCR using primers from the Illumina Nextera XT indexing kit. PCR was performed with 5 μL of each amplicon as a template and Kapa HiFi Hotstart ReadyMix. PCR conditions for this second round were: one cycle of 95 °C for 3 min, then 8 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, followed by one cycle of 72 °C for 5 min. Indexed libraries were then purified, pooled, gel purified, spiked and denatured. Sequencing was performed on the Illumina MiSeq sequencer using 500 cycle MiSeq reagent kits (San Diego, CA, USA). Sequence quality control, pre-processing, amplicon sequencing and data analysis were carried out as in the descriptions in previous studies [16].

3. Results and Discussion

3.1. Overall Performance of Partial Nitrification on Treating Livestock Wastewater

The IASBR and CASBR were operated for 120 days, and the COD removal rate reached a steady state after 15 days. The average COD removal rate was 87.6 ± 1.7% and 89.7 ± 3.4% in IASBR and CASBR, respectively. In the effluent, BOD_5 concentration was only 20 ± 13 mg/L. The effluent was added into a flask with activated sludge taken from the IASBR, and then it was continuously aerated for two days. There was no evident decrease in COD, indicating that most of the remaining COD in the effluent was non-biodegradable organic matter, such as cellulose and hemicelluloses [3].

Nitrite accumulation occurred immediately in the beginning period (Figure 2) due to the activated sludge in IASBR and CASBR seeded from a partial nitrification reactor. However, during this period, it was observed that the effluent NH_4^+-N level rose with a significant pH decrease (from 7.9 in Day 1 to 6.1 in Day 10). It shows that the alkalinity in the livestock wastewater was not sufficient to sustain a stable pH. Thus, an additional 4.5 g/L of alkaline solution ($NaHCO_3$) was added to the digestate liquid from Day 13. The nitrification activity recovered immediately, and after Day 30, the effluent NH_4^+-N concentration dropped to below 10 mg/L with a stable pH of 8.1 ± 0.2.

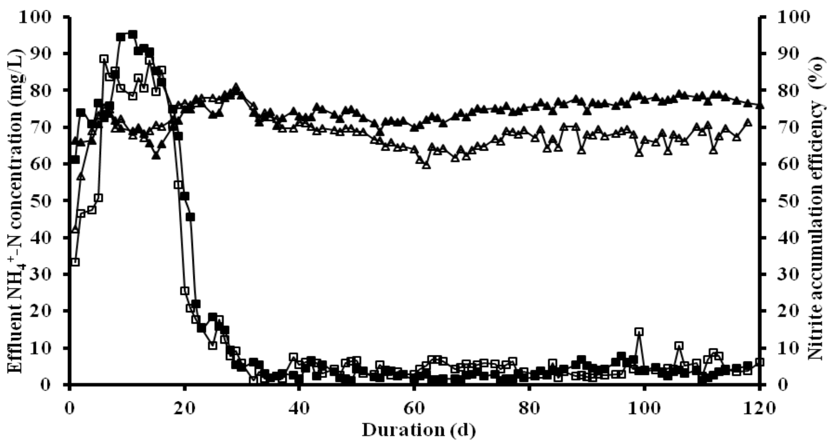

Figure 2. Performance of effluent NH_4^+-N concentration and NO_2^--N accumulation efficiency in SBRs (■: effluent NH_4^+-N in IASBR; □: effluent NH_4^+-N in CASBR; ▲: NO_2^--N accumulation efficiency in IASBR; Δ: NO_2^--N accumulation efficiency in CASBR).

In two SBRs, NO_2^--N accumulated and more than 90 mg/L of NO_2^--N appeared in the effluent as soon as the reactor operation commenced. In the steady state, effluent NO_2^--N concentration increased to 544 ± 36 mg/L and 492 ± 65 mg/L in IASBR and CASBR, and an average nitrite accumulation efficiency of 78.4 ± 1.3% and 64.7 ± 3.2% was achieved, respectively. It was observed that the accumulation efficiency of effluent NO_2^--N in CASBR was lower than that of IASBR. The nitration activity recovered when the partial nitrification sludge experienced continuous aeration. Therefore, the oxidation of NO_2^--N to NO_3^--N was encouraged in CASBR, and NOB activity partly recovered to lead to an increase in the NO_3^--N concentration in the effluent from Day 38.

