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Research article

Optimization of Aeration Strategies in Moving Bed Biofilm Reactor for Hospital Wastewater Treatment

Paranee Sriromreun[1], Yanika Lerkmahalikit[2], Suchira Thongson[3] and Suthida Theepharaksapan[3]*

[1]Department of Chemical Engineering, Engineering Faculty, Srinakharinwirot University, Nakhon Nayok, 26120, Thailand
[2]Central Instruments Center Building, Excellent Center Sustainable, Engineering faculty, Srinakharinwirot University, Nakhon Nayok, 26120, Thailand
[3]Department of Civil and Environmental Engineering, Engineering Faculty, Srinakharinwirot University, Nakhon Nayok, 26120, Thailand

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Abstract

The growing complexity of hospital wastewater presents significant challenges for traditional treatment methods, highlighting the need for innovative and energy-efficient alternatives. This study investigated the effect of different aeration strategies on the biological removal efficiency of carbon and nitrogen in a laboratory-scale moving bed biofilm reactor (MBBR). The system was operated under three aeration conditions: continuous aeration and two intermittent aeration regimes. The results show that intermittent aeration enhanced nitrogen removal through simultaneous nitrification-denitrification (SND) while maintaining high organic matter degradation efficiency. In Phase I (continuous aeration), carbon removal efficiency reached 87.4%, whereas total nitrogen removal remained below 20% due to the absence of anoxic conditions. In contrast, intermittent aeration in Phases II and III significantly improved nitrogen removal to 82.5% and 87.8%, respectively, while achieving comparable carbon removal rates. The most effective operational mode involved a 40-min aeration period followed by 20 min without aeration, facilitating denitrification and minimizing energy consumption. Furthermore, the system maintained stable biofilm development, with mixed liquor suspended solids (MLSS) concentrations ranging from 1.9 to 3.3 g/L. Despite the low MLSS concentration, the system consistently exhibited high treatment performance, underscoring the robustness of biofilm-based processes in MBBR systems. These findings highlight the potential of optimized aeration strategies in MBBR systems to enhance treatment efficiency while reducing operational costs. This study provides valuable guidance for designing sustainable hospital wastewater treatment systems that meet stringent regulatory standards.

Keywords: hospital wastewater; biological treatment; MBBR; aeration; nitrogen removal

*Corresponding author: E-mail: suthidat@g.swu.ac.th
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1. Introduction

The increasing scale of healthcare services and the growing complexity of medical practices have led to a substantial rise in both the volume and complexity of hospital wastewater (Kumari et al., 2020). Hospital wastewater treatment plants (WTP) face critical environmental challenge due to the highly complex composition of the wastewater, which often contains elevated levels of organic matter, pathogens, and refractory substances used for medical purposes in a wide range of concentrations. Additional contributors include waste from laboratory and research activities and the excretion of medicines such as pharmaceuticals, contrast agents, radionuclides, solvents, and disinfectants, many of which are resistant to conventional biological degradation (Verlicchi et al., 2010; Dai et al., 2023).

Traditional wastewater treatment methods often fall short in effectively handling such complex effluents and are typically energy-intensive (Bhandari et al., 2023). Recent studies have emphasized the urgent need for advanced biological treatment systems capable of removing emerging contaminants such as pharmaceuticals and antibiotic residues (Zhang et al., 2020; Bhandari et al., 2023), which pose significant ecological and public health risks (Eapen et al., 2024; Ugoeze et al., 2024). As a result, developing cost-effective and energy-efficient alternatives is crucial for achieving compliance with increasingly stringent environmental regulations.

