INFLUENCE OF SHORT-TERM pH FLUCTUATIONS ON NITROUS OXIDE EMISSIONS IN A SEQUENCING BATCH REACTOR FOR PARTIAL NITRIFICATION

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ABSTRACT

This study aimed to evaluate the impact of short-term pH fluctuations on nitrous oxide (N_2O) emissions in a sequencing batch reactor used for partial nitrification. Despite variations in operational conditions, the reactor consistently demonstrated high nitritation efficiency, with optimal performance at pH 7.0. The highest N_2O emission factor was observed at pH 7.6 (0.46%), followed by pH 7.0 (0.38%), while lower emissions were recorded at pH 8.2 and pH 6.4. High-throughput sequencing of 16S rRNA gene amplicons revealed significant temporal shifts in the microbial community, with *Nitrosomonas* becoming dominant during steady-state partial nitrification, and no nitrite-oxidizing bacteria detected. In the batch cycle, N_2O emissions predominantly occurred in the early phase, peaking between 5 and 15 minutes, likely due to rapid ammonium oxidation. These insights highlight the importance of pH control for minimizing N_2O emissions in wastewater treatment systems.

ẢNH HƯỞNG CỦA SỰ BIẾN ĐỘNG pH NGẮN HẠN ĐẾN PHÁT THẢI KHÍ N_2O Ở BỂ BÙN HOẠT TÍNH THEO MẺ THỰC HIỆN QUÁ TRÌNH NITRATE HÓA BÁN PHẦN

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TỪ KHÓA

Phát thải N_2O
Biến động pH
Nitrate hóa bán phần
Bể bùn hoạt tính theo mẻ
Vi khuẩn oxi hóa amonia

TÓM TẮT

Nghiên cứu này nhằm đánh giá ảnh hưởng của sự dao động pH ngắn hạn lên lượng phát thải khí nitơ oxit (N_2O) trong một bể bùn hoạt tính theo dạng mẻ thực hiện quá trình nitrat hóa bán phần. Mặc dù điều kiện vận hành thay đổi, hệ thống vẫn đạt hiệu suất nitrit hóa cao, với hiệu suất tối ưu ở pH 7.0. Hệ số phát thải khí N_2O cao nhất được ghi nhận ở pH 7.6 (0,46%), tiếp theo là pH 7.0 (0,38%), trong khi đó hệ số phát thải thấp hơn được ghi nhận ở pH 8.2 và pH 6.4. Kết quả phân tích trình tự gen 16S rRNA cho thấy sự thay đổi rõ rệt trong cấu trúc hệ vi sinh vật trong bùn hoạt tính, với *Nitrosomonas* chiếm tỉ lệ cao khi bể bùn hoạt tính đạt tình trạng mức nitrite hóa ổn định, trong khi đó không phát hiên nhóm vi khuẩn nitrate hóa. Trong chu kỳ mỗi mẻ phản ứng, lượng phát thải N_2O chủ yếu xảy ra trong giai đoạn đầu, đạt mức phát thải N_2O cao sau 5 đến 15 phút, có thể do quá trình oxy hóa amonia diễn ra nhanh chóng. Những phát hiện này nhấn mạnh tầm quan trọng của việc kiểm soát pH để giảm thiểu phát thải N_2O trong các hệ thống xử lý nước thải.

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1. Introduction

Nitrogen removal in industrial and domestic wastewaters is an essential process for protection of the receiving water bodies from eutrophication. However, current technologies for nitrogen removal by nitrification and denitrification are costly and energy-intensive due to high energy demand for aeration, external organic supply, alkalinity for pH neutralization, and excess sludge treatment. Hence, development of a cost-effective and compact reactor system for nitrogen removal is important [1]. It is also crucial to consider nitrous oxide (N_2O) emissions during biological nitrogen removal processes. N_2O emission from wastewater treatment plants (WWTPs) has gained considerable interest among policy makers and scientists since it is a strong greenhouse gas with global warming potential approximately 300 times as strong as that of CO_2, and causes destruction of the stratospheric ozone layer [2], [3]. In recent years, WWTPs have gained attention as significant point sources of N_2O emissions. N_2O emission from WWTPs expectedly increases by approximately 13% between 2005 and 2020 [4], and its impact accounts for up to 78.4% of the total CO_2 footprint in WWTPs [5]. WWTPs aim to reduce nitrogen pollution, but N_2O emissions during treatment can contribute to climate change. Identifying factors that increase N_2O production is the key to mitigate emissions and enhance wastewater management sustainability.