Batch experiments show that in the NH_4^+-N oxidation process, the SOUR of IASBR and CASBR was 13.6 and 9.5 mg O_2/(g biomass·h), and as for the NO_2^--N oxidation, the SOUR of IASBR and CASBR was 1.4 and 2.0 mg O_2/(g biomass·h), respectively. In IASBR and CASBR, the SOUR of NH_4^+-N was over more than that of NO_2^--N. This means that the capability of nitrite accumulation was actively maintained under the intermittent aeration and continuous aeration conditions. The additional alkalinity supplement might have been the important factor promoting partial nitrification in two SBRs. It caused the pH values in two SBRs to range from 7.9–8.4. When pH is above 7.5, it may inhibit the growth and activity of NOB [17,18].

It was observed that the partial nitrification efficiency in IASBR was 21.2% higher than that in CASBR, and the SOUR ratio of NH_4^+-N oxidation to NO_2^--N oxidation reached 9.71:1 in IASBR. Under the intermittent aeration condition, the control strategy of alternating aeration and non-aeration could provide a suitable environment for partial nitrification. In order to investigate the influence of intermittent aeration on nitrite accumulation, under the condition of alternating 0, 10, 20, 30 or 40 min non-aeration periods with continuous 50 min aeration period conditions, the capability of partial nitrification was examined through batch experiments (Figure 3).

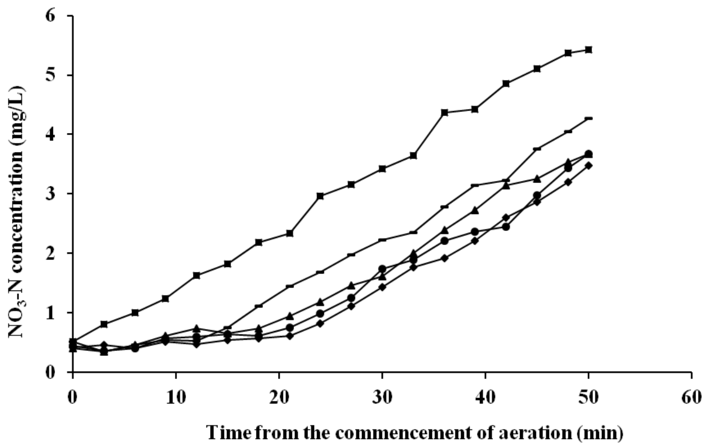

Figure 3. Influence of aeration strategy on partial nitrification (■: 0 min non-aeration duration; -: 10 min non-aeration duration; ▲: 20 min non-aeration duration; •: 30 min non-aeration duration; ⧫: 40 min non-aeration duration).

Besides the 0 min non-aeration condition, after the commencement of aeration, the increase in nitrate was retarded for 12, 18, 18 and 21 min, respectively. NO_2^--N oxidation was not activated immediately after aeration started when the non-aeration period was adopted [19]. After a "lag time" of NO_2^--N oxidation, the activity of the NOB recovered, and the calculated NO_3^--N accumulation rates were 0.0972, 0.0977, 0.0967 and 0.9930 mg NO_3^--N/(L·min), respectively. Compared with 0.1030 mg NO_3^--N/(L·min) in the 0 min non-aeration condition, which could denote continuous aeration condition, there was no evident decrease of NO_3^--N accumulation rates in the intermittent aeration conditions. Thus, the intermittent aeration strategy was discovered to induce a lag reaction for NO_2^--N oxidation in a short time, but had no further effects on the inhibition of NOB activity.

