

Article

Full-Scale Demonstration of Nitrogen Removal from Mature Landfill Leachate Using a Two-Stage Partial Nitrification and Anammox Process

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Abstract: The excessive discharge of nitrogen leads to water eutrophication. The partial nitrification and anammox (PN/A) process is a promising technology for biological nitrogen removal in wastewater treatment. However, applying it to mature landfill leachate (MLL) faces challenges, as the toxic substances (e.g., heavy metal) within MLL inhibit the activity of anammox bacteria. Therefore, most previous studies focused on diluted, pretreated, or chemically adjusted MLL. This study demonstrated at full scale that the two-stage PN/A process can treat raw MLL. Initially, the operational issue of sludge floatation resulted in rapid biomass loss with overflow discharging, which selectively suppresses nitrite-oxidizing bacteria (NOB), promoting the achievement of nitrite accumulation. After that, the NOB suppression was self-sustained by the high in situ free ammonia concentration, i.e., 26.2 ± 15.9 mg N/L. In the subsequent anammox tank, nitrogen removal primarily occurred via the anammox process, complemented by denitrification, achieving total nitrogen removal efficiency exceeding 72%. In addition, the nitrogen removal capacity of this system was significantly influenced by temperature with the nitrogen-loading rate above 0.4 kg N/m³/d at 38 °C and approximately 0.1 kg N/m³/d at 21 °C. The optimization of system operation, such as gradually increasing MLL content, remains necessary to enhance nitrogen removal capacity further.

Keywords: partial nitrification and anammox; mature landfill leachate; full-scale demonstration; seasonal temperature varying



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1. Introduction

Nitrogen in wastewater should be reduced before discharge to prevent water eutrophication [1]. Currently, most nitrogen (mainly ammonia nitrogen) in wastewater is removed using various biological pathways [2,3]. Among these, nitrification/denitrification via the nitrate pathway is widely implemented due to its ease of operation. Specifically, in the nitrification process, ammonia nitrogen is first oxidized to nitrite by ammonia oxidation bacteria (AOB: $\text{NH}_4^+ + 1.5\text{O}_2 + 2\text{OH}^- \rightarrow \text{NO}_2^- + 3\text{H}_2\text{O}$), and then the nitrite is oxidized to nitrate by nitrite oxidation bacteria (NOB: $\text{NO}_2^- + 0.5\text{O}_2 \rightarrow \text{NO}_3^-$). In the denitrification process, nitrate is reduced to nitrogen gas in four steps ($\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$) with the use of organics as the electron donor. Unfortunately, this process is energy-intensive, requiring substantial aeration for nitrification and additional organic carbon for denitrification [4,5]. Anaerobic ammonia oxidation (anammox), discovered in 1995, removes nitrogen without the need for aeration or organic carbon ($\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{HCO}_3^- + 0.13\text{H}^+ \rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- + 0.066\text{CH}_2\text{O}_{0.5}\text{N}_{0.15} + 2.03\text{H}_2\text{O}$) [6]. This

inherent advantage makes anammox-based nitrogen removal processes highly promising. Partial nitrification and anammox (PN/A), which involves partial nitrification (AOB: $\text{NH}_4^+ + 1.5\text{O}_2 + 2\text{OH}^- \rightarrow \text{NO}_2^- + 3\text{H}_2\text{O}$) followed by anammox, can significantly reduce the need for aeration (by close to 60%) and eliminate the requirement for organic carbon entirely when compared to the nitrification/denitrification process [7]. Clearly, the anammox process requires two nitrogenous compounds: ammonium and nitrite. Ammonium is obtained from the wastewater, while nitrite is supplied by AOB. Therefore, the main challenge of the PN/A process is to accumulate nitrite by suppressing NOB, which converts nitrite to nitrate [4]. Indeed, in treating high-ammonium wastewater, NOB can be relatively easily suppressed selectively by the generated free ammonia ($\text{NH}_4^+ \leftrightarrow \text{NH}_3 + \text{H}^+$) at high pH and high ammonia nitrogen conditions [8–11]. In general, the PN/A process is a suitable technology to remove nitrogen from wastewater with high ammonium concentration and a low biodegradable chemical oxygen demand (COD)-to-nitrogen ratio.

The leachate generated from mature landfills is a typical low biodegradable COD-to-nitrogen ratio wastewater [12,13]. The ratio of biodegradable COD to nitrogen in mature landfill leachate (MLL) is normally below 0.3 [14], which is significantly lower than the required minimum ratio (2.74) for denitrification via the nitrate pathway ($\text{NO}_3^- \rightarrow \text{N}_2$) in theory. Simultaneously, the ammonium concentration in typical MLL is about 1000 mg N/L, indicating that a suitable free ammonia level can be easily achieved for NOB suppression [15]. Therefore, the PN/A process is a suitable technology for removing nitrogen from MLL, and many successful demonstrations have been reported [16–20]. For example, a two-stage process—PN/A followed by denitrification—was applied to remove nitrogen from MLL [21]. The results of this lab-scale study showed that this proposed system removed a total nitrogen (TN) of 93.3% from MLL, with the PN/A process being the main contributor. Zhang et al. (2021) [16] also developed a three stage biological process to treat MLL in the lab, including PN, which is followed by fermentation–denitrification, and PN/A. This system achieved a nitrogen removal efficiency (NRE) of $99.2 \pm 0.1\%$ and nitrogen removal rate (NRR) of $0.61 \pm 0.04 \text{ kg N/m}^3/\text{d}$. Li et al. (2021) [20] operated a pilot-scale two-stage PN/A process to remove nitrogen from MLL, resulting in an NRR of about 60.2% and an NRR of about $0.95 \text{ kg N/m}^3/\text{d}$.

