A novel two-stage partial-nitritation/anammox (PN/A) process based on zeolite biological fixed bed reactor for low-strength ammonium wastewater treatment

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Abstract. A novel two-stage partial nitritation/anammox (PN/A) process coupled by a zeolite biological fixed bed reactor (ZBFB) and an anammox reactor were proposed for wastewater containing 30 mg/L NH4+-N by long-term operation. The cycle operational results by adsorption and biological desorption in ZBFB showed adsorption effluent NH4+-N maintained at 3.0-4.0 mg/L and the average biological desorption effluent NO2--N was 42.2 mg/L. In ZBFB, free ammonia inhibition on nitrite oxidizing bacteria was the main reason for stable nitrite accumulation performance with nitrite accumulation ratio as 88.70% during biological desorption step. Total nitrogen in the mixture of influent and biological desorption effluent of ZBFB could be removed to less than 15 mg/L by the subsequent anammox reactor. High-throughput sequencing analysis results presented the enrichment of Nitrosomonas and inhibition of Nitrobactor and Nitrospira in ZBFB, and dominance of Candidatus Kuenenia in anammox reactor. All results revealed desirable feasibility for nitrogen removal from low-strength ammonium wastewater by ZBFB combined with anammox reactor.

1. Introduction
At present, biological nitrogen removal (BNR) processes via nitrite pathway, such as shortcut nitrification-denitrification (SND) and partial-nitritation and anammox (PN/A) process, have become a hot issue due to the economic and energy-saving advantages[1]. Since anaerobic ammonium oxidizing bacteria (AnAOB) can directly oxidize ammonium (NH4+-N) to nitrogen gas (N2) with nitrite (NO2--N) as an electron acceptor without exogenous carbon source[2], PN/A process showed obvious economic and energy-saving superiority in treating ammonium wastewater. The PN/A process can be achieved by either one-stage reactor or two-stage reactor[3]. For low-strength ammonium wastewater treatment, one-stage PN/A process is prone to many problems, such as excess nitrate accumulation, unstable nitrite production and nitrogen removal performance. Miao et al.[4] also discovered that effluent nitrate (NO3--N) increased continuously accompanying with a significant decrease of TNRE from 58.80% to 14.70% in one-stage PN/A process treating low-strength sewage. These results indicated that nitrite...
oxidizing bacteria (NOB) activity was not effectively inhibited in many one-stage PN/A reactors. Compared with one-stage PN/A process, two-stage PN/A process could be operated under optimized control conditions. The reason might be that ammonium oxidizing bacteria (AOB) and AnAOB were cultivated in separated reactor, which was beneficial to the stability of PN and anammox through the reduction of negative impact of DO on AnAOB. Wu et al. indicated that the start-up performance of two-stage PN/A process system (TN\textsubscript{RE}=86.00-92.00%) was higher and more stable than that of one-stage PN/A process system (TN\textsubscript{RE}=81.00-87.00%). Based on these results, two-stage PN/A process should be preferred for treating low-strength ammonium wastewater. Nevertheless, the combined full nitritation with anammox process was rarely reported, especially for low-strength ammonium wastewater.

For low-strength ammonium wastewater treatment, PN process is critical for successful application of two-stage PN/A process. Thus, the oxidation of nitrite must be restricted by accumulation of AOB and wash-out of NOB in reactors. To achieve stable accumulation, strategies such as low DO, intermittent aeration, free ammonia (FA) inhibition, high temperature control were reported. However, low DO might result in a low ammonium removal rate as well as low biomass yield. For intermittent aeration, AOB would negatively affected by short aeration duration due to frequent changes under aerobic and anoxic condition, resulting in the growth of heterotrophic bacteria in reactor. FA inhibition was always proved available for stable nitrite accumulation from high-strength ammonium wastewater, while few focused on low-strength ammonium wastewater due to the rapid decrease of ammonium and loss of NOB inhibition during aeration phase for long-term operation. Thus, it is necessary to find a new way to achieve stable nitrite accumulation for low-strength ammonium wastewater.