Biological treatment has been recognized as a sustainable and practical alternative to conventional chemical approaches, which are often associated with high operational costs and environmental impacts. Within this context, the moving bed biological reactor (MBBR) has received considerable attention due to its high treatment efficiency and operational adaptability (Sindhi & Shah, 2015; Gupta et al., 2022; Madan et al., 2022). Compared to conventional systems such as activated sludge or sequencing batch reactors (SBRs), MBBRs offer advantages including compact design, reduced sludge production, and greater resilience to fluctuations in influent quality, making them particularly suitable for hospital wastewater treatment. Unlike traditional suspended-growth systems, MBBR integrates suspended and attached growth by supporting biofilm development on freely moving plastic carriers within the reactor (McQuarrie & Boltz, 2011; Accinelli et al., 2012).

An additional advantage of MBBR is its ability to facilitate SND within a single reactor. This is made possible by oxygen gradients across the biofilm matrix: dissolved oxygen diffuses inward from the bulk liquid, creating aerobic conditions near the surface for nitrification and anoxic zones deeper within the biofilm where denitrification can proceed (Van Loosdrecht & Jetten, 1998). This stratification allows both processes to occur simultaneously within a single reactor, eliminating the need for separate tanks (Yamagiwa et al., 1998; Hibiya et al., 2003; Satoh et al., 2004). Several studies have demonstrated that MBBR systems can effectively remove both nutrients and micropollutants while maintaining stable treatment performance, even under fluctuating wastewater characteristics (Almomani & Bohsale, 2020; Madan et al., 2022; Nourredine & Barjenbruch, 2024).

Despite these benefits, aeration remains a major operational challenge in MBBR systems. Continuous aeration, though commonly used to maintain dissolved oxygen (DO), often results in high energy costs and limited nitrogen removal due to the lack of anoxic conditions. Intermittent aeration alternating between aerated and non-aerated phases has been proposed as a more energy-efficient alternative that enhances nitrogen removal by enabling SND (Derco et al., 2017). Controlled ON–OFF cycles allow for aerobic nitrification followed by anoxic denitrification, improving overall total nitrogen (TN) removal while

reducing energy demand (Hidaka et al., 2002; Di Bella & Mannina, 2020; Muttaqin et al., 2022). However, limited research has systematically evaluated how different aeration regimes affect both carbon and nitrogen removal in MBBR systems treating real hospital wastewater.

This study aimed to address this gap by systematically analyzing the effect of continuous and intermittent aeration strategies on carbon and nitrogen removal efficiency in an MBBR system used for treating hospital wastewater. Three aeration conditions, continuous aeration and two intermittent aeration regimes, were evaluated to identify the optimal aeration strategy for maximizing pollutant removal while minimizing energy consumption, providing quantitative insights into aeration optimization for the development of more energy-efficient and sustainable hospital wastewater treatment solutions, and supporting regulatory compliance and improved environmental management.

2. Materials and Methods

2.1 Water sample

Hospital wastewater was collected daily from the equalization tank of the wastewater treatment facility at a teaching hospital and medical center in Nakhon Nayok Province, Thailand. The hospital, which is affiliated with the medical faculty in a university, has a capacity of 500 beds and employs approximately 1100 staff members. The influent wastewater was stored in a 70-L tank before being fed into the MBBR.

2.2 Lab-scale MBBR and operating conditions

Laboratory-scale experiments were performed at the Environmental Engineering Laboratory of Srinakharinwirot University, where the research system was installed and operated. Figure 1 illustrates a schematic diagram of the MBBR system. Wastewater was introduced into the first chamber using a peristaltic pump. The second chamber functioned as a combined clarification and settling unit, designed to remove total suspended solids (TSS) and recirculate residual BOD to the first chamber for further treatment. Aeration was provided through a coarse-bubble diffuser located at the base of the reactor, with high-efficiency regenerative blowers supplying oxygen and ensuring adequate mixing of the biomedia carriers. The carriers, composed of polyethylene, were free-floating elements with a specific surface area of 500 m^2/m^3 (25 mm diameter, 12 mm height), supporting biofilm growth and enhancing treatment performance. A peristaltic pump was utilized to control the influent flow entering the system. For reactor start-up, a 10-L sample of seed sludge obtained from the aeration tank of a municipal wastewater treatment facility was introduced into the bioreactor. Biomedia carriers were added to the reactor, maintaining a 33% filling ratio, a value selected based on practical operational parameters observed in full-scale hospital wastewater treatment systems. This filling ratio fell within the recommended range (30-50%) for optimal biofilm development and pollutant removal in MBBR systems, as reported in previous studies (Cao et al., 2017; Eshamuddin et al., 2024; Wang et al., 2024). Maintaining 33% media filling balances surface area availability for microbial attachment and efficient oxygen transfer, preventing excessive clogging while