Although the PN/A process is an attractive, carbon- and energy-efficient technology for nitrogen removal, concerns often arise regarding its application in MLL treatment due to the limited adaptability of anammox bacteria to toxic compounds (e.g., heavy metals and toxic organics) present in MLL as well as the variable environmental conditions encountered in real-world applications (e.g., temperature fluctuations) [22]. Most studies [14,23] reported thus far have demonstrated the PN/A process using either diluted MLL or chemically adjusted MLL. For instance, biodegradable organics (e.g., sodium acetate) have been added to enhance denitrification performance, or bicarbonate/acids have been dosed to adjust the alkalinity-to-ammonium ratio to a suitable range. Understanding the efficiency of the PN/A process in removing nitrogen from raw MLL would accelerate its broader application. However, to date, there have been few demonstrations of raw MLL treatment, let alone at a full-scale level.

This study aims to demonstrate the feasibility of using a two-stage PN/A process to remove nitrogen from raw MLL at a full scale across seasonal temperature variations (ranging from 21 to 38 °C). The system operated continuously for 367 days, during which the performance of the two-stage PN/A process was monitored by regularly measuring concentrations of nitrogenous compounds (ammonium, nitrite, nitrate, and total nitrogen) and COD in both influent and effluent streams. The temperature, nitrogen-loading rate (NLR), and dissolved oxygen (DO) concentration of the process were also recorded. Additionally, biomass samples were collected from each bioreactor for microbial community assessment. The findings from this study could provide significant technological support for developing the PN/A process in treating MLL.

2. Method and Materials

2.1. Full-Scale System Setup, Operation, and Monitoring

The entire system was installed in Xi'an, China, and operated continuously for 367 days (from March 2023 to March 2024) using raw MLL as the feed. The working volume of the PN process (a steel tank) was approximately 896 m³, which was seeded with activated sludge from a local full-scale aerobic nitrification system treating domestic wastewater. The PN process was operated in sequencing mode, 480 min per cycle, including 20 min of feeding, 430 min of aeration, and 30 min of settling. Initially, the effluent was discharged via overflow during feeding. However, sludge floatation caused significant biomass loss. To address this, a pump (65WL65, Lanshen group Co. Ltd., Shanghai, China) was used to discharge the effluent from the middle of the PN reactor. Consequently, the sequencing mode was changed to 20 min of feeding, 385 min of aeration, 30 min of settling, and 45 min of discharging. Additionally, a buffer tank was installed to collect the PN effluent, which also served as the influent tank for the subsequent anammox process.

The anammox reactor, also made of steel, had a working volume of about 982 m³. Initially, approximately 180 m³ of denitrification sludge from a kitchen wastewater treatment plant was inoculated into the anammox reactor to enrich the anammox bacteria in situ. However, the anammox bacteria did not enrich within 100 days, so about 10 m³ of anammox sludge from a process treating amino acid wastewater was inoculated into the anammox reactor. The anammox process was also operated in sequencing mode, 480 min per cycle, including 122 min of feeding, 268 min of anoxic reaction, and 90 min of settling. The effluent was discharged via overflow during feeding.

In this study, all the inoculated sludge was collected using a pump and transported in a 10 m³ steel container, which was immediately tracked to the site and then pumped into the full-scale reactors. The dissolved oxygen (DO) concentration in the PN process was maintained below 1 mg O₂/L during the startup stage and between 1 and 2 mg O₂/L during the steady state using on/off control of the blower (MLG125B, Tianjin Tiangu Machinery Manufacturing Co. Ltd., Tianjin, China). In brief, the blowers were turned on when the DO concentration reduced to the low setpoint, and it turned off when the DO concentration reached the high setpoint. A programmable logic controller system was used to manage all controls. During aeration, several blowers were used to inject compressed air into the PN process at a flow rate of 11.8 m³/min via several microporous air diffusers. pH was measured using a pH meter (pH-100pro, LICHEN Instrument Technology Co. Ltd., Shanghai, China), and DO (LR-DO700, Hangzhou Sinomeasure Automation Technology Co. Ltd., Hangzhou, China) and temperature (WZPB-241 PT100L, Anhui, LAND Automation Equipment Sales CO. Ltd., Anhui, China) were monitored using online meters. The pH of the PN process was controlled by adjusting aeration: if the pH dropped below 7.2, aeration was reduced, and vice versa.