Nitrification, the process through which ammonia (NH_4^+) is oxidized to nitrate (NO_3^-), is a key biological process in the nitrogen cycle of wastewater treatment. It typically occurs in two steps: the oxidation of ammonia to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB), followed by the oxidation of nitrite to nitrate by nitrite-oxidizing bacteria (NOB). In recent years, however, the concept of partial nitrification (i.e., stopping the process at the nitrite stage) has gained interest for applications in wastewater treatment, particularly for systems designed to operate with anammox (anaerobic ammonium oxidation) processes. Partial nitrification and anammox process (or PN/A) have been regarded as the most innovative treatment process in environmental biotechnology recent years [6] - [8]. The PN/A is a remarkable microbial conversion process in which NH_4^+ is oxidized using NO_2^-, as electron acceptor, to produce nitrogen gas by anammox bacteria, which belongs to the order of Planctomycetales [8] - [11].

In comparision with conventional treatment through nitrification and denitrification processes, PN/A process requires no external carbon source and reduces 62% energy use [12], and 90% CO_2 emission [11], [13]. While this method reduces the operational costs of wastewater treatment, it has been shown to increase the likelihood of N_2O emissions. This is particularly the case during the ammonia oxidation phase, where intermediates such as hydroxylamine (NH_2OH) or NO_2^- may undergo incomplete oxidation, leading to N_2O production.

pH is a fundamental operational parameter that influences microbial activity, chemical equilibria, and gas emissions in biological wastewater treatment processes. The performance of nitrifying bacteria is highly sensitive to pH fluctuations, as both AOB and NOB have optimal pH ranges for efficient ammonia and nitrite oxidation [14] - [17]. Outside of these ranges, their activity can be significantly inhibited, leading to incomplete nitrification and the accumulation of intermediates that may result in N_2O emissions [16], [18] - [21]. On the other hand, short-term pH fluctuations are common in WWTPs due to diurnal variations in wastewater composition and process control strategies. Such fluctuations can disrupt the balance of microbial populations, shift nitrogen conversion pathways, and promote the formation of nitrogenous intermediates such as nitrite and hydroxylamine [17], [20] - [23]. These intermediates have been identified as key precursors to N_2O production, particularly in systems performing partial nitrification. Therefore, understanding how short-term pH variations impact N_2O emissions is essential for optimizing process control strategies to minimize the environmental footprint of WWTPs.

The sequencing batch reactor (SBR) is a type of activated sludge process for WWTP that operates in batch mode. However, the batch nature of the process can lead to fluctuating conditions, such as pH changes, which can impact N_2O emissions [24]. Studying and controlling these fluctuations in an SBR offer key insights for optimizing performance and reducing GHG emissions. Numerous studies have investigated N_2O emissions from various biological wastewater treatment processes, including the SBRs [15], [24], [25]. These studies have shown that operational factors such as dissolved oxygen (DO), substrate concentration, and pH can have significant impacts on N_2O production [14], [26] - [29]. For example, low DO conditions have been linked to increasing in N_2O emissions during nitrification, as oxygen limitation leads to incomplete oxidation of NH_4^+ and NO_2^- [15], [30], [31]. Similarly, changes in pH have been shown to affect the activity of AOB and NOB, potentially leading to the accumulation of nitrogenous intermediates that promote N_2O formation [16], [20], [28], [32]. It is also well-known that that pH can vary considerably during a single operational cycle in an SBR. Such short-term pH fluctuations may arise due to changes in aeration patterns, variations in influent composition, or metabolic activities of the microbial community [14], [32], [33]. These short-term pH shifts could have a significant impact on both the nitrification efficiency and the rate of N_2O emissions [26], [32]. However, many of these studies have focused on long-term pH trends or steady-state conditions, and very few studies on the impact of short-term pH changes on N_2O emissions. Given the dynamic nature of SBRs and the occurrence of transient pH variations, further research is needed to elucidate the relationship between pH fluctuations and N_2O emissions in these systems.