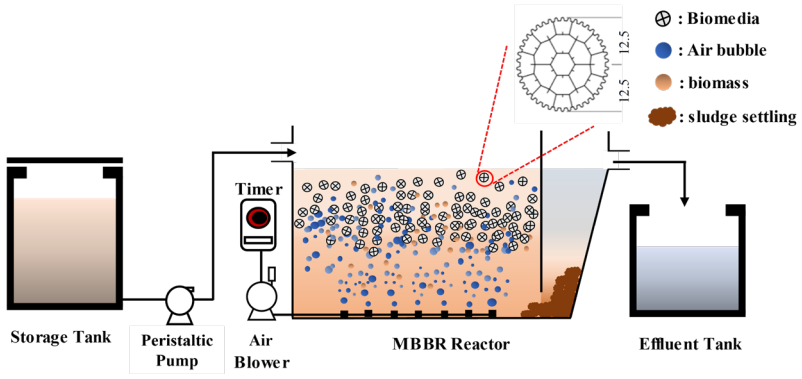


Figure 1. Schematic diagram of the media-containing reactor system

ensuring sufficient biofilm activity. Several operational adjustments were made during the early experimental phase to optimize hydraulic performance. The operational phase was conducted on a bench scale designed to support the development of suspended activated sludge and biofilm on free-floating plastic media, thereby simulating real-scale treatment conditions in hospital wastewater management.

The experimental campaign lasted 97 days, with a hydraulic retention time (HRT) of 12 h and a sludge retention time (SRT) of 15 days, following an initial 14-day cultivation phase in batch mode. After this period, operational conditions were systematically adjusted to compare the performances of continuous aeration and intermittent aeration strategies. A timer controlled the on/off cycles during intermittent aeration to ensure precise regulation of aeration intervals.

The experimental campaign was divided into three phases (Table 1), each with a different aeration time (ta). The first phase lasted 30 days under continuous aeration (CA) conditions. In the second phase, the total cycle time (tc) was 30 min, with 15-min cycles alternating between aeration and anoxic periods, resulting in aeration occurring for 50% of the cycle. In the third phase, the aeration duration was extended to 40 min, yielding a t_a/t_c ratio of 0.66, with 40 min of aeration followed by 20 min of anoxic conditions. These aeration regimes were designed to complete a 60-min operational cycle, which facilitates consistent time-based control and comparative performance analysis. The 15:15 and 40:20 ON/OFF settings also align with intermittent aeration strategies reported in previous studies that successfully enhanced nitrogen removal in MBBR systems treating hospital or high-strength wastewater (Di Bella & Mannina, 2020; Muttaqin et al., 2022).

2.3 Analytical methods

The water samples were analyzed by assessing various parameters, including DO, pH, temperature, conductivity, oxidation-reduction potential (ORP), and total dissolved solids (TDS) using appropriate analytical probes. MLSS was analyzed following the standard method (JSWA, 1997). Ammonium nitrogen (NH_4-N), total Kjeldahl nitrogen (TKN), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and suspended solids (SS) concentrations were measured according to standard methods (APHA, 2017). Nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) analyses were conducted using Hach Lange cuvettes and a DR900 spectrophotometer (Hach Lange, Belgium).