Concentrations of COD, ammonium, nitrite, nitrate, and TN in the influent, PN effluent, and anammox effluent were measured 4–6 times per week. Mixed liquor suspended solids (MLSSs) concentration in the PN and anammox reactors was monitored 3–4 times per week. Biomass samples, from two reactors, were separately collected from the sampling points (in the middle of the reactor) of each reactor, for the microbial community composition analysis, on Day 99, Day 127, Day 163, Day 189, Day 224, Day 249, and Day 310.

2.2. Chemical Analysis

Concentrations of MLSS and COD were measured according to the standard methods [24]. Mixed liquor samples were filtered through 0.45 µm Millipore filters for the determination of ammonium, nitrite, nitrate, and TN concentrations with a spectrophotometer (DR3900 Hach). The used methods are detailed in Table S1.

2.3. Microbial Analysis

The microbial community composition of biomass samples from PN and anammox reactors was analyzed using 16S rRNA amplicon sequencing at Guangdong Magigene Biotechnology Co., Ltd. (Guangzhou, China) via the Illumina NovaSeq™ X Plus. Microbial DNA was extracted with the MOBIO PowerSoil® DNA Isolation Kit (12888-100), following the protocol from MOBIO Laboratories (Carlsbad, CA, USA), and its quality was verified using gel electrophoresis. High-throughput sequencing was performed targeting the V4 region of the 16S rRNA gene with primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3'). Raw sequencing data were processed using fastp, an ultra-fast all-in-one FASTQ preprocessor (v 0.14.1, Shenzhen HaploX Bio-Technology Co. Ltd., Shenzhen, China), to obtain clean tags. Subsequently, sequences were clustered into operational taxonomic units (OTUs), which were annotated by SILVA database (v138, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Bremen, Germany) at a 97% identity threshold.

2.4. Statistical Analysis

The nitrogen load rate (NLR), nitrogen removal efficiency (NRE), nitrogen accumulation rate (NAR), and free ammonia were calculated according to the following equations (Equations (1)–(4)):

$$\text{NLR} = \frac{\text{TN}_{\text{in}} \times Q}{V} \quad (1)$$

$$\text{NRE} = \frac{\text{TN}_{\text{in}} - \text{TN}_{\text{out}}}{\text{TN}_{\text{in}}} \times 100\% \quad (2)$$

$$\text{NAR} = \frac{\text{NO}_2^- - \text{N}}{\text{NO}_2^- - \text{N} + \text{NO}_3^- - \text{N}} \quad (3)$$

$$\text{FA} = \frac{17}{14} \times \frac{\text{NH}_4^+ - \text{N} \times 10^{\text{pH}}}{e^{6344/(273+T)} + 10^{\text{pH}}} \quad (4)$$

where TN_{in} and TN_{out} are the total nitrogen concentration in the influent and effluent (mg N/L), Q is the flow rate (m^3/d), V is the reactor working volume (m^3), and $\text{NH}_4^+ - \text{N}$, $\text{NO}_2^- - \text{N}$, and $\text{NO}_3^- - \text{N}$ are the concentrations of ammonium, nitrite, and nitrate, respectively.

3. Results and Discussion

3.1. Long-Term Operation of PN Process

Figure 1 presents the influent ammonium concentration, influent and effluent COD, ammonium, nitrite, and nitrate concentrations, the nitrite accumulation ratio (NAR), ammonium removal efficiency (ARE), pH, free ammonia, temperature, and nitrogen-loading rate (NLR) of the PN reactor. The average influent COD concentration was approximately 2082 mg/L, falling within the typical range reported for MLL [19] (Table 1). Following treatment in the PN process, the COD concentration decreased slightly to ~1525 mg/L, indicating a removal efficiency of only ~26.8% (Figure 1a). Although the COD concentration in MLL was not exceptionally high compared to other industrial wastewaters, most organics in MLL are difficult to be degraded within the applied activated sludge system. For instance, Ying et al. (2024) [13] employed a two-stage PN/A process to treat MLL with a COD concentration of 777.6 ± 19.2 mg/L. The COD removal efficiency was reported as $27.4 \pm 5.4\%$ in the PN stage and $28.6 \pm 3.6\%$ in the anammox stage.

