

Article

Nitrogen Removal for Liquid-Ammonia Mercerization Wastewater via Partial Nitritation/Anammox Based on Zeolite Sequencing Batch Reactor

Lei Zheng ^{1,2}, Yongxing Chen ^{1,2}, Songwei Zhou ^{1,3}, Yuchen Chen ^{3,4}, Xingxing Wang ^{1,3},
Xiaojun Wang ^{1,2,3}, Lijuan Zhang ⁵ and Zhenguo Chen ^{1,2,3,5,*}

¹ School of Environment and Energy, South China University of Technology, Guangzhou 510006, China; 420052590@163.com (L.Z.); chen Yongxing20@foxmail.com (Y.C.); zhousongwei96@sina.com (S.Z.); 201920246099@mail.scut.edu.cn (X.W.); cexjwang@scut.edu.cn (X.W.)

² The Key Lab of Pollution Control and Ecosystem Restoration in Industry Clusters, Ministry of Education, Guangzhou 510006, China

³ Hua An Biotech Co., Ltd., Foshan 528300, China; e0572685@u.nus.edu

⁴ School of engineering, National University of Singapore, Singapore 138607, Singapore

⁵ School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou 510641, China; celjzh@scut.edu.cn

* Correspondence: chenzg14@scut.edu.cn; Tel.: +86-20-1341-6151-277

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Abstract: Liquid-ammonia mercerization is commonly used to enhance the quality of cotton fabric in the textile industry, resulting in a large amount of liquid-ammonia mercerization wastewater (LMWW) containing high concentration of ammonia to be disposed of. This study proposes a partial nitritation/anammox (PN/A) process based on stable nitritation by a zeolite sequencing batch reactor (ZSBR) for the nitrogen removal of LMWW. The ZSBR could quickly achieve stably full nitritation with a nitrite accumulation ratio higher than 97% and an ammonia removal rate of $0.86 \text{ kg N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ for the raw LMWW with an ammonia level of 1490 mg/L. In order to avoid anammox inhibition by free nitrous acid, the ZSBR was successfully changed to PN operation with diluted LMWW for effluent meeting anammox requirements. The next anammox reactor (an up-flow blanket filter (UBF)) realized a total nitrogen removal efficiency of 70.0% with a NLR (nitrogen loading rate) of $0.82 \text{ kg N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ for LMWW. High-throughput sequencing analysis results indicated that *Nitrosomonas* and *Candidatus Kuenenia* were the dominant bacteria in ZSBR and UBF, respectively. All results revealed that the PN/A process based on ZSBR as the PN pretreatment process was feasible for LMWW, facilitating cost-effective and low-carbon nitrogen removal for LMWW treatment in the textile industry in the future.

Keywords: liquid-ammonia mercerization wastewater; zeolite; SBR; nitritation; anammox

1. Introduction

Since pure cotton is well-known to have greater breathability and hygroscopicity than chemical fiber materials, clothes made from cotton are softer and more comfortable. However, these kinds of clothes always have poor wrinkle resistance and are likely to deform or shrink after long wear and numerous washes. This problem can be greatly improved by mercerizing cotton fabric [1]. Though caustic soda liquid is involved in the traditional process of mercerization, it causes damage to the cotton fiber and requires a post-pickling process, which is an extra potential pollution source. Compared to the traditional mercerization process, the new mercerized finish technological process, employing liquid-ammonia, helps to improve tactile impression, abrasive resistance, and wrinkle

resistance [2]. At present, cotton fabric processed by liquid-ammonia mercerization is known as a high-end product in the market.

The process of liquid-ammonia mercerization treatment of cotton fabric often fails to completely recycle ammonia. To handle ammonia emission in this process, sulfuric acid is adopted to absorb the emission gas. In this situation, wastewater (WW) with high $\text{NH}_4^+\text{-N}$ (1500–3000 mg/L), high SO_4^{2-} (1000–3000 mg/L), and high pH (9.0–9.3) but low alkalinity (1300–2000 mg/L, calculated as CaCO_3) and low Chemical oxygen demand (COD_{Cr}) (150–250 mg/L) is generated. For textile enterprises, in order to impact the high-end clothing market, large-scale liquid-ammonia mercerization of cotton fiber is indispensable. However, the textile industry is faced with the issue of the nitrogen pollution from liquid-ammonia mercerization wastewater (LMWW) generated in mercerization processes. Nonetheless, due to the lack of organic matter and alkalinity, resolving the nitrogen pollution in LMWW through complete nitrification and denitrification leads to high operational cost, high energy consumption, and troublesome sludge disposal. Thus, finding a resource-efficient and eco-friendly process to treat the LMWW is urgent and necessary.

Anammox was discovered in the 1980s and can be regarded as the most economical biological nitrogen removal technology, with the advantages of high efficiency, no carbon source input, and no waste sludge production [3,4]. Based on the characteristics of LMWW, anammox is very suitable for nitrogen removal from LMWW due to its low C/N ratio. The prerequisite for anammox is firstly converting a part of ammonia nitrogen in the WW to nitrite by ammonium-oxidizing bacteria (AOB). Then, anaerobic ammonia oxidizing bacteria (AnAOB) can remove both ammonia and nitrite synchronously, resulting in a small amount of nitrate produced [5]. Thus, to provide the necessary substrate for the requirement of anammox, it is indispensable to achieve partial nitritation (PN) with certain concentration of nitrite, and to avoid the final oxidation of nitrite to nitrate.