Table 1. Aeration mode of MBBR system

Condition	Experimental Phases		
	I	II	III
Operational period	day 0-29	day 30-62	day 63-97
Duration (day)	30	33	35
Aeration Condition	continuous	intermittent	intermittent
Cycle Time (min)	-	30	60
Aeration Time (min)	continuous	15	40
Anoxic Time (min)	-	15	20

2.4 Biomass of attached biofilm

During each experimental phase, ten biomedia carriers were sampled from the reactor and placed in pre-weighed aluminum cups. These samples were dried at 105°C overnight to obtain the initial dry weight. The attached biomass was then removed by treating the carriers with 2 M NaOH, followed by thorough rinsing with deionized water, and subsequently re-drying under the same conditions. Biomass accumulation was calculated as the difference in dry weight before and after biomass removal and normalized by the surface area of each carrier. The decision to use ten biomedia carriers per sampling event was based on methodologies reported in previous MBBR studies, in which analyzing a representative subset of 5-15 biomedia carriers provided reliable estimates of biofilm biomass while minimizing disruption to reactor operation (McQuarrie & Boltz, 2011; Fonseca & Bassin, 2019).

2.5 Statistical analysis

Data were collected from triplicate experiments and are presented as mean±standard deviation (SD). Statistical analyses were conducted using SPSS for Windows, ver. 20.0 (IBM; Armonk, NY, USA). A one-way analysis of variance (ANOVA) was conducted to assess the effects of different aeration conditions on treatment performance, with significance determined at $p<0.05$. Pearson's correlation was used to examine the relationship between DO levels and TN removal efficiency. All tests were carried out at a 95% confidence level.

3. Results and Discussion

3.1 Hospital wastewater compositions

The characteristics of the hospital wastewater used in this study are summarized in Table 1. The pH value of 6.73±0.61 falls within an acceptable range for biological treatment but may require buffering to support optimal microbial activity (Tchobanoglous et al., 2003). The high TDS concentration (725.33±538.15 mg/L) indicates the presence of dissolved ions that could potentially inhibit microbial metabolism (Verlicchi et al., 2010; Eshamuddin et al., 2024). The TSS level (80.50±43.44 mg/L) suggests a moderate amount of particulate matter, which may necessitate effective clarification to avoid excessive biomass accumulation in the bioreactor (Eshamuddin et al., 2024). The organic load, represented by BOD_5 (140.83±108.54 mg/L) and COD (569.33±443.04 mg/L), reflects the presence of

both biodegradable and refractory organic compounds. A low BOD_5:COD ratio (<0.3) suggests poor biodegradability (Metcalf & Eddy, 2014), consistent with the presence of pharmaceutical residues and disinfectants typically found in hospital effluents (Kumari et al., 2020; Bhandari et al., 2023). Although this study did not quantify specific antibiotics or disinfectants, their presence was expected and may have influenced microbial activity and treatment performance. Regarding nitrogen content, the Total Kjeldahl Nitrogen (TKN) concentration (89.38±60.60 mg/L), primarily consisting of ammonium nitrogen (NH_4-N) (72.52±50.80 mg/L), indicates a significant nitrogen load, which requires efficient nitrification-denitrification processes for effective removal (Ruiz et al., 2006; Pan et al., 2022). The nitrate-nitrogen (NO_3-N) concentration (9.78±6.41 mg/L) further suggests some level of prior biological conversion but highlights the need for enhanced nitrogen removal strategies, such as SND in MBBR systems (Hidaka et al., 2002). Several influential parameters exhibited high standard deviations, reflecting the inherent variability in hospital wastewater resulting from daily fluctuations in patient services, laboratory activities, and pharmaceutical usage. Despite this variability, the MBBR system demonstrated stable treatment performance across all phases, indicating strong resilience of the biofilm community to influent shock loads.