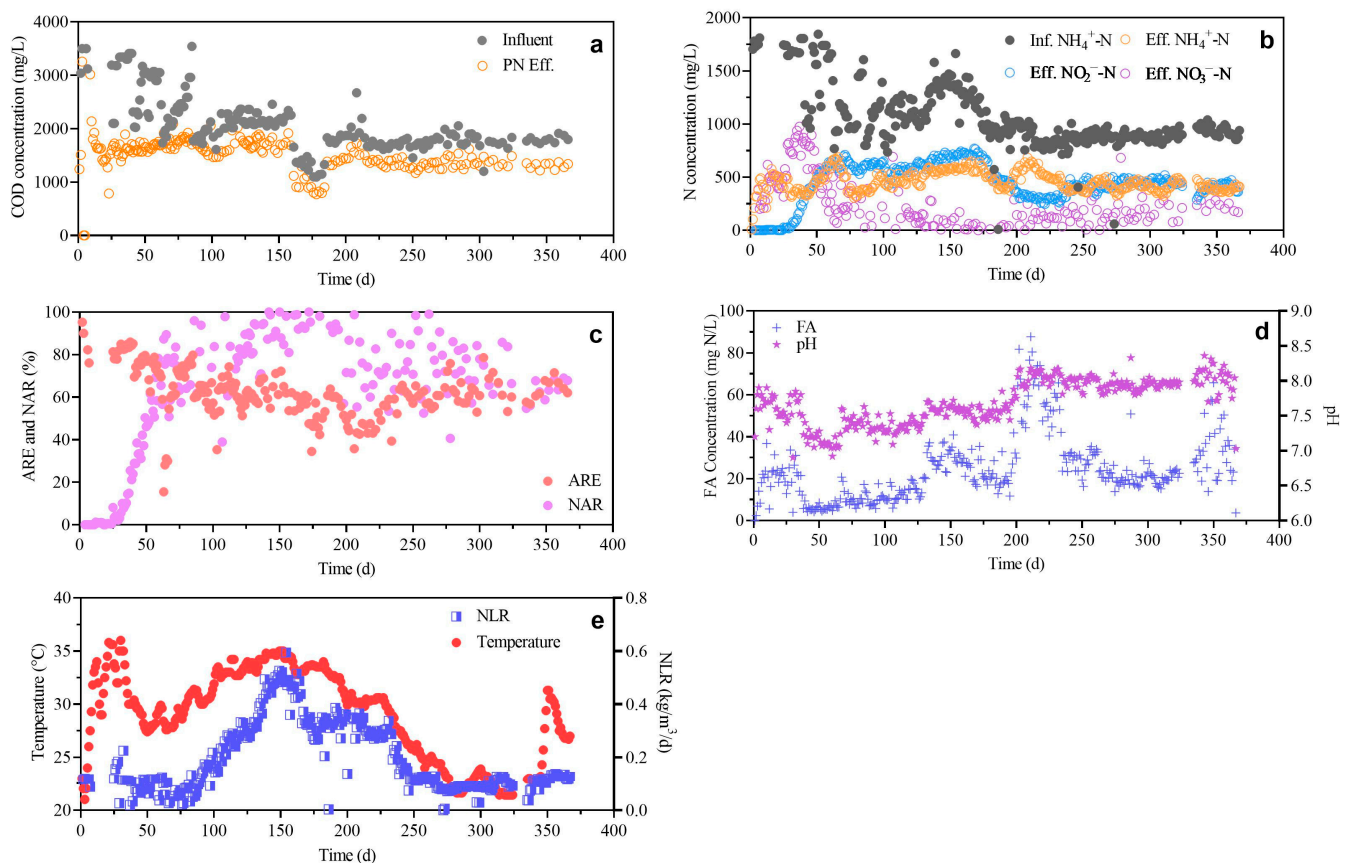


Figure 1. The performance of the PN process. COD concentration in the influent and effluent (a). Influent ammonia nitrogen concentration and effluent ammonia nitrogen, nitrite, and nitrate concentrations (b). Ammonia nitrogen removal efficiency (ARE) and nitrite accumulation ratio (NAR) (c). The free ammonia (FA) concentration and pH (d). The nitrogen-loading rate (NLR) and temperature (e).

Table 1. Key characteristics of the investigated mature landfill leachate.

Parameter	Unit	Value
Ammonia nitrogen	mg N/L	1042.7 ± 267.1
Total nitrogen	mg N/L	1311.0 ± 375.5
COD	mg O ₂ /L	2082.7 ± 506.0
pH		8.5 ± 0.1

In the investigated MLL, the average concentrations of ammonium and TN were 1042 ± 267 mg N/L and 1311 ± 375 mg N/L, respectively. Within the PN process, about $61.7 \pm 11.3\%$ of the influent ammonium was oxidized (Figure 1b,c). Initially, the oxidation product was primarily nitrate with minimal nitrite accumulation (i.e., NAR close to zero). An operational accident caused sludge floatation, which results in significant biomass loss with the effluent in the PN process, leading to a decrease in MLSS concentration from nearly 3000 mg/L to below 500 mg/L over 10 days (from Day 25 to Day 35) (Figure S2). Interestingly, the biomass loss accident led to nitrite accumulation, which was evident by a substantial increase in NAR from 8.1% on Day 25 to 69.3% on Day 57. Subsequently, from Day 57 to Day 367, the NAR sustained at a high level, averaging $76.9 \pm 13.6\%$. During this period, the effluent nitrite and nitrate concentrations were 499.0 ± 129.0 mg N/L and 165.5 ± 125.1 mg N/L, respectively, indicating significantly higher activity of AOB compared to NOB. The pH and free ammonia concentrations during this PN process period

were 7.7 ± 0.3 and 26.2 ± 15.9 mg N/L (Figure 1d), respectively, which as reported in the literature sustained a level sufficient for NOB suppression [25].