In recent years, zeolite was widely used as a superior adsorbent for ammonium and served as an excellent biofilm carrier facilitating enrichment of AOB. The equilibrium of ammonium adsorption between zeolite and liquid phase could offer an appropriate FA range for inhibition on NOB instead of AOB by maintaining a favorable NH\textsubscript{4}\textsuperscript{+}-N concentration in reactor. In previous study, Chen et al. verified that zeolite biological fixed bed reactor (ZBFB) was proved available to achieve PN with influent NH\textsubscript{4}\textsuperscript{+}-N of 50 mg/L via cycles of adsorption and biological desorption under 36.0 °C, showing promising advantages of fast star-up, easy control in promoting nitrite accumulation for low-strength ammonium wastewater. Based on these, this study supposed that ZBFB should be available in a two-stage PN/A process system for low-strength ammonium wastewater treatment. In this proposed process, full nitritation for low-strength ammonium wastewater could be achieved more stable and easier. Therefore, a novel two-stage PN/A process based on ZBFB could be developed, which extended the application of novel biotechnology in nitrogen removal for low-strength ammonium wastewater treatment.

This study aimed to demonstrate the feasibility of using two-stage PN/A process to treat low-strength ammonium wastewater by a ZBFB combined anammox reactor. Different low-strength ammonium wastewater was firstly fed into parallel ZBFB reactors with nitritation performance test to determine the feasible ammonium concentration of influent. Then long-term operation of ZBFB reactor for the feasible lowest ammonium concentration wastewater was carried out for further evaluation of stability of nitrite accumulation. Then, nitrite accumulated effluent from ZBFB was mixed with the original influent of ZBFB to feed the subsequent anammox reactor for the next nitrogen removal. In addition, the potential mechanisms were further discussed and verified by analyzing the microbial community structure and functional bacteria variations under different operational stages.

2. Materials and methods

2.1. Reactors

Three parallel ZBFB reactors were made by polymethyl methacrylate columns (φ=70 mm) filled with 1.0 L zeolite biofilm. The zeolite biofilm was picked from a zeolite biological aerated filter (Height:
2.0 m, inner diameter: 10.0 cm, zeolite particle diameter: 1.0-2.0 mm, up-flow velocity: 2.0 m/h) continuously treating wastewater containing 10 mg/L NH₄⁺-N with effluent NH₄⁺-N, NO₂⁻-N and NO₃⁻-N of ZBAF was 0.1 mg/L, 0.8 mg/L and 9.1 mg/L, respectively. A perspex column (φ=60 mm, H=700 mm) with working volume of 1.0 L was inoculated by anammox sludge as the anammox reactor for further nitrogen removal from mixture of synthetic low-strength ammonium wastewater (NH₄⁺-N=30 mg/L) and biological desorption effluent from ZBFB. (Figure 1). The mixture was pumped into the bottom of the anammox reactor and the effluent came out at the top. The anammox reactor was started up with mature anammox sludge (Mixed Liquor Suspended Solid = 3000 mg/L) from a plug-flow reactor (50 L) in our lab. The specific activity of the anammox sludge was 0.287 g N/g VSS/day. Sponge was set at the outlet to avoid sludge loss and no sludge discharge was employed during the whole study. Nitrogen loading rate (NLR) was controlled by adjusting the influent nitrogen concentration and hydraulic retention time (HRT). Temperature was kept at 30.0 ± 1.0 °C by heater.

Figure 1 The schematic diagram of ZBFB combined with ANAMMOX for low strength ammonium wastewater: 1. Influent tank (NH₄⁺-N=30 mg/L); 2. Influent/circulating pump; 3. Zeolite biological fixed bed (ZBFB); 4. Adsorption effluent (AE) tank; 5. Desorption effluent (DE) tank; 6. Air pump; 7. Anammox influent tank; 8. Anammox influent pump 2; 9. Anammox reactor.

2.2. Wastwater and and chemical reagent
Low-strength ammonium wastewater used in this study was made by tap water and NH₄Cl, with certain ammonium-nitrogen (NH₄⁺-N) concentration (15, 30 and 50 mg/L) as needed. In the biological desorption step, Na₂CO₃ was offered as alkalinity and other necessary elements were supplied with NaH₂PO₄, 10 mg/L; CaCl₂ꞏ2H₂O, 5.6 mg/L; MgSO₄ꞏ7H₂O, 300 mg/L; FeSO₄ꞏ7H₂O, 24.0 mg/L; and 1.0 mg/L trace element solution[18]. All reagents used in this study were AR grade with purity higher than 99.70%.