Overall, the substantial variability in wastewater characteristics as indicated by the large standard deviations in Table 2 reflects the highly fluctuating nature of hospital discharges, which presents significant challenges for consistent treatment performance. Elevated concentrations of key pollutants, particularly COD, TKN, and NH_4^+-N, underscore the need for robust biological processes to ensure adequate removal prior to discharge. Given the likely presence of persistent contaminants, conventional treatment methods may prove insufficient. This reinforces the need for advanced biological technologies tailored to handle such complex effluents. In particular, the high ammonium load highlights the importance of precise aeration control to support effective nitrification. Meanwhile, implementing intermittent aeration can promote denitrification, enabling more complete nitrogen removal. This study highlights the critical role of optimized aeration strategies in enhancing MBBR performance. By balancing treatment efficiency with energy conservation, such strategies offer a sustainable and practical solution for hospital wastewater management.

Table 2. Typical compositions of the hospital wastewater

Parameters	Unit	Average Value
pH	-	6.73±0.61
Total Dissolved Solids (TDS)	mg/L	725.33±538.15
Total Suspended Solids (TSS)	mg/L	80.50±43.44
Total Kjeldahl Nitrogen (TKN)	mg/L	89.38±60.60
Ammonium nitrogen (NH_4-N)	mg/L	72.52±50.80
Nitrate-nitrogen (NO_3-N)	mg/L	9.78±6.41
BOD_5	mg/L	140.83±108.54
COD	mg/L	569.33±443.04

Note: Values are presented as mean±standard deviation (SD), based on 10 influent samples (n = 10).

3.2 Operating conditions of the MBBR

The MBBR system maintained stable physicochemical conditions throughout the experimental period, supporting consistent microbial activity and reliable treatment performance. Operational parameters including pH, DO, temperature, organic loading rate (OLR), nitrogen loading rate (NLR), and MLSS were routinely monitored to assess system stability. As shown in Figure 2, the variations in these parameters reflect the influence of aeration strategies on reactor behavior and provide insight into operational optimization for environmental management.

The pH remained relatively stable in Phases I and II, averaging 7.3±0.4, which is within the optimal range for biological treatment. In Phase III, however, a significant increase in pH was observed. This may be attributed to the longer aeration period (40 minutes) and reduced anoxic phase (20 min), which altered the system's alkalinity balance. Increased nitrification during extended aeration resulted in greater NH_4^+-N oxidation to NO_3^--N, a process that consumes alkalinity. Nonetheless, the stripping of CO_2 during aeration may have offset the acidifying effect, leading to a net rise in pH. Phases II and III, which employed intermittent aeration, allowed a more balanced nitrification–denitrification cycle and contributed to pH stabilization. These pH trends align with biochemical mechanisms: nitrification under aerobic conditions releases hydrogen ions (H^+), lowering pH, while denitrification under anoxic conditions consumes H^+, restoring alkalinity and raising pH (Tchobanoglous et al., 2003; Henze et al., 2006). In Phase III, the shorter anoxic duration likely reduced denitrification efficiency and limited the pH-buffering effect.

DO levels ranged from 3.5 to 7.1 mg/L, with a mean of 5.3±1.6 mg/L. In Phase I (continuous aeration), DO remained consistently high due to uninterrupted oxygen supply. In contrast, intermittent aeration in Phases II and III resulted in marked fluctuations, with DO levels dropping during non-aerated periods and recovering during aeration. This pattern facilitated SND, enhancing nitrogen removal efficiency. Although the average DO concentration in Phase III was slightly lower than in Phase I due to the intermittent aeration pattern, it remained within the optimal range (4.5-6.0 mg/L) to support both nitrification and denitrification without compromising microbial activity (Cao et al., 2017; Wang et al., 2020). Meanwhile, temperature remained within a narrow and favorable range of 27.8-31.2°C (average: 29.6±1.4°C), providing a stable environment for microbial communities and biofilm processes. These stable conditions likely minimized external environmental influence on system performance. The OLR during the experimental period ranged from 0.026 to 0.032 kg COD/m³·d (average: 0.030±0.002), while the NLR varied between 0.015 and 0.024 kg N/m³·d (average: 0.019±0.0003). These loading rates fall within operational ranges reported to support effective biofilm formation and stable pollutant removal in MBBR systems (Di Bella & Mannina, 2020; Boavida-Dias et al., 2022). The relatively consistent OLR across all phases ensured a steady supply of substrate for microbial metabolism, promoting biofilm stability and sustained COD removal. This operational consistency likely contributed to the system's reliable performance throughout the study. A progressive decrease in MLSS concentration was observed (Figure 2f), dropping from 5.0 g/L to a stable range of 1.9-3.3 g/L, which could be attributed to the extended hydraulic retention time (HRT) of 12 days, low organic content in influent wastewater, and the potential inhibitory effects of pharmaceuticals and disinfectants in hospital wastewater (Jiang et al., 2018; Kawan et al., 2022).