Given these contexts, the goal of this study was to unveil the influence of short-term pH fluctuations on N_2O emissions in an SBR performing partial nitrification. The specific objectives of the study include (1) to evaluate the effect of short-term pH changes on the performance of partial nitrification in an SBR; (2) to quantify N_2O emissions under different pH fluctuation scenarios and identify the key possible operational conditions that lead to increased N_2O production through observing the microbial community structure in the reactor. This research was expected to provide valuable insights into the mechanisms of N_2O production during partial nitrification, particularly under dynamic conditions, while also contributing to the development of more effective process control strategies for minimizing GHG emissions from wastewater treatment.

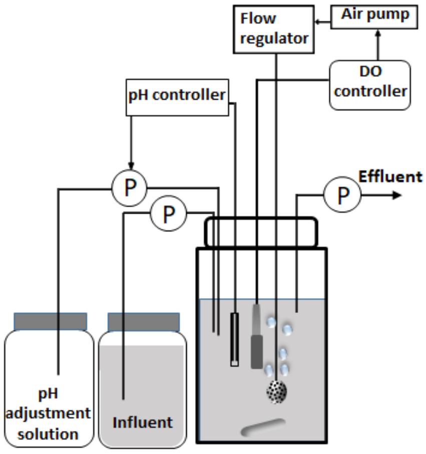

Figure 1. *Scheme of the sequencing batch reactor for partial nitrification*

2. Methodology

2.1. Operation conditions for sequencing batch reactor

A sequencing batch reactors (SBR) for partial nitrificationn was operated for 150 days. The working volume of the lab scale SBR was 8L. Inoculum biomass was activated sludge from wastewater treatment plant treating domestic wastewater. MLVSS was controlled at around 2500mg/L. Biomass in the reactor was not intentionally withdrawn during the experiment. The reactor was mixed well by an mixer setting at around 150 - 200 rpm. pH of the reactor was controlled at 7.0, and DO was set at 0.5-0.75mg/l in the start-up period. Temperature of the reactor was maintained at 25 ± 2°C. The reactor received the synthetic inorganic wastewater mimicking high-strength nitrogenous wastewater, consisting of (in mg/L): $NaHCO_3$ (11,878), $MgSO_4 \cdot 7H_2O$ (280), KH_2PO_4 (27), $CaCl_2 \cdot 2H_2O$ (120), $MnSO_4 \cdot H_2O$ (3.3), $CuCl_2 \cdot 2H_2O$ (0.8), NaCl (600), $FeSO_4 \cdot 7H_2O$ (3.3), $ZnSO_4 \cdot 7H_2O$ (1.7), and $NiSO_4 \cdot 6H_2O$ (0.3). The amount of $(NH_4)_2SO_4$ added in the synthetic wastewater was adjusted to ensure an initial NH_4^+-N concentration summarized in Table 1. Automatic DO controller was installed for setting the expected DO levels. During the feeding and aeration periods, aeration was maintained at a constant airflow rate of 0.2 L/min, and the DO controller was adjusted to regulate the dissolved oxygen level. pH was adjusted by using a pH controller. Saturated $NaHCO_3$ or 1M HCl solutions was used as a neutralizing agents for adjustment pH of the SBR (Figure 1).

Table 1. *Operation condition for the sequencing batch reactor for partial nitrification*

Parameters	Phase 1	Phase 2	Phase 3	Phase 4
Operation time (day)	0 - 35	34 – 41	42 - 60	61 – 150
DO (mg/L)	0.5	0.75	0.5	0.5
pH	7.0	7.0	7.0	7.0 and change to pH 7.6 (day 95), pH 8.2 (day 111), and pH 6.4 (day 125)
HRT (day)	2.0	2.0	1.5	1.0
Influent NH_4^+ (mgN/L)	600 - 900	1000	1000	1000

Synthetic influent feeding to reactors was stepwise increase from 600 $mgNH_4^+$-N/L to 1000 $mgNH_4^+$-N/L. During the initial start-up phase (0–41 days), the hydraulic retention time (HRT) of the reactor was maintained at 2 days. This was progressively reduced to 1.5 days (42–60 days) and finally to 1 day (from day 61 to day 150). Based on influent characteristics and operational conditions, the reactor was divided into four distinct phases, as outlined in Table 1. One cycle of the SBR operation was 8 hours, consisting of 0.05 h (3 min) for filling influent, 6.0 h for aeration, 0.5 h for settling, 0.45 h for decanting, 1 h for idling. For each cycle, one third of the liquid volume was discharged and replaced with a influent synthetic wastewater explained afterwards, ensuring a hydraulic retention time of 1 day.