2.3. Experimental procedures
The ZBFB reactors (named as ZBFB1, ZBFB2, ZBFB3) were applied for adsorption with synthetic low-strength ammonium wastewater (NH₄⁺-N = 15, 30 and 50 mg/L) by inflow rate of 1.0 L/h for 8, 16 and 24 h, resulting in adsorption effluent NH₄⁺-N as 4.23, 4.11 and 3.94 mg/L, respectively. Then ZBFBS were operated by cycle of adsorption and biological desorption for different synthetic low-strength ammonium wastewater (NH₄⁺-N = 15, 30 and 50 mg/L), respectively. The adsorption temperature was set at ambient temperature without any control and biological desorption operational temperature was controlled by water bath as 36.0 °C. For each cycle, low-strength ammonium wastewater was pumped into the bottom of ZBFBS for adsorption with inflow rate of 2.0 L/h for 1 h (ZBFB1 and ZBFB2), and for 3 h (ZBFB3), respectively. Then ZBFBS were shifted to biological desorption for 20 h with aeration of 300 mL/min (DO: 5.0-6.0 mg/L), inner reflux of 3.0 L/h, Na₂CO₃ addition (1 g/L for ZBFB1 and ZBFB2, 2g/L for ZBFB3) and other necessary elements. All ZBFBS were carried out for 15 cycles in this study to compare the nitrite accumulation performance. The
ZBFB2 was applied for long-term cycle operation for synthetic low-strength ammonium wastewater (NH$_4^+$-N=30 mg/L). The operational parameters were listed in Table 1. Biological desorption effluent from each cycle was stored in the biological effluent tank for the feed of the anammox reactor. The anammox reactor was carried out for 97 days divided into 3 phases by controlling different influent nitrogen concentration and NLR (Table 2). The feed for anammox reactor was made manually in phase I and II, and then changed to the mixture by low-strength ammonium influent and biological desorption effluent from ZBFB2 operation.

| Table 1 Operational cycles of ZBFB2 in this study |
|-----------------------------------------------|
| **Cycle** | **Adsorption** | **Biological desorption** |
|           | Q (L/h) | t (h) | Na$_2$CO$_3$ (g/L) | Aeration (mL/min) | Temp. (°C) | DO (mg/L) | Reflux (L/h) | HRT (h) |
| 1-102     | 2.0     | 1.0   | 1.0               | 300              | 36.0    | 5.0-6.0  | 3.0        | 12.0   |

| Table 2 Experimental scheme of Anammox reactor for nitrogen removal |
|-----------------------------------------------|
| **Phase** | **Cycle** | **Influent NH$_4^+$-N (mg/L)** | **Influent NO$_2^-$-N (mg/L)** | **NLR (kg N·m$^{-3}$·day$^{-1}$)** |
|           |           |                               |                               |                                   |
| I         | 1-3       | 61.4 ± 2.4                    | 65.2 ± 0.9                     | 0.256 ± 0.007                     |
|           | 4-13      | 53.7 ± 4.9                    | 68.3 ± 6.5                     | 0.403 ± 0.069                     |
|           | 14-34     | 51.4 ± 3.6                    | 67.0 ± 7.2                     | 0.572 ± 0.043                     |
| II        | 35-41     | 14.8 ± 0.7                    | 20.1 ± 1.2                     | 0.661 ± 0.021                     |
|           | 42-53     | 15.3 ± 0.5                    | 20.9 ± 2.5                     | 0.462 ± 0.029                     |
| III       | 54-72     | 15.4 ± 0.5                    | 21.0 ± 2.1                     | 0.312 ± 0.018                     |
|           | 73-82     | 15.6 ± 0.3                    | 21.4 ± 1.7                     | 0.472 ± 0.024                     |
|           | 83-97     | 15.6 ± 0.2                    | 21.3 ± 1.3                     | 0.318 ± 0.011                     |

2.4. Chemical analysis and calculations  
During the whole experiment, ammonium-nitrogen (NH$_4^+$-N), nitrite-nitrogen (NO$_2^-$-N), nitrate-nitrogen (NO$_3^-$-N) were detected by standard methods, respectively$^{[19]}$. Total nitrogen (TN) was calculated by the sum of NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N. The temperature and DO were both measured by a digital DO meter (HQ30d, HACH, USA) and pH of every sample was measured by a pH meter (PHS-3C, INESA Scientific Instrument Co. Ltd, China). The nitrite accumulation ratio (NAR), the nitrite production rate (NPR), the nitrogen loading rate (NLR), the nitrogen removal rate (NRR), the total nitrogen removal efficiency (TN$_{RE}$), and FA concentration was calculated as followed, respectively.