This full-scale demonstration spanned over a year and encompassed four seasons, experiencing temperatures ranging from 21 to 35 °C (Figure 1d). Notably, changes in the PN reactor's NLR were closely associated with temperature fluctuations. Specifically, from Day 50 to Day 150, as the operating temperature increased from ~27 to ~35 °C, the NLR rose fourfold from around 0.1 kg N/m³/d to about 0.5 kg N/m³/d. Subsequently, the NLR gradually decreased to about 0.1 kg N/m³/d with declining temperatures. During winter, the average PN reactor temperature and NLR were about 21 °C and 0.1 kg N/m³/d, respectively.

3.2. Long-Term Operation of Anammox Process

Figure 2 displays the concentrations of COD and TN in the influent and effluent along with parameters including the TN removal efficiency (TNRE), pH, free ammonia concentration, NLR, and reactor temperature of the anammox process. The anammox process exhibited limited ability to further reduce the COD concentration, with influent and effluent concentrations averaging 1524 ± 251 mg/L and 1363 ± 246 mg/L, respectively (Figure 1a). The COD removal efficiency was notably low at approximately 10%. The poor biodegradability of organics in the influent resulted in weak denitrification performance, causing nitrogen removal primarily through the anammox process.

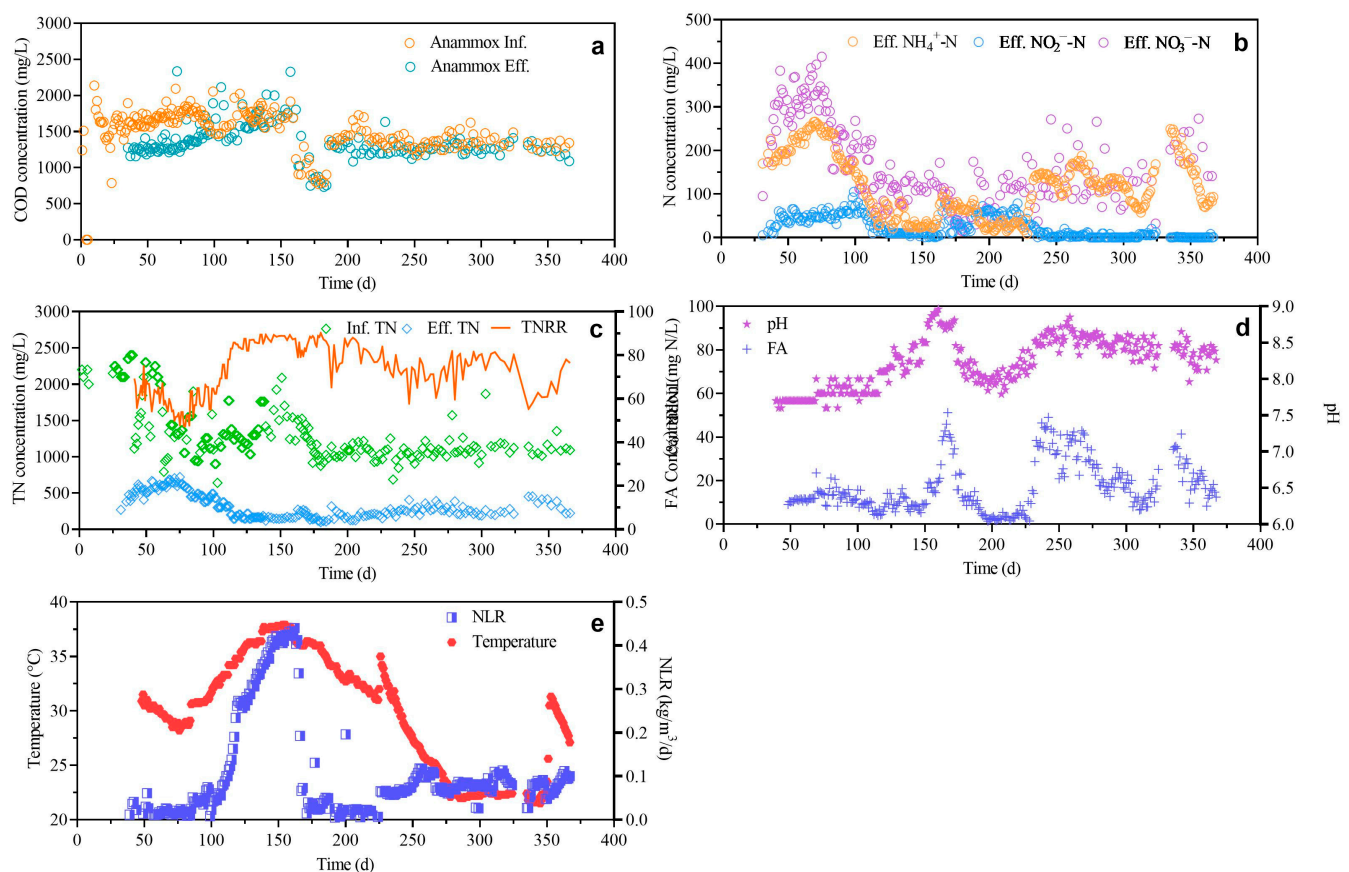


Figure 2. The performance of anammox process. COD concentration in the influent and effluent (a). Effluent ammonia nitrogen, nitrite, and nitrate concentrations (b). Influent and effluent total nitrogen (TN) concentrations (c). The free ammonia (FA) concentration and pH (d). The nitrogen-loading rate (NLR) and temperature (e).