The average ratios of NO_2^--N accumulation rate to NO_3^--N accumulation rate within the 50 min aeration period were investigated (Figure 4). In 10, 20, 30 and 40 min non-aeration conditions, the ratios were 1.28, 1.44, 1.51 and 1.35 times of that in 0 min condition, respectively. As for the 40 min non-aeration condition, the NH_4^+-N oxidation rate was only 0.294 mg NH_4^+-N/(L·min), compared with 0.358, 0.364, 0.356 and 0.346 mg NH_4^+-N/(L·min) in 0, 10, 20 and 30 min non-aeration conditions, respectively.

This means that a slight inhibition of ammonium-oxidizing bacteria (AOB) could be carried out in the 40 min non-aeration condition. Thus, the optimal alternating non-aeration duration was 20–30 min for partial nitrification treatment of livestock wastewater.

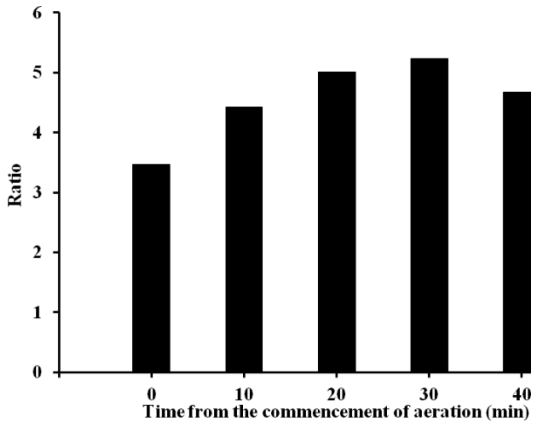

Figure 4. Ratio of NO_2^--N accumulation rate to NO_3^--N accumulation rate under the different non-aeration durations.

Sludge samples were taken from two SBRs to investigate the microbial population by 16S rRNA gene sequencing on Day 45 (partial nitrification set-up stage) and Day 109 (partial nitrification steady-state stage) compared with seed sludge (Figure 5).

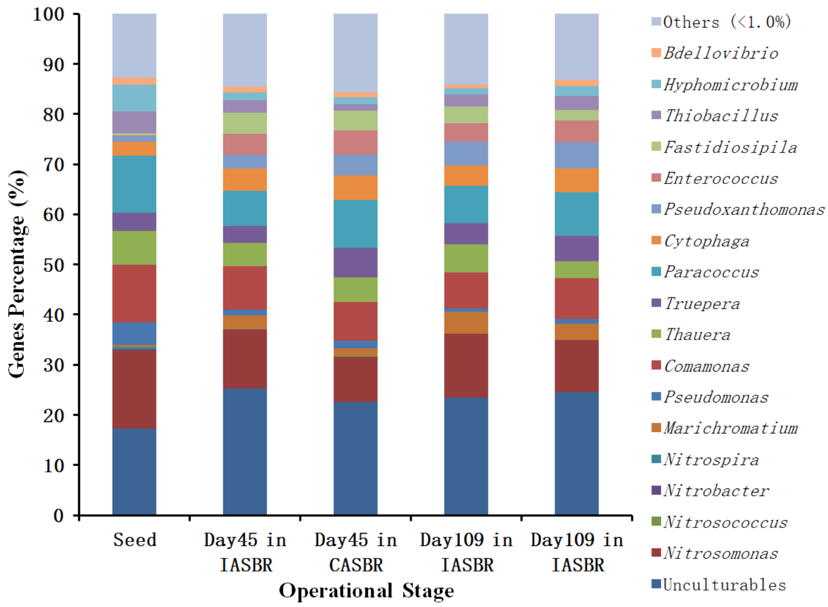

Figure 5. Microbial population analysis of partial nitrification sludge by 16S rRNA gene sequencing.