Many strategies for achieving stable PN have been reported, such as low dissolved oxygen (DO) [6], high temperature [7], free ammonia (FA), and free nitrous acid (FNA) [8,9]. For LMWW treatment, elevating the WW temperature above 25 °C [7] to achieve stable PN means additional energy consumption and increased operational cost. A reactor under oxygen-limited operational conditions can favor nitritation while limiting the growth of nitrite-oxidizing bacteria (NOB). However, low DO concentration inherently limits the AOB activity with potentially low ammonia removal rate. In a previous study, NOB was more sensitive to FA than AOB. NOB could be inhibited by FA ranging in 0.1–1 mg/L while the FA threshold concentration for AOB was 10–150 mg/L [10]. Based on the high ammonia concentration of LMWW, it is feasible to realize stable inhibition of NOB through high FA concentration suppression. Nevertheless, as the AOB converts the ammonia to nitrite, the FA level in the reactor should decrease and the repression of NOB should decrease. Zeolite, an ammonia absorbent as demonstrated by Jung et al. [11], may keep a dynamic equilibrium ammonia concentration and an appropriate FA level, which can favor a stable nitritation performance. Recently, Yang et al. [12] found that zeolite biological aeration filter (ZBAF) filling was suitable to accumulate nitrite and could yield high nitrite production when treating WW with high ammonium concentration. Nevertheless, compared to activated sludge processes, ZBAF requires higher aeration intensity because of the pressure due to the high filter biofilm layer. In addition, after long-term operation of the ZBAF, the filter is inevitably worn by the flow of water and air. Thus, in practical operation, building an LMWW treatment faculty including ZBAF requires higher equipment investment and running energy consumption. In relative terms, combining the zeolite with a sequencing batch reactor (SBR) might be more convenient and cost-effective than ZBAF due to its simple operation and required equipment. Furthermore, comparing a SBR to a zeolite sequencing batch reactor (ZSBR), Chen et al. [13] found that the ZSBR was able to bear larger nitrogen loading and could realize more resilient nitritation. Regrettably, we found no relevant studies investigating the stable nitritation on LMWW via ZSBR or evaluating whether PN/A is a feasible process to treat LMWW.

Until now, there is no satisfactory solution for economical and efficient nitrogen removal from LMWW. Hence, this study aimed to achieve the stable nitritation of LMWW through a lab-scale ZSBR

and investigate the PN performance of ZSBR. Then, the ZSBR was combined with an anammox reactor to realize further nitrogen removal for LMWW via a partial nitrification/anammox (PN/A) process. Moreover, high-throughput sequencing technology was applied to identify the microbial community variation in the ZSBR and anammox reactor. The obtained results are expected to provide theoretical guidance for the application of ZSBR in achieving stable nitrification of LMWW and further nitrogen removal via anammox in the future.

2. Materials and Methods

2.1. Reactor Set Up and Operation

A ZSBR was applied for partial nitrification in order to produce suitable feed for anammox, and conducted in a Perspex reactor with an effective volume of 9 L filled with 10 g/L zeolite powder (size: 80–200 mesh, ammonium adsorption capacity: 7.1–8.6 mg/g). An air blower supplied the oxygen to the ZSBR through an aeration stone with a constant airflow of 0.25–1.5 L/min to keep the DO higher than 4 mg/L for a sufficient oxygen supply. ZSBR was operated in a cycle of 7 h, consisting of feeding (10 min), aeration (360 min), settling (30 min), and drainage and idling (20 min). The aeration time was changed to 180 min when the ZSBR was running in PN mode.

An up-flow blanket filter (UBF) was used for the anammox reactor (working volume of 2.25 L) and a heating rod was used to maintain the temperature (30 ± 2 °C). An internal circulation line was set up to carry out the internal circulation in a ratio of 1:1. The schematic diagram is shown in Figure 1.

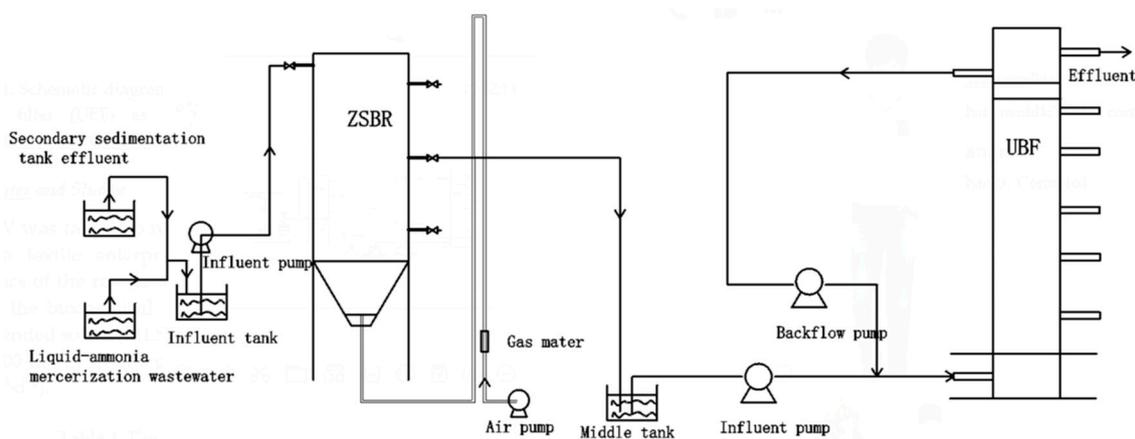


Figure 1. Schematic diagram of a zeolite sequencing batch reactor (ZSBR) combined with an up-flow blanket filter (UBF) as a partial nitrification/anammox (PN/A) process for liquid-ammonia mercerization wastewater.

2.2. Wastewater and Sludge

LMWW was taken from a liquid-ammonia mercerization workshop in the water purification center of a textile enterprise (Named as Group Y) in Guangdong province, China. The characteristics of the raw and diluted LMWW are listed in Table 1. The seed sludge of ZSBR was taken from the biochemical pool in the water purification center of Group Y, where the mixed liquor suspended solids (MLSS) was 5000 mg/L, and the UBF was inoculated with anammox sludge (MLSS = 3200 mg/L) from a pilot anammox reactor in our laboratory (nitrogen removal rate = $0.8\text{--}1.0$ kg N·m⁻³·d⁻¹).

Table 1. The characteristics of liquid-ammonia mercerizing wastewater.