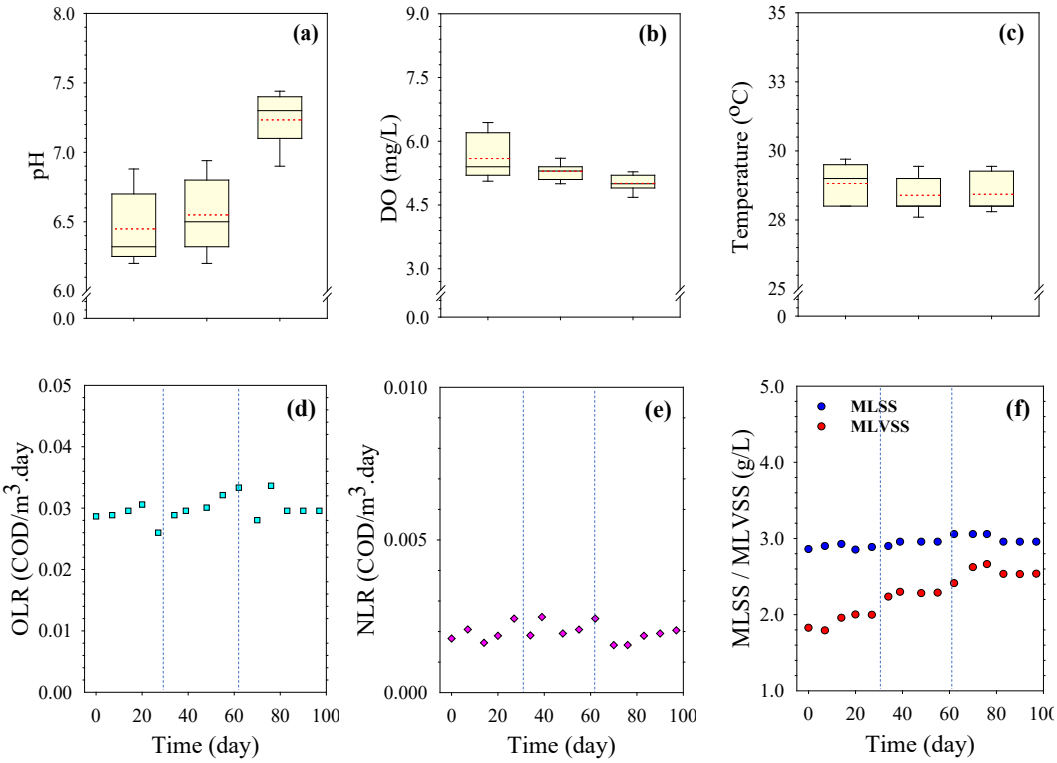


Figure 2. The operational parameters of the MBBR system include: (a) pH, (b) DO, (c) temperature, (d) OLR, (e) NLR, and (f) MLSS concentration. In each plot, the box boundaries represent the interquartile range, which includes the 25th and 75th percentiles. The whiskers extend to the 5th and 95th percentiles. The central horizontal line within the box indicates the median, while the red dashed line represents the mean value.