Except for unculturable bacteria, the determined microbial analysis results show that the dominant genus in the sludge samples was recognized to be capable of degrading a wide range of refractory organic pollutants; for example, *Comamonas* (7.2–11.5% of total genera), *Thauera* (3.4–6.7% of total genera), *Truepera* (3.4–5.9% of total genera), and *Pseudomonas* (0.7–4.5% of total genera), or of degrading celluloses, including *Cytophaga* (2.8–4.9% of total genera) and *Pseudoxanthomonas* (1.1–5.1% of total genera) [20–23]. *Paracoccus* (7.0–11.4% of total genera) was found to be the main denitrifier in two reactors [24]. Besides the nitrification genera (Figure 6), about 6.5–8.5% of the other genera might be species of bacteria indigenous to livestock manure, such as *Enterococcus* and *Fastidiosipila*.

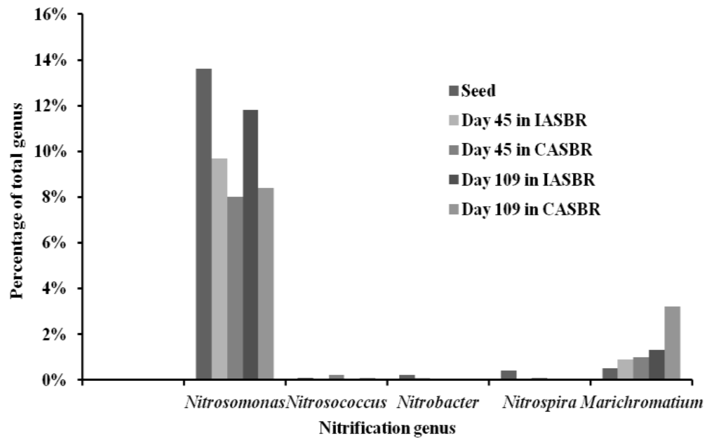

Figure 6. Microbial population analysis of nitrification genus in two SBRs.

Nitrification genus analysis (Figure 6) shows that *Nitrosomonas* was the key bacteria for NH_4^+-N oxidation in two SBRs, and was recognized as the main AOB species in IASBR and CASBR. Common NOB genera (including *Nitrobacter* and *Nitrospira*) were not detected evidently. It shows NOB was inhibited by partial nitrification conditions. However, favorable conditions for NO_2^--N oxidation actually occurred in IASBR and CASBR (Figure 2). One of the reasons might be that the concentration of common NOB genera in the SBRs was too low to be identified by 16S rRNA gene-sequencing equipment; another reason was that the special NOB species, such as *Marichromatium* which was considered as a kind of heterotrophic NO_2^--N oxidation bacteria [25], presented in two SBRs to induce the oxidization of NO_2^--N. The persistent high-COD environment in the reaction cycle was considered to be one of the reasons for the growth of *Marichromatium* bacteria.

According to Figure 6, the percentage of AOB and NOB decreased in both of IASBR and CASBR on Day 45 compared with the seed sludge. The high concentration of SS in the original livestock wastewater could dilute the density of bacteria in the reactors and inhibit the propagation of AOB and NOB in the start-up stage. In this period, the decreased pH could also affect the activity of AOB and NOB [26]. In the steady-state stage (Day 109), the quantity of *Nitrosomonas* in IASBR rapidly recovered from 9.3% to 11.8%, but only a 0.4% increase appeared in CASBR. It shows that the growth of AOB could be accelerated in the intermittent aeration condition. As for *Marichromatium*, 1.0% and 2.2% of the increase was observed in IASBR and CASBR from Day 45 to Day 109, respectively. It shows that the NOB activity recovered in the long-term livestock wastewater treatment process. However, in IASBR, due to the inhibition of the intermittent aeration strategy, the recovery of NOB was lower than that in CASBR.

3.2. Cultivation and Performance of ANAMMOX for Treating Partial Nitrification Effluents

ANAMMOX was considered as a potentially useful technology for treating NO_2^--N containing wastewater without an organic carbon source. In order to investigate the performance of NO_2^--N reduction from the effluents of the partial nitrification reactor and the effects of non-biodegradable organic matter on the activity of ANAMMOX biomass, a UASB reactor experiment was conducted.