Items	NH ₄ ⁺ -N (mg/L)	pH	Alkalinity (as CaCO ₃ mg/L)	COD _{Cr} (mg/L)
Raw LMWW	1500~2500	8.90~9.15	1300~2000	150~250
Diluted LMWW	300~330	7.35~7.55	750~1000	50~100

2.3. Experimental Procedure

Operational conditions for ZSBR are described in Table 2. In phase I (cycle 1~55), influent was produced from LMWW and diluted by tap water with 100~600 mg/L ammonia, and the mass ratio of alkalinity (calculated as mg CaCO₃/L) to ammonia was kept at 7:1 by adding NaHCO₃. In phase II (cycle 56~85), raw LMWW was fed to ZSBR with a ratio of alkalinity to ammonia of 3:1. In phase III (cycle 86~125), considering the actual situation of the enterprise sewage plant and the inhibition threshold of the influent base concentration on AnAOB (NO₂⁻-N < 140 mg/L) [14], LMWW diluted by the effluent from the secondary sedimentation tank, with an ammonia concentration of about 300 mg/L, was chosen as the influent of ZSBR. The UBF was fed with ZSBR effluent in phase III, and the operating strategy of the UBF was to adjust the inlet flow according to the effluent nitrogen concentration.

Table 2. Detailed operational conditions during different stages of the ZSBR.

Phase	Cycle	Airflow (L/min)	Influent Ammonium (mg NH ₄ ⁺ -N/L)	HRT (h)	Alkalinity Ratio (Alkalinity: NH ₄ ⁺ -N)	NaHCO ₃ (g/L)
I	1~55	0.25~1.50	100~600	6	7:1	1.17~7.0
II	56~85	1.20~1.50	1490	6	7:1	17.4
III	86~125	1.00~1.50	300~330	3	3:1	1.5~1.65

2.4. Analytical Methods

Ammonia (NH₄⁺-N), nitrite (NO₂⁻-N), and nitrate (NO₃⁻-N) were analyzed daily by Nessler's reagent spectrophotometer method, N-(1-naphthalene)-ethylenediamine dihydrochloride spectrophotometry, and ultraviolet spectrophotometry respectively according to the Chinese standard for the examination of water and wastewater (China, 2004) [15]. A multifunctional portable instrument (HQ30d, HACH, USA) was used to determine DO and temperature. A pH meter (PHS-3C, INESA Scientific Instrument Co. Ltd., China) was used to measure the pH value.

FA and FNA concentrations were calculated by Equations (1) and (2) [12]. The nitrite accumulation ratio (NAR), nitrite production rate (NPR), and ammonia removal rate (ARR) of ZSBR were estimated using Equations (3)–(5), respectively.

$$FA = \frac{17}{14} \times \frac{NH_4^+ - N \times 10^{pH}}{\exp[6334/(273 + T)] + 10^{pH}} \quad (1)$$

$$FNA = \frac{46}{14} \times \frac{NO_2^- - N}{\exp[-2300/(273 + T)] \times 10^{pH}} \quad (2)$$

$$NAR = \frac{NO_2^- - N_{eff}}{NO_2^- - N_{eff} + NO_3^- - N_{eff}} \times 100\% \quad (3)$$

$$NPR = \frac{NO_2^- - N_{eff} \times V - NO_2^- - N_{rest} \times (1 - R) \times V}{V \times 1000 \times t} \times 24 \quad (4)$$

$$ARR = \frac{NH_4^+ - N_{inf} \times R \times V + NH_4^+ - N_{rest} \times (1 - R) \times V - NH_4^+ - N_{eff} \times V}{V \times 1000 \times t} \quad (5)$$

where V is the effective volume of the ZSBR (L); R is the volumetric exchange ratio; t is the cycle time (h); T is the water temperature in the reactor (°C); NH₄⁺-N_{inf}, NH₄⁺-N_{rest} and NH₄⁺-N_{eff} are the influent, rest and effluent ammonium concentrations in one cycle (mg/L), respectively; NO₂⁻-N_{eff} are the influent and effluent nitrite concentrations before and after the aerobic stage in the ZSBR (mg/L), respectively; NO₂⁻-N_{rest} is the rest nitrite in the last cycle (mg/L); and NO₃⁻-N_{eff} is the nitrate concentration after the aerobic stage in the ZSBR (mg/L).

The nitrogen removal efficiency (NRE) and total nitrogen loading rate (NLR) of the UBF were calculated by Equations (6) and (7).

$$\text{NRE}_{\text{nitrogen}} = \frac{\text{Nitrogen}_{\text{inf}} - \text{Nitrogen}_{\text{eff}}}{\text{Nitrogen}_{\text{inf}}} \times 100\% \quad (6)$$

$$\text{NLR} = \frac{\text{TN}_{\text{inf}}}{\text{HRT} \times 1000} \times 24 \quad (7)$$

where the subscript “nitrogen” can be ammonia, nitrite, or TN; $\text{Nitrogen}_{\text{inf}}$ and $\text{Nitrogen}_{\text{eff}}$ are nitrogen in the influent and effluent; TN_{inf} is the total nitrogen in the influent; and HRT is the hydraulic retention time (h).

The chemical costs for PN/A and complete nitrification and denitrification were calculated according to the chemical dosage for 1 kg N removal and the price of chemical. NaHCO_3 and anhydrous glucose were used for alkalinity and carbon source supply.

2.5. DNA Extraction, PCR Amplification, and High-Throughput Sequencing Analysis

The seed sludge sample of ZSBR and UBF (SEED1, SEED2), the mature ZSBR nitritation sludge at cycle 50, and the UBF sludge at day 75 were chosen for high-throughput sequencing analysis. All biological samples were extracted for total genomic DNA using a Bacterial DNA Isolation Kit (50, D3350-01, OMEGA, USA) according to the manufacturer’s instructions.

The amplicon generation of 16S rRNA for 16S V4 region was accomplished using the primers 319F and 806R with the corresponding barcode on the Illumina Miseq PE250 platform. The PCR reaction was carried out with a total volume of 50 μL , and 30 ng genome DNA, 4 μL of PCR Prime Cocktail, and 25 μL of Phusion High-Fidelity PCR Master Mix (New England Biolabs) was used. The detailed operation of PCR included initial denaturation at 98 °C for 3 min, 30 cycles of denaturation at 72 °C for 45 s, extension at 72 °C for 7 min, and finally holding at 4 °C. The PCR products were purified with AmpureXPbeads (AGENCORT) to remove the nonspecific products.