To assess the impact of short-term pH changes on N_2O emissions in the SBR, gas concentrations were measured during a single cycle on day 89 (pH 7.0), day 95 (pH 7.6), day 111 (pH 8.2), and day 125 (pH 6.4). The reactor was adjusted for four cycles at each pH level before sampling for N_2O and water quality during the 5th cycle. Each short-term pH set point was set across four cycles of the SBR (resulting less than 2 days for each pH set point). Thirteen gas samples were collected to measure N_2O emission rates at -5, 0, 5, 15, 30, 60, 120, 180, 240, 300, 360, 420 and 480 minutes during each SBR cycle. The average N_2O emission rate for each pH condition was calculated based on the emission rates from the nine collected samples per cycle.

2.2. Analytical methods on nitrogen constituents

NH_4^+, NO_2^-, and NO_3^- concentrations in influent and effluent were regularly measured 2-3 times/week and analyzed according to standard methods for the examination and wastewater [34].

Partial nitrification efficiency (PNE) was defined as:

$$PNE = \frac{C_{NO_2^- - N\cdot eff}}{C_{NO_2^- - N\cdot eff} + C_{NO_3^- - N\cdot eff}} \times 100\% \qquad (1)$$

where $C_{NO2^- -N\cdot eff}$ and $C_{NO3^- -N\cdot eff}$ represent NO_2^- and NO_3^- concentrations in effluent, respectively.

Nitrification efficiency (NE) was calculated by:

$$NE = \frac{C_{NO_2^- \cdot eff} + C_{NO_3^- \cdot eff}}{C_{NH_4^+ \cdot \inf} + C_{NO_2^- \cdot \inf} + C_{NO_3^- \cdot \inf}} \times 100\% \qquad (2)$$

where $C_{NH4^+ -N\cdot inf}$, $C_{NO2^- -N\cdot inf}$ and $C_{NO3^- -N\cdot inf}$ represent NH_4^+, NO_2^- and NO_3^- concentrations in influent.

2.3. Analysis on N_2O gas emission

Gaseous samples taken from the headspace of the SBR were collected using 1L gas collection bags. Quantitative detection of gaseous N_2O was performed using gas chromatography (GC-14B, Shimadzu, Kyoto, Japan) and an electron capture detector (ECD). The N_2O emission factor of oxidized NH_4^+ in the reactor was calculated using the equation (3):

$$N_2O \text{ emission factor} = (m_{N2O}/m_{load}) \times 100 \qquad (3)$$

where mN_2O is the quantity of N_2O emission (g), acquired by integration of N_2O as a function of time during one cycle; and m_{load} is the mass of NH_4^+ loading during SBR cycle.

2.4. Deep sequencing of 16S rRNA gene amplicons

DNA was extracted from nitrifying biomass in the SBR reactor by a FastDNA^{TM} Spin Kit (MP Biomedicals, CA) according to the instructions of manufacturer, followed by measurements of DNA concentrations and purities by a spectrophotometer (Nanodrop2000c, Thermo Scientific, MA). PCR was conducted by the primer set 515f-806r to amplify the V4 hypervariable region of 16S rRNA gene [35]. The PCR amplicons were cleaned up with an AMPure XP kit (Beckman Coulter, CA), followed by electrophoresis by 0.7% agarose gel and 1.15% Synergel (Diversified Biotech, MA). The DNA amplicon on the gel was excised and purified by a Wizard® SV Gel and PCR Clean-Up system (Promega, WI), and the purified DNA was quantified by a fluorometer (Qubit, Life Technologies, CA). Deep sequencing of 16S rRNA gene amplicons and the subsequent data analysis were performed by a MiSeq DNA sequencer (Illumina, CA) as previously described [36]. Operational taxonomic units (OTUs) were set at 97% identity as a threshold.

3. Results

3.1. Partial nitrification performance of the SBR

Time courses of nitrogen constituent concentrations in effluent are shown in figure 2A. It is obvious that the NH$_4^+$ was oxidized with higher rate and remained lower concentrations in effluent by time, and higher NO$_2^-$ was accumulated in later time, especially in phase 3 (42-60 days) and phase 4 (day 61 to 150). Even though HRT of the reactors was reduced from period 3 to 4, high nitritation efficiency was achieved. The partial nitrification (nitritation) and nitrification efficiencies of the reactor were shown in figure 2B. It shows that high nitritation and nitrification was achieved since day 36 onward. Partial nitrification efficiency (PNE) in phase 3 (HRT 1.5 days, from day 42-60) fluctuated from 59.0-92.4%, while that of phase 4 (from day 61 onward) was in the range 67.1-99.8%.