3.3 Carbon and nitrogen biological removal

The performance of the MBBR over all experimental periods is summarized in Table 3. This table presents the influent and effluent quality values, along with the corresponding average removal efficiencies for each period. The COD removal efficiencies remained consistent across all three phases: 87.4% in Phase I (continuous aeration), 88.8% in Phase II (intermittent aeration with 15 min ON and 15 min OFF), and 89.2% in Phase III (intermittent aeration with 40 minutes ON and 20 minutes OFF). These results align with previous findings that biofilm-based systems can maintain stable organic degradation under varying aeration strategies (Zhang et al., 2020). The stability of MLSS across phases supports the idea that biofilms help maintain microbial activity and prevent biomass washout (McQuarrie & Boltz, 2011). Although the COD to BOD_5 ratio of the hospital wastewater was approximately 4, indicating the presence of poorly biodegradable compounds, the system consistently achieved COD removal efficiencies above 85% across all phases. This performance is likely due to the advantages of the biofilm-based MBBR configuration, which supports microbial communities capable of co-metabolizing recalcitrant organics. The extended hydraulic retention time (12 h) and stable organic

loading rate further enhanced the conditions for effective biodegradation. These results align with previous studies where similar treatment efficiency was reported for hospital wastewater using MBBR systems (Verlicchi et al., 2010).

Table 3. Influent and effluent qualities. (n=5)

Phase	Parameter	Influent (mg/L)	Effluent (mg/L)	Removal (%)
I	COD	563±29.9	70.7±6.7	87.4±1.7
	TKN	38.3±5.4	15.0±2.6	60.8±6.6
	TN	45.3±5.0	15.7±2.4	65.5±5.3
II	COD	604±32.8	67.2±2.7	88.8±0.6
	TKN	42.3±4.9	7.9±1.2	81.2±2.5
	TN	48.9±4.3	8.6±1.2	82.5±2.2
III	COD	590±36.9	63.6±6.2	89.2±1.0
	TKN	35.1±3.9	4.2±0.4	87.7±2.4
	TN	40.9±3.9	5.0±0.3	87.8±1.8

In contrast to carbon removal, nitrogen removal was strongly influenced by aeration strategy (Figure 3). During Phase I (continuous aeration), NH_4^+-N concentrations decreased significantly, indicating efficient nitrification (>60%), but TN removal remained low (~20%) due to the absence of anoxic conditions required for denitrification (Hidaka et al., 2002). Interestingly, despite the lack of a defined anoxic phase in Phase I, a TN removal efficiency of approximately 65% was observed. This removal is likely attributed to microbial assimilation, where nitrogen is incorporated into newly formed biomass during cell growth. Additionally, the biofilm matrix may have developed anoxic zones in its deeper layers, facilitating limited SND even under bulk aerobic conditions. These mechanisms are supported by previous studies on biofilm-based systems (Henze et al., 2006; McQuarrie & Boltz, 2011), which highlight the role of internal oxygen gradients in supporting partial denitrification without explicit anoxic operation. The introduction of intermittent aeration in Phases II and III significantly improved TN removal efficiency. In Phase II (15 min ON/15 min OFF), denitrification was enhanced, leading to a total nitrogen removal of 82.5%. The further extension of the aeration phase in Phase III (40 min ON/20 min OFF) improved nitrification efficiency, reaching 87.8% TN removal, while maintaining sufficient anoxic conditions for denitrification (Table 3). These results are consistent with literature indicating that well-balanced aeration cycles promote effective nitrogen removal in MBBR systems (Wang et al., 2020). This finding aligns with our Phase III results, where the 40:20 min ON–OFF aeration regime provided optimal conditions for SND, leading to 87.8% TN removal.

The DO levels remained within the ideal range (4.5-6.0 mg/L), allowing both nitrifying and denitrifying microbial populations to thrive. Wang et al. (2020) similarly reported that appropriately timed intermittent aeration promotes oxygen stratification within the biofilm, enabling both aerobic and anoxic reactions to proceed in parallel. This operational consistency supports the validity of our aeration strategy in achieving enhanced nitrogen removal.