The low-quality reads which contained more than one ambiguous base or were contaminated by adapter (>10 bp overlap) were removed in an initial quality filtering. FLASH (Fast Length Adjustment of Short reads, v1. 2. 11) [16] was used for merging the paired-end reads from original DNA fragments in order to obtain clean tags which were used for operational taxonomic unit (OTU) clustering. The sequence data were processed using the (Quantitative Insights Into Microbial Ecology) QIIME software package [17]. 16S rRNA gene sequences more than 97.0% similarity were classified into the same OTU). Annotation of the taxonomic information of each representative sequence for each OUT was performed using the RDP Classifier (version 2.2) [18]. All sequences obtained from high-throughput sequencing were compared with the Greengenes 16S rRNA gene database [12].

3. Results and Discussion

3.1. Performance of ZSBR

3.1.1. Start-Up Period

During phase I, a gradually increasing NO_2^- -N accumulation was observed (Figure 2), while the share of NO_3^- -N in the effluent declined to 10%. Based on real-time control of blower frequency and pH, Gu et al. [19] spent 40 days elevating the NAR of a pilot SBR from 19.8% to 90%. Zhang et al. [20] proposed a novel pH-DO control strategy and observed apparent NOB inhibition and the promotion of AOB after 33 days of operation, while the NAR increased to 90%. In this study, the NAR increased stepwise and reached 90.0% at the 23rd cycle, indicating that NOB inhibition in the ZSBR was successful. Compared to a conventional SBR, achieving stable nitritation in the ZSBR was significantly faster. A similar conclusion was put forward by Chen et al. [13], who conducted a parallel nitritation experiment between a ZSBR and a SBR.

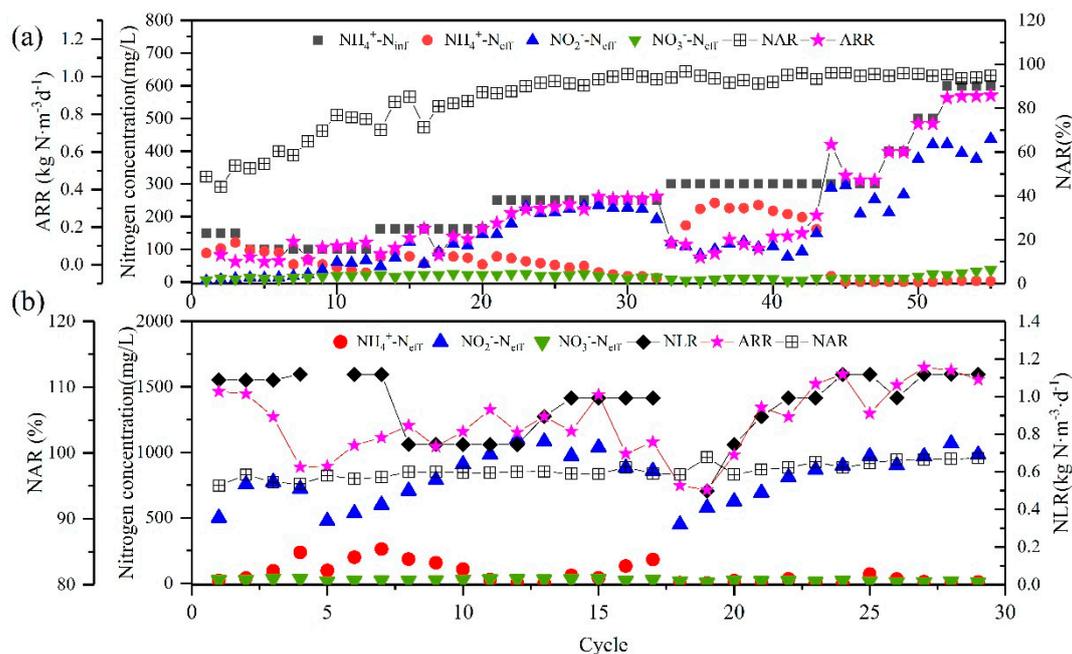


Figure 2. Operational performance of nitritation by a zeolite sequencing batch reactor during phase I and II for liquid-ammonia mercerization wastewater: (a) ammonia removal in the zeolite sequencing batch reactor during phase I; (b) ammonia removal in the zeolite sequencing batch reactor during phase II.

In Figure 2a, the increase of ARR stopped when the influent ammonia increased to 300 mg/L, while the effluent ammonia increased with decreasing ammonia removal performance by the ZSBR. For the proliferation of AOB, amplified aeration was employed in the ZSBR and the DO was increased to 4.5 mg/L. As a result, the AOB activity increased rapidly after the aeration was amplified, and increased ARR was observed with effluent NO₃⁻-N lower than 15 mg/L. NOB suppression would be easily eliminated by suddenly improving aeration for high DO. Jiang et al. [21] increased the DO to 5.0 mg/L in a nitritation SBR, and the NAR dropped rapidly to below 1% after 45 days' operation. Researchers in [22] found that high a DO concentration (2–4 mg/L) could damage the nitrite accumulation immediately since the NOB favored high DO. On the contrary, this study found that high DO (>4 mg/L) could not promote NOB activity and efficient nitrite accumulation was obtained in ZSBR.

In phase II (Figure 2b), raw LMWW with 1490 mg/L ammonia was fed to the ZSBR with a volumetric exchange ratio of 20% to 25%, leading to an NLR of 1.0~1.25 kg N·m⁻³·d⁻¹ for the ZSBR. Under such a high NLR, the ZSBR ran successfully with average NAR and ARR of 97.3% and 0.86 kg N·m⁻³·d⁻¹, respectively. Pacek et al. [23] achieved nitritation in an SBR after 23 days of operation with a low NLR (0.3 kg N·m⁻³·d⁻¹), but the nitritation was not stable and was eliminated in 180 days. Dachao et al. [20] spend 60 days to achieve stable nitritation in high-strength ammonia synthesis WW with an NLR of 0.78 kg N·m⁻³·d⁻¹ and an NAR > 95.0%. Compared to their study, achieving nitritation of LMWW via a ZSBR required only 7 days of operation and the highest ARR of the ZSBR was 1.12 kg N·m⁻³·d⁻¹. In summary, in contrast to conventional SBR, ZSBR shows visible advantages in nitrogen loading, removal efficiency, and start-up velocity.