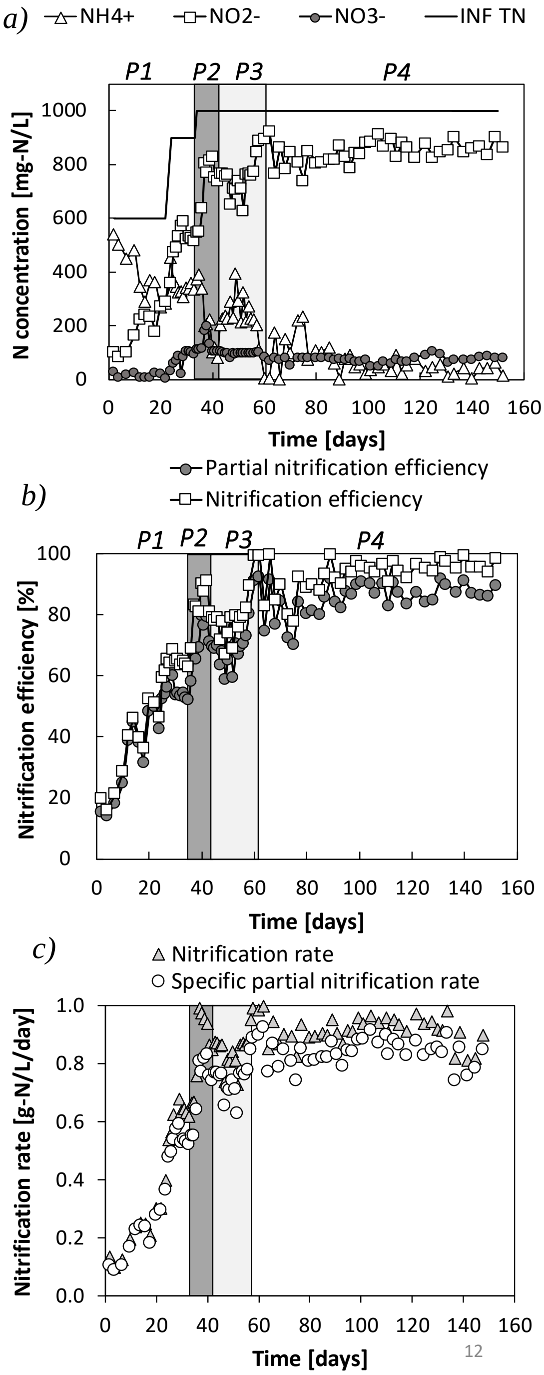

Figure 2. *Nitrogen constituent concentrations in effluent (a), partial nitrification and nitrification efficiency (b), and specific nitrification rate of partial nitrification reactor (c) by time course*

During the period when the pH of the SBR was adjusted for short-term changes, the nitritation and nitrification efficiencies at pH 7.6 were high, at 84% and 88%, respectively (on day 95). These figures were similarly high at pH 8.2 (on day 111) and pH 6.4 (on day 125). Specifically, nitritation efficiencies at pH 8.2 and pH 6.4 were both 83%, while nitrification efficiencies were 87% and 90%, respectively. Under these pH conditions, the reactor demonstrated the highest nitritation and nitrification efficiencies at pH 7.0, as observed on day 89, achieving rates of 87% and 99%, respectively. Generally, the short-term fluctuations of pH (from 7.0 to 6.4, 7.6, or 8.2) did not affect nitrification rate and PNE.

The ammonium oxidation rate and specific partial nitrification rate of the reactor were shown in Figure 2c. It showed that NH_4^+ oxidation rate was high from day 36. Although the HRT of the reactor was reduced to 1 day in period 4 from period 3 (HRT 1.5 day), NH_4^+ oxidation rate was also high (0.91 ± 0.02 g-N/L/d averagely). Partial nitrification rate in phase 4 was at 0.82 ± 0.02g-N/L/d. In comparison with other researches, relatively high NH_4^+ oxidation rate was achieved in this study, e.g. 0.65 g-N/L/d in Kampschreur *et al* [37], and 0.89 g-N/L/d in Gabarro *et al.* [38]. Generally, relatively high partial nitrification was achieved in the PN reactor. Overall, despite short-term pH changes, the specific nitrification rate of the SBR remained high and relatively stable under the varying pH conditions.