The feeding of the anammox process contained ammonium, nitrite, and nitrate at concentrations of 469.0 ± 86.3 mg N/L, 500.0 ± 127.8 mg N/L, and 181.4 ± 142.9 mg N/L, respectively. The ratio of ammonium to nitrite was approximately 1.07, which is lower than the theoretical value of 1.32 typically expected for efficient anammox processes. Consequently, the effluent retained a residual ammonium concentration of about 112 mg N/L (Figure 1b). Due to unstable anammox activity during the operational debugging phase (Day 0 to Day 240), the effluent frequently contained nitrite (around 20 to 50 mg N/L). However, from Day 240 to Day 367, the anammox process stabilized, resulting in significantly reduced effluent nitrite concentrations, reaching approximately 3 mg N/L. During the period of stable anammox performance, the ratio between removed nitrite and removed ammonium was approximately 1.53, exceeding the theoretical value of 1.32. Concurrently, unlike typical anammox processes that produce nitrate, the effluent nitrate concentration (143.8 ± 91.7 mg N/L) was lower than the influent concentration (178.6 ± 142.9 mg N/L). These findings suggest the involvement of denitrification in the second stage, aiding in the removal of nitrite and nitrate. Overall, the two-stage PN/A process achieved a total nitrogen removal efficiency of $72.2 \pm 7.3\%$, which was attributable to the combined contributions of the anammox and denitrification processes (Figure 1c).

In the second stage, the pH initially increased from about 7.9 to nearly 9.0 and then decreased to around 8.5 during the debugging phases. During the stable operation phase (from Day 240 to Day 367), the average pH of the anammox process was maintained at 8.5 ± 0.2 , which was significantly higher than the PN process's pH of about 8.0 during the same phase due to alkalinity generated during the anammox and denitrification processes (Figure 1d). This elevated pH led to a high free ammonia concentration of 23.8 ± 10.4 mg N/L in the anammox tank during the stable operation. Studies have shown that free ammonia concentrations exceeding 2 mg N/L can impair the nitrogen removal performance of anammox processes [26]. The elevated free ammonia concentration inhibited anammox activity, resulting in a relatively low NLR in this system.

Operating temperature has a well-documented impact on anammox activity (Figure 1e). Throughout this full-scale study, the operating temperature varied between 22 and 38 °C. The NLR was manually adjusted to prevent anammox inhibition caused by excessive nitrite accumulation. Initially, the NLR increased notably from below 0.1 kg N/m³/d to above 0.4 kg N/m³/d as the temperature rose from approximately 28 °C to around 38 °C during the debugging phases. Subsequently, a decrease in temperature led to a significant reduction in the NLR to a very low level (below 0.03 kg N/m³/d). After operating at a low NLR for about two months, during the stable operating phase, the anammox bacteria gradually adapted to the lower temperature conditions. Eventually, the NLR could be sustained at approximately 0.1 kg N/m³/d even at a temperature as low as around 21 °C during stable operating phase. Clearly, the activity of anammox bacteria was significantly impacted by the operating temperature.

3.3. Microbial Community Composition Assessment

A total of 12 biomass samples were collected (six from the PN reactor and six from the anammox reactor) at different time points (Day 99, Day 127, Day 163, Day 189, Day 224, Day 249, and Day 310) for microbial community composition analysis using 16S rRNA gene amplicon sequencing. Good's coverage value was 0.999, indicating sufficient library sizes. Figure 3 displays the top five phyla at each sample of the PN reactor, representing more than 90% of the reads. Proteobacteria dominated on Day 99, accounting for 31.2%, followed by Planctomycetes (23.5%), Bacteroidetes (14.9%), Patescibacteria (8.9%), and Calditrichaeota (6.9%) (Figure 3a). The composition of these main phyla remained relatively stable over the 367 days of operation, with Proteobacteria and *Bacteroidetes* consistently ranking in the top three, representing over 60% of total reads.