3.1.2. Mechanism of the Rapid Nitritation in ZSBR

In order to investigate the cause of the rapid start of ZSBR nitritation and the role played by the zeolite in the ZSBR, typical nitrogen concentration variations, pH values, and FA in the 55th cycle were continuously monitored (Figure 3b). As shown in Figure 3b, the ammonia concentration at 0.25 h was less than 150 mg/L. Similarly, in Figure 3a, the concentration of FA before aeration was lower than the FA in an SBR without zeolite. This phenomenon might be explained by zeolite's ammonia absorption

ability, which was connected with the FA concentration. That is, compared to a conventional SBR, the FA concentration at the initial stage in the ZSBR was lower. In analogy, a high FA would lead to a decreased AOB activity or even complete inhibition of the AOB [24]. It could be concluded that the zeolite in the ZSBR could reduce the repression to AOB by influent ammonia.

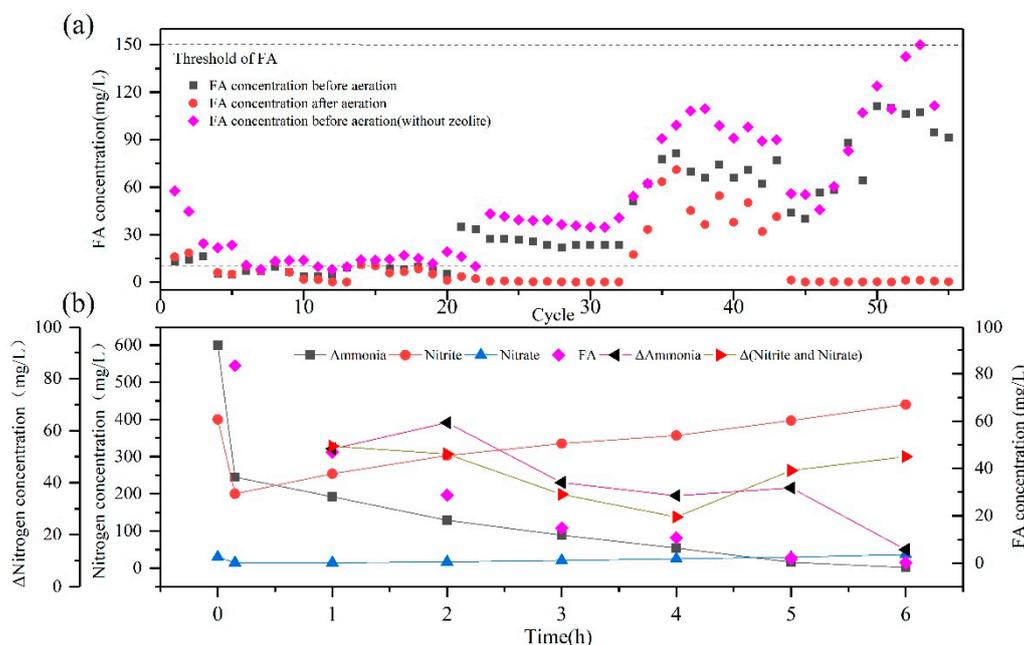


Figure 3. Free ammonia (FA) variations in zeolite sequencing batch reactor: (a) The variation of free ammonia concentration during starting period, (b) Dynamics of free ammonia and nitrogen in a typical cycle.

As shown in Figure 3b, the change of ammonia (Δ Ammonia) was higher than the change of nitrite and nitrate (Δ (nitrite and nitrate)) in the first four hours, indicating that the zeolite slowly absorbed ammonia at the beginning of the reaction. However, small amount of ammonia generally desorbed from the zeolite into the liquid phase during the last two hours, which meant zeolite could help to maintain high FA levels in the later reaction phase. The discrepancy between Δ ammonia and Δ (nitrite and nitrate) could support the conclusion that the zeolite played the role of ammonia absorption–desorption in the ZSBR.

In the first 20 cycles, the ZSBR was operated at 19~23 °C (ambient temperature) with a DO of 1.2~4.5 mg/L. Obviously, the temperature and DO were not the keys to the rapid start-up of nitrification in this study. The average FA concentration before aeration in the first 20 cycles was 8.2 mg/L, which is far beyond the inhibition threshold of NOB (0.1~1.0 mg/L). Therefore, maintaining the system at a high FA concentration via the absorption and desorption equilibrium of ammonia by zeolite was the main reason that nitrification was achieved so rapidly in the ZSBR.

3.1.3. Partial Nitrification of ZSBR

To operate the ZSBR in PN mode, the dosage of feeding alkalinity was decreased (alkalinity ratio = 3:1) and the HRT was 3 h (Table 3). Figure 4a shows that the effluent ratio of $\rho(\text{NO}_2^- \text{-N})$ to $\rho(\text{NH}_4^+ \text{-N})$ was between 1.1 and 1.4, meeting the requirement for anammox [25]. Since nitrification is a process consuming alkalinity, it is feasible to control the nitrification process by controlling the alkalinity dosage and HRT. As shown in Figure 4b, when running in nitrification mode, the NPR of the ZSBR was almost stable at $0.86 \text{ kg N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ and the average NAR was higher than 97.5%. In contrast, in phase III, the NPR was only $0.66 \text{ kg N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ for the ZSBR influent with 300 mg/L ammonia, while the PN performance was strongly stable with an effluent NAR > 99%. During start-up stage (phase I), the average FA concentration of the effluent was 11.85 mg/L, while the average FA concentration was

1.44 mg/L during PN operation. The low FA concentration might contribute less to NOB inhibition while the effluent FNA could be as high as 0.11 mg/L—higher than the FNA threshold of NOB [26]. Hence, rather than FA, FNA was the primary inhibitor of NOB in the last reaction phase.

Table 3. Effects of different influent substrate ratios on the anammox reactor.