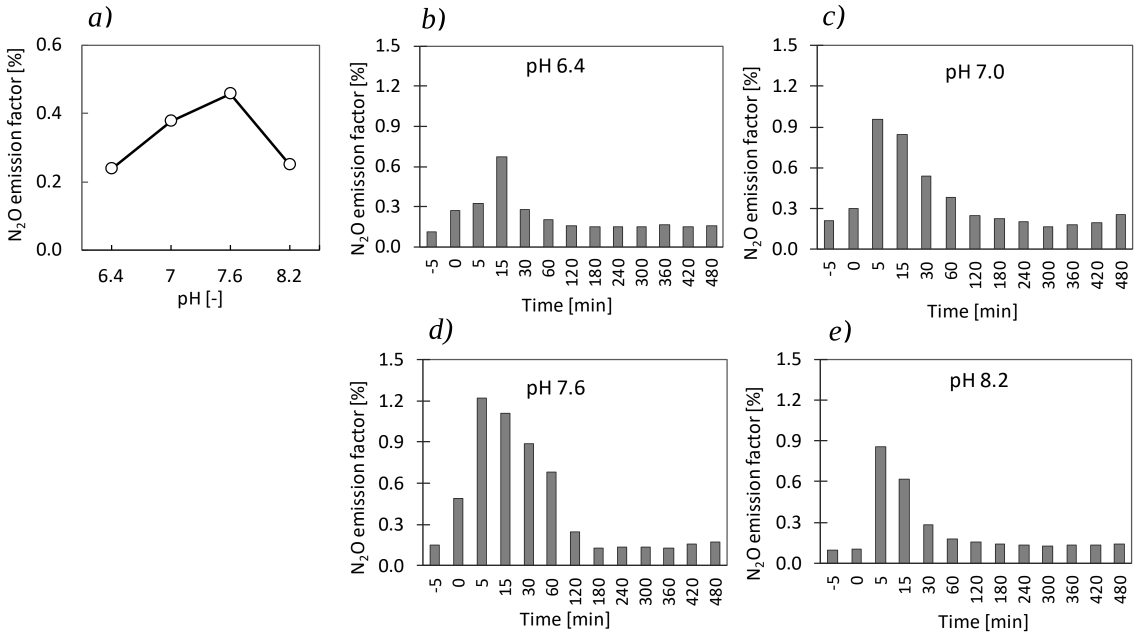

Figure 3. *The N_2O emission factors of PN reactor under short-term change of pH (a) and time course profiles of N_2O emission factor by sequencing batch reactor cycle at pH 6.4 (b), pH 7.0 (c), pH 7.6 (d), and pH 8.2 (e)*

3.2. N_2O gas emission with short-term pH fluctuations

The N_2O emission factors of PN reactor under short-term change of pH are shown in Figure 3. N_2O emission factor was the highest at pH 7.6 (0.46%), followed by the value of 0.12 % at pH 7.0, then at pH 8.2 (0.25%) and then at pH 6.4 (0.24%). Additionally, the results of one cycle profile of N_2O gas emission with short-term fluctuations was shown in Figure 3a. In the batch test of the SBR, N_2O gas concentrations were measured at different time intervals throughout a cycle. The results revealed that N_2O concentrations were highest within the first 60 minutes of each cycle. The peak concentrations were consistently observed between the 5- and 15-minute marks, suggesting that the early stages of the cycle contributed significantly to N_2O emissions. After 60 minutes, the concentrations began to gradually decline in all four tested cycles.

3.3. Identification and transition of predominant bacterial species

The transitions of community composition at genus level by deep sequencing of 16S rRNA amplicons were summarized in Figure 4. The microbial community structure in the sequencing batch reactor (SBR) showed significant shifts throughout the experimental period. The abundance of *Nitrosomonas* genus, a key AOB, increased dramatically from 1% in the inoculum to 48% by day 60 in phase 3, and 17% by day 125 in phase 4 (Figure 4a). This trend aligned with the high nitritation efficiency of the reactor during these phases. Another notable genus observed was *Ellin6067* (from the *Nitrosomonadaceae* family), suggesting possible involvement in nitritation process (Figure 4B). Extremely low NOB were detected in biomass samples in day 60 and 125, which supported the partial nitrification process. The genus *Nitrospira* (from the *Nitrospiraceae* family) is the only genus of NOB, which showed low abundance at the early stages (1.6% on day 0 and 2.5% on day 20) (Figure 4b). These shifts indicate that the community adapted to the operational conditions of the reactor, promoting nitritation and limiting NO_2^- oxidation.