NAR(%) = \frac{\text{NO}_2^- - \text{N}}{\text{NO}_2^- - \text{N} + \text{NO}_3^- - \text{N}} \times 100%  

\text{NPR}(\text{kgN} \cdot \text{m}^{-3} \cdot \text{day}^{-1}) = \frac{\text{NO}_2^- - \text{N} \times 24}{t \times 1000}  

\text{NLR}(\text{kgN} \cdot \text{m}^{-3} \cdot \text{day}^{-1}) = \frac{\text{TN}_N - \text{TN}_N}{t \times 1000} \times 24  

\text{NRR}(\text{kgN} \cdot \text{m}^{-3} \cdot \text{day}^{-1}) = \frac{\text{TN}_N - \text{TN}_N}{t \times 1000} \times 24  

\text{TN}_{RE}(\%) = \frac{\text{TN}_N - \text{TN}_N}{\text{TN}_N} \times 100\%  

\text{FA}(\text{mg/L}) = \frac{17}{14} \times \frac{\text{NH}_4^- - \text{N} \times 10^{14}}{6334 \exp \left( \frac{6334}{273 + T} \right) + 10^{14}}  

where t was the biological desorption operational time (h) and T was the Celsius temperature (°C).
3. Results and discussion

3.1. ZBFB performance for different influent ammonium wastewater

In order to investigate the feasibility of ZBFB for nitrite accumulation from the low-strength ammonium wastewater, synthetic wastewater containing 15, 30 and 50 mg/L NH$_4^+$-N was disposed by parallel ZBFB reactor (ZBFB1, ZBFB2, ZBFB3), respectively. Figure 2 showed the comparison of biological desorption results for different low-strength ammonium wastewater. It was observed that nitrite accumulation was not sufficient in ZBFB1, with NAR remaining at the range of 47.40-60.60% and highest NO$_2^-$-N concentration as 15.8 ± 5.6 mg/L in the desorption effluent. For ZBFB2, the desorption effluent NO$_2^-$-N presented in an obvious rising trend within 12 h, resulting in an average NPR of 0.086 kg Nꞏm$^{-3}$ꞏd$^{-1}$. Subsequently, the desorption effluent NO$_2^-$-N slightly increased from 43.3 ± 1.8 mg/L at 12 h to 45.7 ± 3.0 mg/L at 16 h and then descended after 16 h. Meanwhile, desorption effluent NO$_3^-$-N kept rising, leading to continuous decrease of NAR during the whole biological desorption process in ZBFB2. As the influent NH$_4^+$-N increased to 50 mg/L, NO$_2^-$-N kept increasing apparently to as high as 175.2 ± 7.6 mg/L at 20 h while NO$_3^-$-N stayed in a relative low level from 3.35 ± 0.80 to 18.1 ± 5.78 mg/L in the desorption effluent of ZBFB3. The NAR maintained higher than 90.70% in the desorption effluent from ZBFB3, which was in accord with the previous study that excellent nitrite accumulation performance could be achieved by adsorption and biological desorption of ZBFB for ammonium wastewater (NH$_4^+$-N=50 mg/L)[7]. Generally, nitrite accumulation only occurred under the condition that seletive suppression on NOB rather than AOB. Since DO levels were 5.0-6.0 mg/L in this study, both of AOB and NOB could gain enough oxygen for their growth[20]. Therefore, DO levels should not be the reason for nitrite accumulation in this study. According to the results by Chen et al.[11], persistently available FA inhibition on NOB under 36 °C was the key for nitrite accumulation during desorption in ZBFB for low-strength ammonium wastewater. In literatures, FA suppression levels on NOB were first reported to be 0.1-1.0 mg/L[21], and researchers further found that the inhibition concentration of FA on NOB should be higher than 0.62 mg/L[22]. However, other researchers also found that increasing AOB activity, instead of FA inhibition on NOB, should be the key for achieving nitritation in low ammonium wastewater. In fact, high temperature utilized in this study facilitated AOB growth compared to NOB[11] and could also enhanced FA levels in ZBFB. The FA concentrations hardly kept higher than 0.62 mg/L (except for the first 4 h) in ZBFB1, accompanied by an inefficient nitrite accumulation. Although the desorption effluent NO$_3^-$-N also gradually increased in ZBFB2, 43.3 ± 1.8 mg/L NO$_2^-$-N could be obtained and the NAR was still as
high as 86.60 ± 1.60% along with FA concentration as 0.76 ± 0.10 mg/L at 12 h. In addition, FA concentrations in ZBFB3 during the whole biological desorption maintained higher than 1.64 ± 0.53 mg/L, resulting in a continuously desired nitrite production. These results further revealed building beneficial condition for AOB cultivation and effective FA level for NOB inhibition was of great importance for the nitrite accumulation for low-strength ammonium wastewater by ZBFB. Moreover, ZBFB was suitable for nitrite accumulation from wastewater containing 30 mg/L NH₄⁺-N by controlling biological desorption operational time.