Influent Substrate Ratio ($\text{NO}_2^- \text{-N}/\text{NH}_4^+ \text{-N}$)	NLR ($\text{kg N}/\text{m}^3/\text{day}$)	$\text{NRE}_{\text{ammonia}}$ (%)	$\text{NRE}_{\text{nitrite}}$ (%)	NRE_{TN} (%)
1.1	0.851	78.19	81.55	69.04
1.2	0.852	77.69	86.24	71.30
1.3	0.815	87.19	73.61	69.84

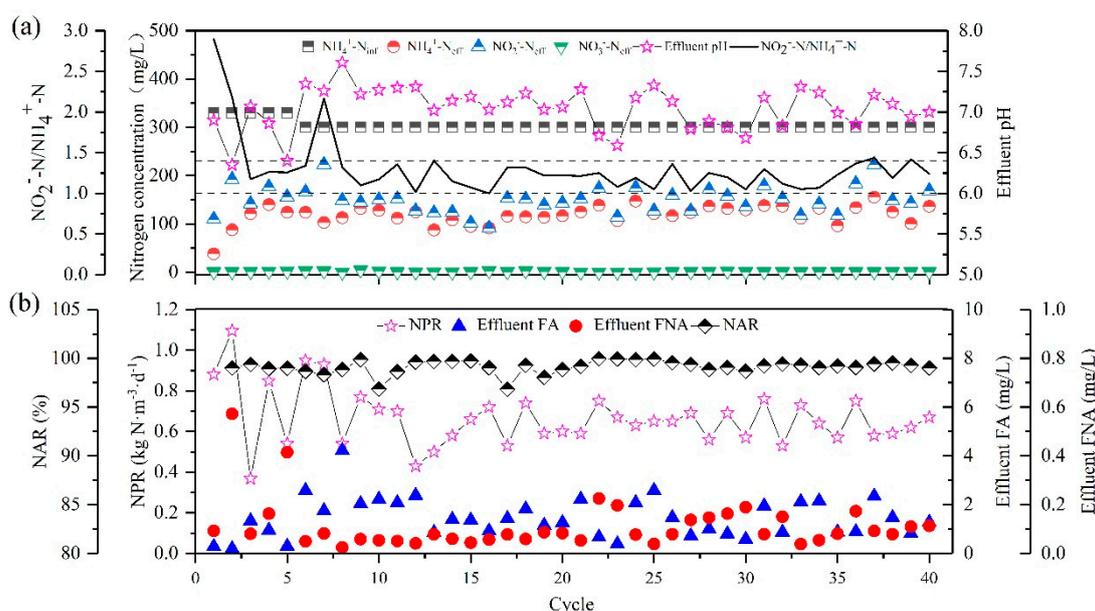


Figure 4. Operational performance of partial nitrification by the zeolite sequencing batch reactor for diluted liquid-ammonia mercerization wastewater in phase III: (a) ammonia removal performance, (b) variations of nitrite accumulation ratio (NAR), nitrite production rate (NPR), effluent free ammonia (FA) and effluent free nitrous acid (FNA).

3.2. Performance of UBF

3.2.1. Start-Up Period

As shown in Figure 5b, the NLR was $0.20 \text{ kg N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ and the total nitrogen removal efficiency was 50% in the UBF during the first 10 days. During days 14–21, the removal efficiency of nitrite and total nitrogen continuously reduced, while the removal efficiency of ammonia nitrogen remained stable. The reason for this might be that the influent ratio of $\rho(\text{NO}_2^- \text{-N})$ to $\rho(\text{NH}_4^+ \text{-N})$ was 1.35, higher than the theoretical value (1.32). Note that the influent still contained oxygen that could be utilized by the AOB to convert ammonia to nitrite. Therefore, AnAOB could be inhibited by the remaining nitrite [27]. To rescind the inhibition of nitrite, the UBF was stopped for 12 h at the 21st day and a small amount of ammonium chloride was added to feed in order to adjust the influent ratio of $\rho(\text{NO}_2^- \text{-N})$ to $\rho(\text{NH}_4^+ \text{-N})$. After relieving the nitrite inhibition at the 24th day, the anammox performance of the UBF improved gradually while the NLR constantly increased. In days 35–45, the NLR stayed at $0.82 \text{ kg N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ with the NRE_{TN} maintained at about 70.0%, and the average ammonia and nitrite of the effluent were respectively 20 mg/L and 40 mg/L (Figure 5a). From Figure 5b, it can be seen that the removal rate of nitrite rose when the influent ratio was under 1.32, while the removal rate of ammonia was stable. Thus, the nitrogen removal of UBF might be better when the inlet ratio of $\rho(\text{NO}_2^- \text{-N})$ to $\rho(\text{NH}_4^+ \text{-N})$ is slightly lower than the theoretical value of 1.32.