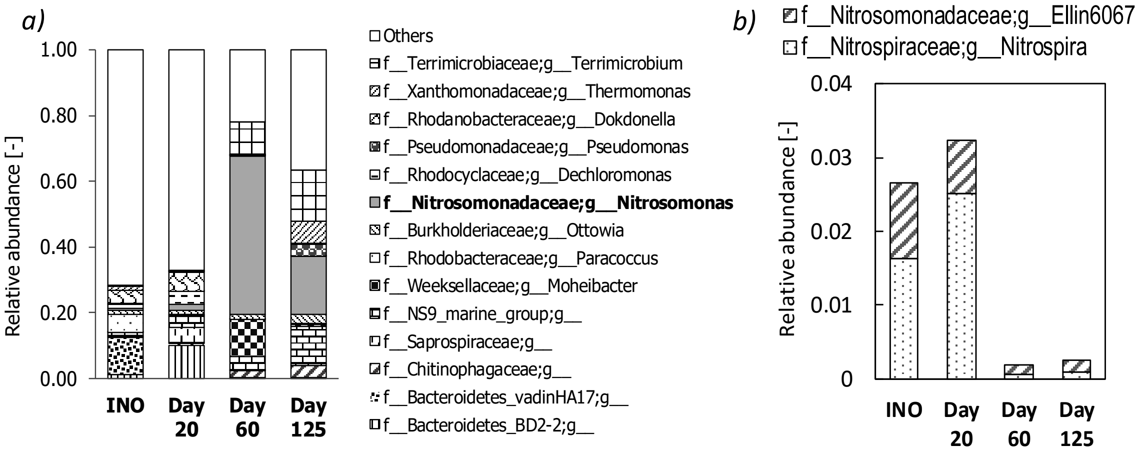

Figure 4. *Microbial community composition at genus level (filtering genera with a minimum relative abundance of 4% in any sample) (a) and relative abundance of the genus Ellin6067 and Nitrospira (b) by time course of the partial nitrification reactor*

3.4. Impact of short-term pH fluctuations and nitrification microbial community on N_2O emission

SBRs are widely used for nitrogen removal because they offer enhanced control over oxygen supply and retention times, which are crucial for driving nitrification and denitrification. However, the batch nature of the process can lead to fluctuating conditions, such as pH changes, which can impact N_2O emissions [24], [39], [40]. The results of this study provide key insights into how short-term pH fluctuations influence N_2O emissions in a SBR for partial nitrification. As noted, the nitrification rate remained relatively stable across different pH levels. However, significant variations in N_2O emission factors were observed, with the highest emission occurring at pH 7.6 (0.46%), followed by pH 7.0 (0.38%), pH 8.2 (0.25%), and pH 6.4 (0.24%). These findings highlighted the complex interplay between pH, microbial activity, nitrogen constituent concentrations, and N_2O production in partial nitrification processes. The N_2O emission factors observed in this study were consistent with findings from other studies. Study by Kampschreur et al. [41] found that N_2O emissions were highest at neutral to slightly alkaline pH levels, with a sharp decline in emissions at more acidic and alkaline pH values. Similarly, Law et al. and Kinh et al. [4], [19] reported that N_2O production peaked at pH 7.6 in a nitrifying biofilm reactor. These studies supported the hypothesis that N_2O production is highest at near-neutral pH levels, where both NH_2OH oxidation and nitrifier denitrification can occur simultaneously. The relatively low N_2O emissions observed at pH 6.4 and 8.2 in this study were also in line with previous research [4], [19].