Before introduction of IASBR effluents into the UASB, synthetic wastewater was fed to cultivate and enrich ANAMMOX bacteria. When the effluent NO_2^--N concentration in the UASB was below 15 mg/L within 15 days, the concentration of influent synthetic wastewater increased as follows: 30 mg/L NH_4^+-N and 40 mg/L NO_2^--N from Day 1 to Day 100 (Stage 1); 60 mg/L NH_4^+-N and 80 mg/L NO_2^--N from Day 101 to Day 223 (Stage 2); and 90 mg/L NH_4^+-N and 120 mg/L NO_2^--N from Day 223 to Day 289 (Stage 3). In the conventional ANAMMOX process, the theoretical ratio of consumed NO_2^--N to consumed NH_4^+-N is 1.32, and the theoretical ratio of consumed NO_3^--N to consumed NH_4^+-N is −0.26 [10]. In the cultivation period of ANAMMOX biomass, these ratios were calculated compared with the theoretical values in order to determine the ANAMMOX activity (Figure 7).

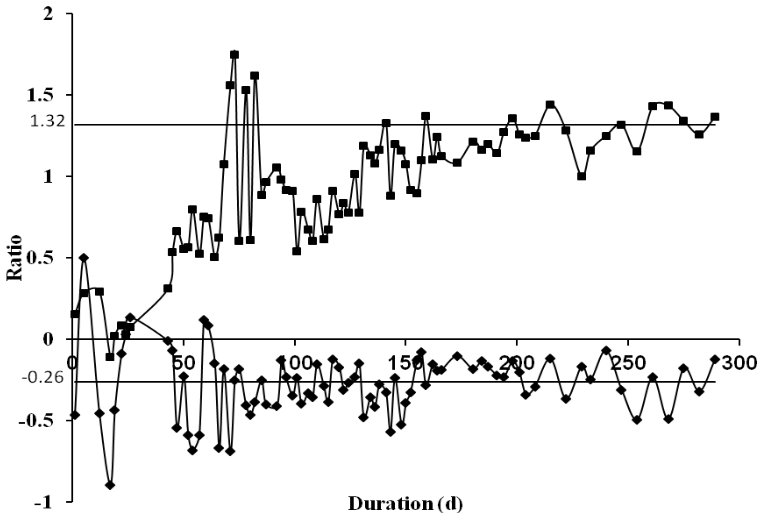

Figure 7. Performance of ANAMMOX biomass cultivation (■: ratios of consumed NO_2^--N to consumed NH_4^+-N; ♦: ratios of generated NO_3^--N to consumed NH_4^+-N).

In Stage 1, almost no NO_2^--N was removed, and NH_4^+-N was partly consumed by microbial anabolism during the start-up periods. After operation for 38 days, the phenomenon of NO_2^--N removal gradually appeared. However, it took 40–50 days for the profile of NO_2^--N to reach a pseudo-steady state. In this period, 66.2% of NO_2^--N removal was observed, and the ratio of consumed NO_2^--N to consumed NH_4^+-N reached 0.95. In Stage 2, with the increase in influent synthetic wastewater concentrations, accumulated NO_2^--N appeared again in the UASB. From Day 131, the concentration of NO_2^--N began to decrease, and satisfactory ANAMMOX performance with an average ratio (consumed NO_2^--N to consumed NH_4^+-N) of 1.24 was observed from Day 180 to Day 223. In Stage 3, no evident changes of ANAMMOX activity were obtained with higher nitrogen loading rates. This shows that a stable ANAMMOX reactor was established with an average NO_2^--N removal rate of 81.5%.