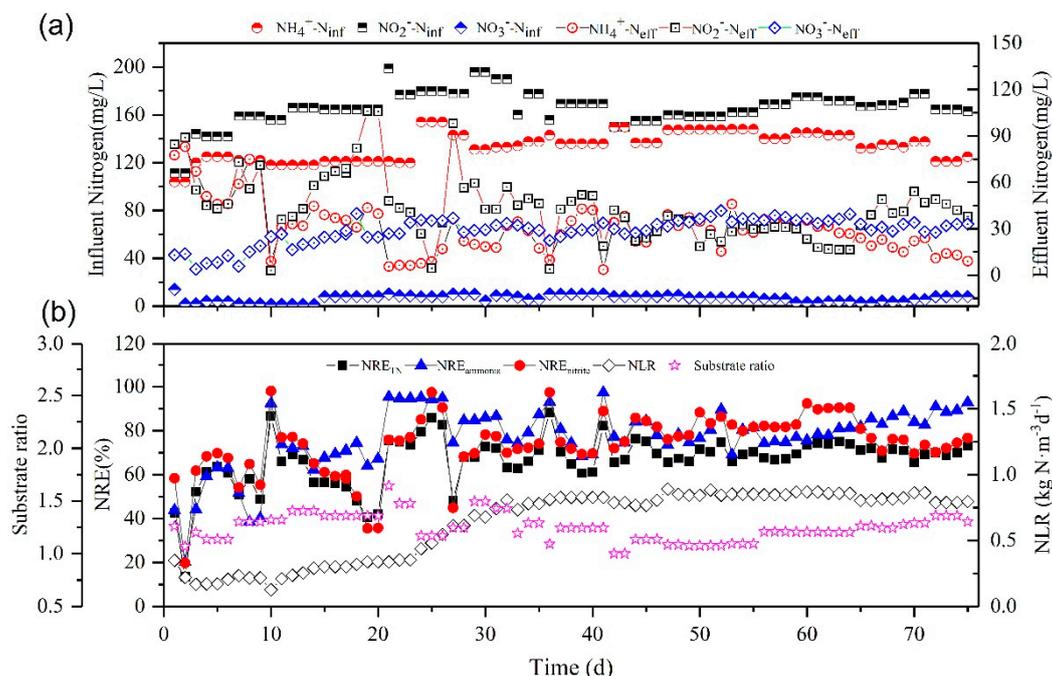


Figure 5. Performance of up-flow blanket filter and the influence of substrate ratio on anammox: (a) influent and effluent nitrogen variations, (b) variations of nitrogen removal performance and substrate ratio in anammox reactor.

3.2.2. The Influence of Influent Substrate Ratio

From Figure 5 (day 45–75) and Table 3, different influent ratios barely influenced the NRE_{TN} of the UBF. The removal rate of ammonia nitrogen increased with increasing substrate ratio. When the influent substrate ratio was 1.3, the effluent $\text{NO}_2^-\text{-N}$ concentration increased, and the average $\text{NO}_2^-\text{-N}$ removal efficiency decreased from 86.3% to 75.2%. These data indicate that there was indeed a weak nitrification in the UBF reactor. The utilized substrate ratio of the UBF was lower when the influent ratio was 1.3, which should be attributed to the higher activity of the AOB due to lower FA concentration in the first 10 days. The experimental data of 30 days were sorted out and the average values of each parameter or index were calculated (Table 3). As can be seen in Table 3, with the influent substrate ratio of 1:1.2, the $\text{NRE}_{\text{nitrite}}$, NRE_{TN} , and NLR of the UBF were higher, but the advantages in reactor performance were not obvious. Moreover, the total nitrogen removal loading was the lowest when the influent substrate ratio was 1.3. This might be a result of the inhibition of increasing nitrite concentration on the AnAOB in the reactor.

3.3. Microbial Community Analysis

High-throughput sequencing technology was used to identify the microbial community variation and functional bacteria in the ZSBR and UBF at the phylum and genus levels (Figure 6). From the seed sludge samples (SEED_1 , SEED_2), ZSBR, and UBF samples, 1436 OTUs, 235 OTUs, 898 OTUs, and 271 OTUs were collected. The main phyla in the SEED_1 sample were *Proteobacteria* and *Bacteroidetes*, among which the relative abundance of *Proteobacteria* (closely related to nitrification) increased in the ZSBR operation process, reaching 94.50% at the end. According to Wang et al. [28], *Proteobacteria* appeared to be responsible for ammonia removal. In the UBF, the microbial community at the phylum level did not change significantly while *Proteobacteria*, *Chloroflexi*, *Bacteroidetes*, and *Planctomycetes* finally stabilized at 38.44%, 25.93%, 22.06%, and 4.22%, respectively.

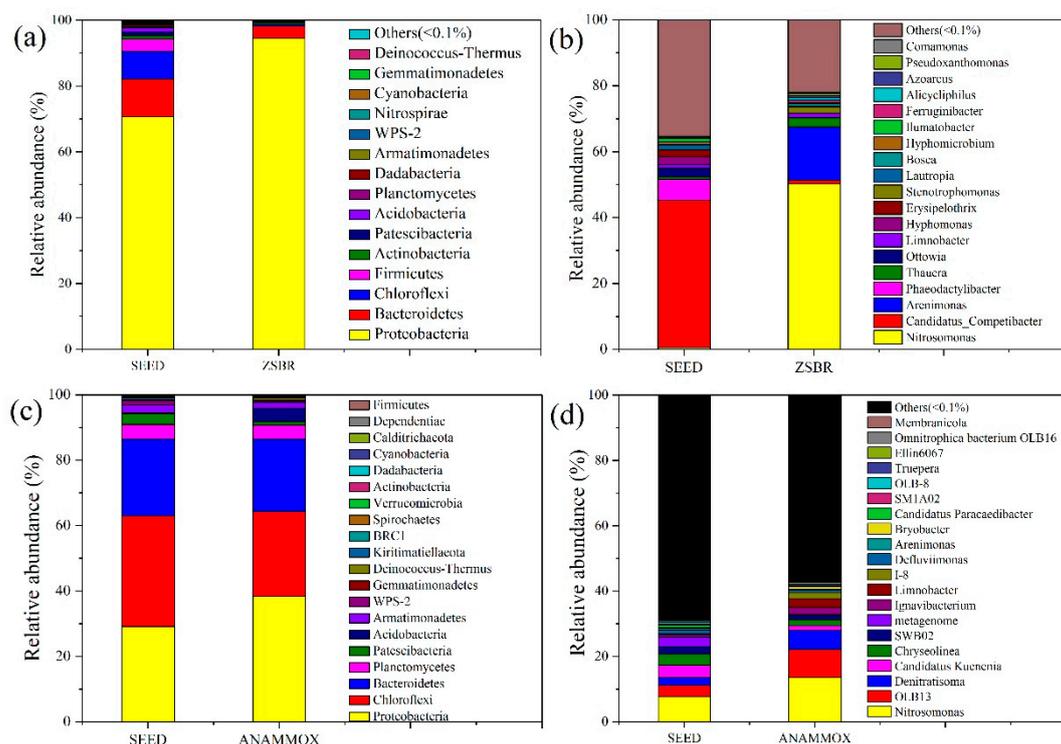


Figure 6. Taxonomic classification of the bacterial communities for SEED and 56 cycles in the zeolite sequencing batch reactor at (a) phylum level and (b) genus level; taxonomic classification of the bacterial communities for SEED and day 70 in the up-flow blanket filter at (c) phylum level and (d) genus level.