3.2. Cycle operations for stable nitrite accumulation by ZBFB

In this study, in order to further investigate the stability and high-efficiency of ZBFB for nitrite accumulation in low-strength ammonium wastewater, the ZBFB2 was operated by cycles of adsorption and biological desorption for synthetic wastewater containing 30 mg/L NH₄⁺-N. In the ZBFB reactor, low-strength ammonium was firstly absorbed by zeolite, then the absorbed ammonium was desorbed into the liquid phase and converted to nitrite by biofilm during biological desorption, which could guarantee enough adsorption capacity of zeolite for the next adsorption. As shown in Figure 3, the adsorption effluent NH₄⁺-N could be stably lower than 5.0 mg/L during the whole experiment. This result indicated that the adsorption performance after biological desorption was hardly affected by biofilm covered on zeolite fillings and ammonium adsorption capacity could be recovered to an appropriate level for the next adsorption[7]. For biological desorption, NO₂⁻-N in effluent stably maintained in the range of 37.5-49.6 mg/L, with an average NAR of 88.70% while effluent NH₄⁺-N and NO₃⁻-N stayed less than 2 mg/L and 10 mg/L, respectively. These results showed an extremely stable and high-efficient cycle operation performance by ZBFB. It should be also noted that the FA concentrations of desorption effluent maintained at the range of 0.197-0.836 mg/L, contributing to the persistent and effective inhibition of NOB. All findings mentioned above demonstrated that after 102 cycle operations, stable nitrite accumulation could be obtained in the treatment of synthetic wastewater containing 30 mg/L NH₄⁺-N in ZBFB at 36 ℃. The efficient nitrite accumulation performance should be ascribed to the preponderant growth rate of AOB than NOB, faster ammonium desorption from zeolite fillings, which enhanced the FA levels for persistently inhibition of NOB under a high temperature during the biological desorption step[11].