AOB and NOB, which responsible nitrification process, are highly sensitive to environmental conditions, particularly pH, which affects their enzymatic activity and growth. For AOB, the optimal pH for ammonia oxidation tends to be in the range of 7.0 to 8.0 [42], [43]. Similarly, NOB, responsible for the conversion NO_2^- to NO_3^-, also have an optimal pH range of 7.0 to 8.0 [4]. In this study, the stable nitrification rates observed across different pH levels suggest that the

PN process was able to proceed effectively, even under short-term pH fluctuations. This indicates that the operational pH conditions were within the tolerable range for AOB and NOB, allowing for consistent nitrification performance. However, the changes in N_2O emissions suggest that small shifts in microbial processes, depending on pH, may affect N_2O production.

Additionally, it was also well known that free ammonia (FA) and free nitrous acid (FNA) display inhibitory effects to AOB and NOB metabolisms [4], [19], [28]. Temperature and pH exert the changes in the equilibriums of NH_4^+/NH_3 ($NH_4^+ + OH^- \rightleftarrows NH_3 + H_2O$) and NO_2^-/HNO_2 ($NO_2^- + H_2O \rightleftarrows HNO_2 + OH^-$) [44]. The FA can inhibit NOB, resulting in the accumulation of NO_2^-, a precursor to N_2O formation. Meanwhile, FNA inhibits both AOB and NOB, potentially reducing nitrification efficiency and altering the pathways through which nitrite is processed. In this study, at short-term pH changed to 7.6, FA concentrations would be moderately high, favoring the inhibition of NOB more than AOB. This creates an imbalance, leading to a build-up of NO_2^-. AOB, partially inhibited, may not completely convert ammonia to nitrite, which then becomes available for the production of N_2O through the nitrifier-denitrification pathway. At pH 8.2, FA concentrations are high, but the higher pH might reduce the activity of the nitrifier-denitrification pathway. At pH 6.4, FNA concentrations significantly rise. FNA is highly inhibitory to both AOB and NOB, causing a sharp reduction in nitrification rates. Furthermore, the low pH might decrease the efficiency of nitrifier-denitrification pathway, leading to the lowest N_2O emission at this pH level.

Short-term pH fluctuations had a significant impact on both the microbial community and N_2O emissions in the SBR. *Nitrosomonas* is known to produce N_2O during ammonia oxidation, particularly under stressed conditions such as suboptimal oxygen or pH fluctuations [45]. The low abundance of NOB allowed NO_2^- to accumulate, which can be reduced to N_2O, especially during pH shifts [14], [33]. At pH 7.0, the reactor demonstrated high nitritation efficiency, with *Nitrosomonas* dominating the microbial community and moderate N_2O emissions (0.12%). When the pH increased to 7.6, the N_2O emission factor rose sharply to 0.46%, experienced a peak in AOB activity enhancing ammonia oxidation. Lowering the pH to 6.4 reduced nitrification efficiency but resulted in moderate N_2O emissions (0.24%), possibly due to less favorable conditions for AOB or slight inhibition of AOB activity. At pH 8.2, N_2O emissions remained low (0.25%), suggesting that both high and low pH levels create conditions conducive to N_2O production. In steady stage of SBR, the microbial community adapted to the operational conditions of the reactor, with *Nitrosomonas* consistently dominating and extremely low NOB detected. This results highlight that N_2O emissions are highly sensitive to these fluctuations, with optimal pH control at around 7.0 necessary to minimize emissions. The relationship between pH and N_2O emissions in PN has been explored in several studies [19], [26], [28]. Law et al. [28] found the highest N_2O emission rate at pH 8.0 in a PN system, linking it to NH_4^+ oxidation rate. Similarly, Lv et al. [26] showed that N_2O emissions increased as pH decreased from 8.5 to 7.5, due to higher AOB activity. The findings of this study suggest important implications for designing and operating WWTPs using PN processes. The observed link between pH and N_2O emissions indicates that precise pH control could effectively minimize N_2O production in these systems.

4. Conclusions

This study demonstrated the influence of short-term pH fluctuations on N_2O emissions and microbial community dynamics in a sequencing batch reactor for partial nitrification. The findings reveal that while partial nitrification efficiency remained stable across different pH conditions, N_2O emissions were sensitive to pH changes. The highest N_2O emissions occurred at pH 7.6, while pH 7.0 provided the most balanced conditions for efficient nitrification with relatively lower emissions. The microbial community, particularly the dominance of *Nitrosomonas*, played a key role in driving ammonia oxidation and influencing emission patterns. These findings highlighted the need to keep pH stable within the optimal range to reduce N_2O emissions in wastewater treatment, promoting more sustainable and eco-friendly practices.

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