Based on the genus-level analysis results, the inoculated sludge of the ZSBR and the nitrification sludge varied tremendously. Before the ZSBR operation, 890 genera were detected (SEED) while 725 genera were detected from the ZSBR sample. In SEED₁, the relative abundance of community compositions relative to nitrifying bacteria was only 0.78%, with the *Nitrosomonas* (AOB) accounting for 0.54%. However, *Nitrosomonas* became the most abundant genus, whose relative abundance was 54.78% while the relative abundance of *Nitrospira* and *Nitrolancea* belonging to the NOB dropped from 0.24% to 0.06% in the ZSBR. This demonstrated the main functional bacteria for the stable nitrification process in the ZSBR.

The main genera in the UBF (anammox) were *Nitrosomonas* (13.57%), *OLB13* (8.57%), *Denitratisoma* (5.91%), *Limnobacter* (2.64%), *Ignavibacterium* (2.04%), I-8 (2.08%), and *Candidatus Kuenenia* (1.42%). The only anammox genus detected was *Candidatus Kuenenia*. *Limnobacter* is a heterotrophic bacterium belonging to the *Proteobacteria*, and its relative abundance increased from 0.13% to 2.64% and was associated with the presence of small amounts of organic matter in the influent. The proliferation of *Nitrosomonas* also confirmed the hypothesis that there was a nitrification process occurring in the UBF reactor. Remarkably, there was a strong positive interaction between *Candidatus Kuenenia*, *OLB13*, and *Denitratisoma* [29]. *Denitratisoma* has been reported in a partial denitrification-anammox (PDN/A) system [30], and there might be a PDN/A system in the UBF for nitrogen removal.

3.4. Significance of ZSBR Coupling with Anammox for LMWW

This study proposes a PN/A process based on stable PN performance by a ZSBR for the removal of nitrogen from LMWW. Compared to a conventional SBR, the ZSBR shows advantages such as faster start-up, high NLR, and stability, which suggests that ZSBR is a more promising process for practical engineering of PN/A treatment of LMWW. At present, to our knowledge, complete nitrification and denitrification is commonly applied for LMWW treatment, which consumes more energy for aeration as well as more carbon, and leads to frequent sludge disposal (mainly generated from denitrification). As listed in Table 4, compared to the complete nitrification and denitrification process, a PN/A process

based on a ZSBR could save about 0.668 dollars/kg N [31], showing a high potential to reduce the nitrogen removal operation cost for LMWW. In the factory of group Y, there is about 100 m³ LMWW that needs to be treated every day. However, this WW is currently disposed of by complete nitrification and denitrification. Due to the high daily operational cost for LMWW, group Y intends to find a more cost-effective nitrogen removal process. Besides, since sulfate is high in LMWW, the denitrification unit always produces a smelly gas in the factory, generated by sulfate reduction bacteria under anaerobic conditions. Based on the results of this study, a PN/A process consisting of a ZSBR and anammox could be a solution for the situation faced by group Y, and could save about 134 dollars (the average ammonia concentration in LMWW is 2000 mg/L) from the reagent costs of the current complete nitrification and denitrification process. That is to say, the problem of LMWW pollution restricting the promotion of liquid-ammonia mercerization in the textile industry can be addressed via a mature PN/A process based on a ZSBR.

Table 4. Comparison of reagent costs between the process proposed in this study and complete nitrification and denitrification.

Process		ZSBR–UBF		Complete Nitrification–Denitrification	
		ZSBR	UBF	Complete Nitrification ³	Denitrification ⁴
Dosage (kg/kg N)	Alkalinity ¹	1.50	0	1.90	0
	Glucose ²	0	0	0	0.90
Cost (dollars/kg N)	Alkalinity	0.543	0	0.689	0
	Glucose	0	0	0	0.522
Total (dollars/kg N)		0.543		1.211	

¹: Dilute LMWW was picked (NH₄⁺-N = 300 mg/L) and NaHCO₃ was chosen for comparison. The price of NaHCO₃ was about 0.362 dollars/kg. ²: Glucose was chosen for the denitrification carbon source for comparison. The price of glucose was about 0.580 dollars/kg. ³: Complete nitrification of 1 kg NH₄⁺-N consumes 7.14 kg alkalinity theoretically. ⁴: Denitrification of 1 kg TN consumes 3 kg glucose theoretically.

4. Conclusions

Due to FA inhibition, stable nitrification for LMWW could be performed in a ZSBR with an average NAR of 97.3% and an ARR of 0.86 kg N·m⁻³·d⁻¹, even though the influent ammonia concentration was 1490 mg/L. In order to prepare a suitable influent for the further anammox reactor and avoid FNA inhibition of anammox bacteria, the ZSBR was changed to run in PN mode with diluted LMWW, which realized efficient PN performance with a stable NPR of 0.66 kg N·m⁻³·d⁻¹ and an average NAR higher than 97.5%. Anammox was successfully applied for the further nitrogen removal after PN treatment, and the most appropriate substrate ratio was 1.2. High-throughput sequencing indicates that the AOB was dominant in ZSBR and *Candidatus Kuenenia* was the prime AnAOB in the UBF. This PN/A process based on a ZSBR could be an economical and efficient way to resolve the nitrogen pollution of LMWW, which could promote LMWW treatment technology and promote the development of the textile industry in the future.

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Abbreviations

AnAOB	anaerobic ammonia oxidizing bacteria
AOB	ammonia oxidizing bacteria
ARR	ammonia removal rate
DO	dissolved oxygen
FA	free ammonia
FNA	free nitrous acid
LMWW	liquid-ammonia mercerization wastewater
MLSS	mixed liquid suspended solids
NAR	nitrite accumulation ratio
NLR	nitrogen loading rate
NOB	nitrite oxidizing bacteria
NPR	nitrite production rate
NRE	nitrogen removal efficiency
NRR	nitrogen removal rate
OTU	operational taxonomic unit
PN	partial nitrification
PN/A	partial nitrification/anammox
TN	total nitrogen
UBF	up-flow blanket filter
WW	wastewater
ZBAF	zeolite biological aerated filter
ZSBR	zeolite sequencing batch reactor

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