In order to investigate the effects of non-biodegradable organic matter on the activity of ANAMMOX biomass in livestock wastewater, the non-biodegradable organic matter was centrifuged from the effluent of IASBR after two days' continuous aeration in a flask with partial nitrification sludge. Influent synthetic wastewater in Stage 3 was fed into 100 mL flasks with different dosages of non-biodegradable organic matter (the initial COD concentrations were 50, 100, 150, 200, 250, 300, 350 and 400 mg/L in the flasks). Then, ANAMMOX biomass taken from the UASB was added into the flasks, and the biomass concentrations in the flasks were adjusted to close to those inside the UASB. Then, the flasks were continuously stirred with magnetic stirrers after stripping DO out for 3 min by using argon gas. Lastly, the effluents were withdrawn and influent synthetic wastewater with non-biodegradable COD was fed in again at a HRT of 8 h. After 1 day and 5 days, the effluent NH_4^+-N, NO_2^--N and NO_3^--N concentrations were measured to evaluate the effects of non-biodegradable organic matter on ANAMMOX (Figure 8).

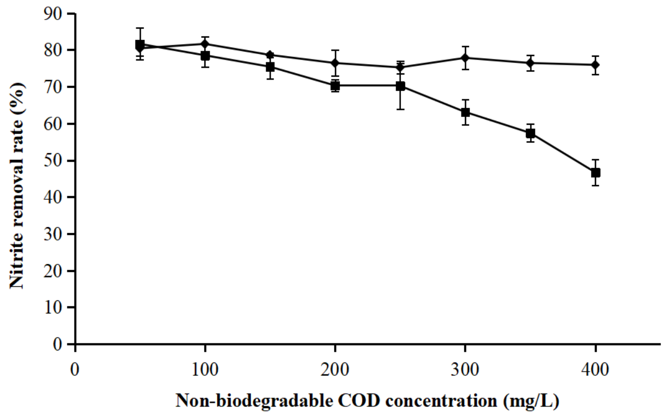

Figure 8. Influence of non-biodegradable organic matter on ANAMMOX activity (⧫: nitrite removal rate after 1 day; ■: nitrite removal rate after 5 days).

The results show that short-term exposure to non-biodegradable organic matter did not affect the activity of ANAMMOX biomass evidently. After 1 day's reaction, the NO_2^--N removal rates were all kept at above 76% under the different non-biodegradable COD concentrations. However, according to the results after 5 days' reaction, high concentrations of COD could further inhibit ANAMMOX activity with the extension of exposure reaction time. When the non-biodegradable COD concentration was more than 200 mg/L, the NO_2^--N removal rate decreased rapidly from 71.2% to 49.8%. It was found that non-biodegradable organic matter in the livestock wastewater had potential inhibition ability against ANAMMOX activity under high concentrations and long-term exposure conditions. The reason might be that the non-biodegradable organic matter, including cellulose and hemicelluloses in livestock wastewater, could disrupt the structure of the biofacies and lead to changes in extracellular polymeric substances (EPS) contents and granulation of ANAMMOX biomass [27].

The long-term performance of the treatment efficiency of the livestock wastewater after partial nitrification by using ANAMMOX technology were investigated at the different non-biodegradable COD concentration gradients. The effluents of IASBR with NH_4Cl adjustment (containing 1240 ± 45 mg/L of COD, 26 ± 3 mg/L of BOD_5, 420 ± 7 mg/L of NH_4^+-N and 552 ± 28 mg/L of NO_2^--N) were fed into the UASB after diluting 10 times from Day 1 to Day 17 (118 ± 13 mg COD/L), diluting 7.5-times from Day 18 to Day 35 (168 ± 9 mg COD/L) and diluting 5 times from Day 36 to Day 50 (242 ± 17 mg COD/L), respectively (Figure 9).