3.3. Nitrogen removal performance by Anammox

In order to further estimate the feasibility of economical and energy-saving treatment for low-strength ammonium wastewater, the anammox reactor was carried out for 97 days dividing into 3 phases. Figure 4 elucidated nitrogen removal performance by the anammox reactor during the entire experiment. In
phase I (day 1-34), the anammox reactor was first fed with synthetic wastewater containing about 50 mg/L NH₄⁺-N and 65 mg/L NO₂⁻-N, respectively. As hydraulic retention time (HRT) gradually shortened from 12 h to 5 h, NLR increased from 0.253 kg N m⁻³ d⁻¹ to 0.575 kg N m⁻³ d⁻¹. Meanwhile, the effluent TN displayed an obvious downward trend, accompanying with an average NRR of 0.374 kg N m⁻³ d⁻¹ and TNRE of 75.77%. These results demonstrated that desirable nitrogen removal performance could be obtained under the condition of influent NH₄⁺-N to NO₂⁻-N ratio of about 1:1.32, an appropriate mole rate for anammox reaction. After the feeding of 15 mg/L NH₄⁺-N and 20 mg/L NO₂⁻-N made manually in phase II (day 35-53), the anammox reactor was then fed with the mixture of low-strength ammonium influent and biological desorption effluent from ZBFB2 operation at a ratio of 1:1 in phase III (day 54-97). As shown in Fig. 4, with HRT of 3 h, both the effluent NH₄⁺-N and the NO₂⁻-N could decreased to 1.0-5.0 mg/L and TN could stably kept less than 15 mg/L in this phase. An average NRR of 0.214 kg N m⁻³ d⁻¹ and TNRE of 67.85% was obtained, which showed great feasibility for anammox in treating this mixed low-strength wastewater. Meanwhile, it should be noted that on days 73-82, HRT was shortened to 2 h with an NLR increase from 0.312 ± 0.018 up to 0.472 ± 0.024 kg N m⁻³ d⁻¹. Although the anammox reactor achieved an higher NRR of 0.271 kg N m⁻³ d⁻¹, the average TNRE decreased from 67.85% to 57.50% with effluent TN higher than 15 mg/L. Under this circumstance, both the average NH₄⁺-N and NO₂⁻-N concentrations in the effluent rose to 5.33 and 6.60 mg/L, respectively. These results might be responsible to the limited activity of AnAOB at a shorter HRT, leading to relatively less NH₄⁺-N oxidation and NO₂⁻-N utilization and slight deterioration of the reactor performance. Subsequently, the stable performance recovered since the HRT extended to 3 h again. Combined with all these results of operations discussed above, it was conclusively proved that nitrogen removal via full nitritation pathway for low-strength ammonium wastewater by adsorption, biological desorption and anammox process was feasible by ZBFB and anammox reactor.

3.4. Potential application and issue to be addressed
For low-strength ammonium wastewater treatment, controlling NOB growth is quite difficult in PN process. The failure of NOB inhibition will lead to a high nitrate concentration and undesirable nitrite production in effluent as well as deteriorate nitrogen removal performance[4, 23]. In this study, ZBFB reactor could produced adsorption effluent with NH₄⁺-N < 5.0 mg/L as well as desorption effluent with 37.5-49.6 mg/L NO₂⁻-N. And then persistent nitrite supply was guaranteed for the next anammox reactor for nitrogen removal. That was to say, difficulty of stable nitrite accumulation could be solved by applying ZBFB as PN reactor for two stage PN/A process. Thus, a novel two-stage PN/A process coupled by ZBFB and anammox was proposed for disposal of low-strength ammonium wastewater. As large energy and cost were still needed for nitrogen removal from domestic sewage, this new process showed highly particular potential for domestic sewage treatment in the future. However, according to Fig. 3, the effluent NO₂⁻-N maintained in the range of 37.5-49.6 mg/L with a low NPR. So how to find a way for higher nitrate production rate during biological desorption by ZBFB is still a tough nut to crack. Besides, as shown in Fig. 4, the highest NRR of anammox process fed with the mixed wastewater was 0.271 kg N m⁻³ d⁻¹ with an average TNRE of only 57.50%. Some operational conditions, such as the mass transfer during biological desorption, should be further investigated to obtain higher standard effluent. In addition, this study was done with synthetic wastewater, which could only provide theoretical feasibility of ZBFB and anammox reactor for low-strength ammonium wastewater. Therefore, it is necessary to carry out the actual domestic sewage treatment study with this combined process in the following study.

4. Conclusions
The ZBFB reactor coupled with anammox reactor were suitable for nitrogen removal as a two-stage PN/A process for wastewater containing 30 mg/L NH₄⁺-N. Long-term operation showed that, due to the effective free ammonia inhibition on NOB during biological desorption step in ZBFB, nitrite accumulation was stable with 37.5-49.6 mg/L NO₂⁻-N and an average NAR of 88.70% in effluent by
the biological desorption of ZBFB. The subsequent anammox reactor could removed TN to less than 15 mg/L with highest NRR of 0.271 kg N·m⁻³·d⁻¹. The *Nitrosomonas* (as AOB) turned into the dominant nitrifiers for nitrite accumulation in ZBFB and *Candidatus Kuenenia* (as AnAOB) was responsible for nitrogen removal in anammox reactor.

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