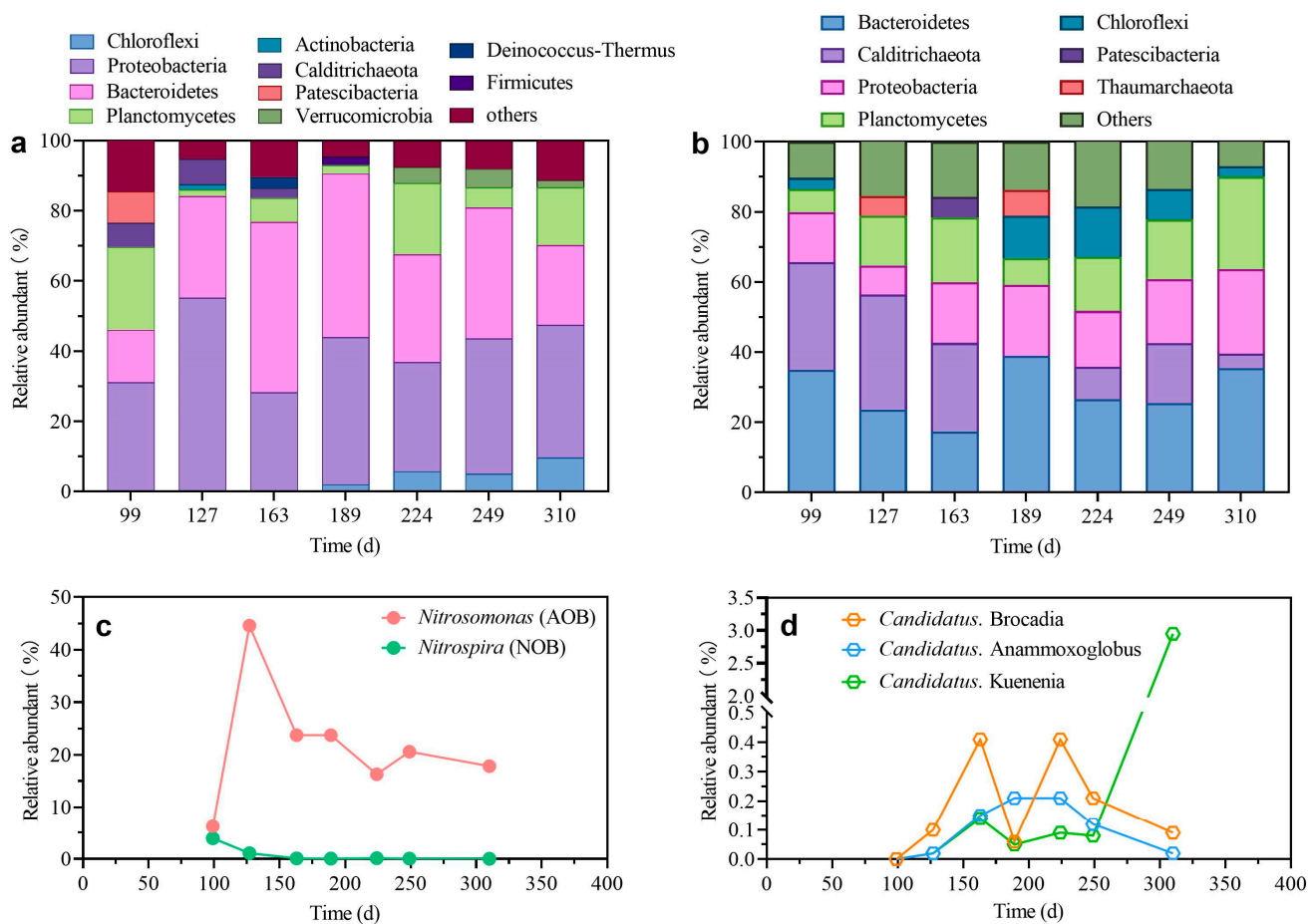


Figure 3. The microbial community composition of the PN and anammox process. Relative abundance of detected microorganisms of PN process (a) and anammox process (b) at the phylum level. Relative abundance of detected AOB and NOB of PN process (c) and anammox bacteria of anammox process (d).

At the genus level, *Nitrosomonas* and *Nitrospira* were the only detected AOB and NOB [27], respectively (Figure 3c). On Day 99, *Nitrosomonas* (6.2%) showed slightly higher abundance than *Nitrospira* (3.9%). As the PN process matured, the relative abundance of *Nitrosomonas* increased significantly to 44.6% on Day 127, while *Nitrospira* decreased to 1.1%. Throughout the subsequent operational period, the relative abundance of *Nitrosomonas* ranged from 16.3% to 23.7%, which was consistently higher than *Nitrospira*. Interestingly, despite a significant temperature reduction from 38 to 21 °C, the relative abundance of *Nitrosomonas* remained unaffected.

In the anammox reactor, the main phyla were Bacteroidetes, Calditrichaeota, and Proteobacteria, collectively representing 50 to 80% of total reads (Figure 3b). Anammox bacteria were not detected in the Day 99 sample, indicating that anammox bacteria was not enriched from the denitrification biomass in 99 days. After seeding with some anammox biomass to the reactor, three anammox bacteria—*Candidatus* (Ca.) Brocadia, Ca. Anammoxoglobus, and Ca. Kuenenia [28]—were identified. Before Day 249, each anammox bacteria's relative abundance was below 0.5%, with Ca. brocadia dominating, followed by Ca. Anammoxoglobus and Ca. Kuenenia (Figure 3d). The shift to lower temperatures altered the composition of anammox bacteria, with Ca. Brocadia becoming the dominant species (2.95%), which was followed by Ca. Brocadia (0.09%) and Ca. Anammoxoglobus (0.02%).