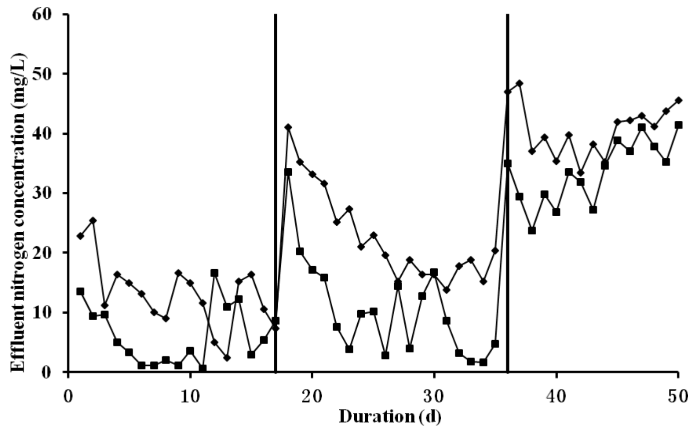

Figure 9. Profiles of ANAMMOX reactor treating the diluted effluents of IASBR (■: NH_4^+-N concentration; ⧫: NO_2^--N concentration).

In the condition of 10-times dilution, 80.1% of NO_2^--N was removed immediately after 3 days. It shows that the treatment efficiency of cultured ANAMMOX bacteria was stable under the low concentration of non-biodegradable organic matter condition. In the condition of 7.5-times dilution, with the increases in influent COD and NO_2^--N concentrations, the performance of effluent NO_2^--N in UASB became worse from Day 18. However, the resumption of ANAMMOX activity was confirmed after 4 days and an average NO_2^--N removal rate of 87.4% was achieved in this period. At this COD loading rate, non-biodegradable organic matter did not severely affect the activity of ANAMMOX. In the third stage, effluent NO_2^--N and NH_4^+-N gradually increased and the NO_2^--N removal rate decreased to 60.5%. Potential inhibition of ANAMMOX biomass appeared with the increase in COD concentrations. One of the reasons was that ANAMMOX activity became worse under high concentrations of non-biodegradable organic matter according to Figure 8; another reason might be that heterotrophic denitrifiers existed in the effluents of IASBR which could compete with ANAMMOX bacteria at a high loading rate [28]. The long-term experimental results demonstrate that when the concentration of non-biodegradable organic matter was lower than 168 $\pm$ 9 mg COD/L, the application of ANAMMOX technology on nitrogen removal from the effluents of partial nitrification treating livestock wastewater was feasible.

4. Conclusions

The performance of nitrogen removal through partial nitrification coupled with ANAMMOX technology treating livestock wastewater was studied to evaluate the influences of non-biodegradable organic matter and microbial communities in this research. The obtained results are as follows:

Partial nitrification was successfully achieved for treating the livestock wastewater. At loading rates of 3.08 kg COD/(m^3·d) and 0.93 kg NH_4^+-N/(m^3·d), COD removal efficiency was 87.6 $\pm$ 1.7% and 89.7 $\pm$ 3.4%, and average nitrite accumulation efficiencies of 78.4 $\pm$ 1.3% and 64.7 $\pm$ 3.2% were obtained in IASBR and CASBR, respectively.

The optimal alternating non-aeration duration was 20–30 min for partial nitrification in IASBR, and the intermittent aeration strategy was discovered to induce a lag reaction for NO_2^--N oxidation in a short time, but had no further effects on the inhibition of NOB activity.

Nitrosomonas was the key bacteria for NH_4^+-N oxidation in the partial nitrification process. Under the intermittent aeration strategy, the quantity of *Nitrosomonas* increased from 9.3% to 11.8%, but only a 0.4% increase appeared in CASBR. Heterotrophic *Marichromatium* was the main NO_2^--N oxidation bacteria under the high-concentration non-biodegradable organic matter condition.

Feeding the effluents of partial nitrification into the ANAMMOX reactor, non- biodegradable organic matter showed potential inhibition effects on ANAMMOX activity with the extension of exposure reaction time and the increase in COD concentrations. When the concentration of non-biodegradable organic matter was lower than 168 $\pm$ 9 mg COD/L, an average NO_2^--N removal rate of 87.4% was achieved.

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