3.4. Implications and Limitations

As a promising biotechnology, the PN/A process has been widely adopted for removing nitrogen from high-strength wastewater. Over 100 full-scale applications have been established globally to address ammonium removal in sidestreams of wastewater treatment plants (WWTPs), indicating a potential increase in PN/A process applications in the near future [29]. However, new applications often require a relatively lengthy startup period to suppress NOB. This full-scale application observed a significant biomass loss, from approximately 3000 mg/L to about 350 mg/L, over a short period (two weeks), leading to a sharp increase in NAR from around 10% to over 70%. Indeed, it has been reported that reducing sludge age can selectively wash out NOB while preserving AOB, making sludge wasting a suitable option for establishing the PN process in a two-stage PN/A setup [30,31]. For example, Wang et al. (2021) [30] selectively inhibit NOB successfully by reducing the reactor's MLSS concentration from about 5000 mg/L to below 2500 mg/L. Once established, stable PN performance can be sustained through self-sustained high free ammonia or free nitrous acid concentrations. It should be noted that the biomass lost from the PN reactor was collected by the subsequent anammox system, which did not appear to impact its performance. However, at that time, microbial community assessment showed that the anammox bacteria were not enriched in the reactor (Figure 3d). Therefore, the impact of lost biomass from the PN process on the subsequent anammox process remains unclear and requires further investigation.

In this full-scale demonstration, raw MLL was fed directly to the two-stage PN/A process without any conditioning. The results showed that functional microorganisms such as AOB, anammox, and denitrifiers could tolerate the toxic compounds present in MLL. However, the achieved NLR at 21 °C was around 0.1 kg N/m³/d, which is lower than reported in some full-scale applications [12]. This lower rate may be attributed to the direct feeding of raw MLL initially, allowing less time for the functional microorganisms to adapt to the pure MLL conditions. Periodically increasing the content of MLL would induce microorganisms' adaptation and then achieve high NLR. For example, Wu et al. (2024) [21] increased the nitrogen removal rate of an anammox-based MLL treatment process from 0.02 kg N/m³/d to over 1 kg N/m³/d by gradually increasing the proportion of MLL from 10% to 100%.

This full-scale PN/A process was operated for a year across four seasons. It was clear that changes in operating temperature significantly impacted the system's nitrogen removal capacity with lower temperatures leading to lower nitrogen removal capacity (Figures 1e and 2e). Indeed, the low temperature is a major challenge for the PN/A process [7,32–34]. This study inoculated the PN/A process with floc sludge for demonstration purposes. It has been reported that systems using granule sludge and biofilm can retain more biomass in the reactor, thereby offsetting the impact of low temperature [32,35].

It took about 240 days to stabilize the performance of this full-scale system, during which some operational issues were successfully resolved. For example, biomass loss was avoided by optimizing the discharging mode, and anammox performance was increased by inoculating with a small amount of anammox biomass (~1% of the working volume). This also suggested the importance of periodically increasing the MLL loading for performance stabilization in real applications.

This study mainly focused on the overall performance of the two-stage PN/A process in treating MLL, specifically regarding the removal of organic and nitrogenous compounds. Although some biomass samples were taken and analyzed, understanding this full-scale two-stage PN/A system at the microbial level is still limited. In our future study, more representative biomass samples will be collected and analyzed using advanced microbial methods (e.g., metagenomic assessment).

Overall, this study successfully demonstrated the feasibility of treating raw MLL using a two-stage PN/A process across seasonal temperature variations (from 21 to 38 °C) at full scale. However, further operation optimization is still necessary to increase the NLR of this system.

4. Conclusions

In conclusion, this study demonstrated the effectiveness of a two-stage PN/A process for removing nitrogen from pure MLL across a range of temperatures (from 21 to 38 °C). Initially, biomass loss led to nitrite accumulation. Subsequently, the high concentration of free ammonia (above 25 mg N/L) inhibited NOB, thereby sustaining stable PN performance, achieving an NAR close to 77%. In the second stage, nitrogen removal primarily occurred via the anammox process, complemented by denitrification, resulting in an overall nitrogen removal efficiency of above 72%. Notably, the study found that the NLR of this two-stage system was significantly affected by the operating temperature. The system achieved an NLR exceeding 0.4 kg N/m³/d at higher temperatures (approximately 38 °C), while the achievable NLR decreased to around 0.1 kg N/m³/d at a temperature as low as ~21 °C.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/pr12071307/s1>, Figure S1: Schematic diagram of the two-stage PN/A process; Figure S2: The MLSS concentration and nitrite accumulation ratio (NAR) of the PN process; Table S1: The detection methods used in this study.

Author Contributions: Conceptualization, R.D. and C.Z.; Project Supervision, R.D. and D.L.; System Operation, D.L. and C.Z.; Data Collection, D.L. and Z.Z.; Data Analysis, Z.H., D.L. and R.Z. Writing, Z.H. and X.L. All authors have read and agreed to the published version of the manuscript.

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