The TN removal efficiency observed in Phase III (~87.8%) aligns closely with the findings of Di Bella and Mannina (2020), who reported ~90% TN removal in a hybrid MBBR under a 40:20 min aeration regime. While both studies applied a 40:20-minute intermittent aeration strategy, Di Bella and Mannina (2020) reported slightly higher COD (93%) and TN

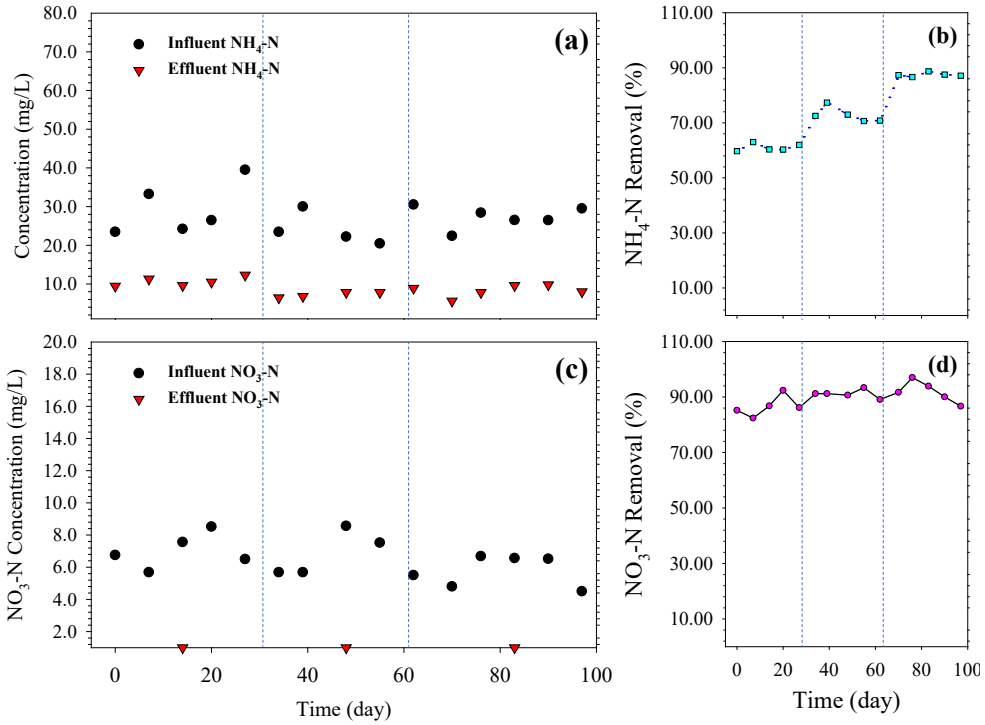


Figure 3. Concentrations of NH_4^+-N and NO_3^--N in the influent and effluent under different aeration modes, along with nitrogen removal efficiencies in the MBBR system.

(90%) removal efficiencies. However, their system treated synthetic wastewater under controlled conditions, whereas the present study was operated under real hospital wastewater influent, which is typically more variable and contains complex pharmaceutical residues. The comparably high removal efficiencies achieved in our study (89.2% COD, 87.8% TN) highlight the robustness and practicality of this aeration approach for real-world applications. The effectiveness of the intermittent aeration strategy can be attributed to the alternation of aerobic and anoxic phases. During the ON period, ammonium is oxidized to nitrate by autotrophic nitrifiers. During the OFF phase, the environment becomes anoxic, enabling heterotrophic denitrifiers to reduce nitrate to nitrogen gas using residual organic carbon. The biofilm matrix further facilitates SND by maintaining oxygen gradients that support both reactions within a single reactor volume.

Although high frequency DO profiling was not performed, routine measurements confirmed that DO levels during OFF phases often dropped below 0.5 mg/L, suggesting the development of anoxic conditions. The substantial improvement in TN removal in Phases II and III compared to Phase I provides additional indirect evidence for effective denitrification. This phenomenon can be attributed to SND occurring within the biofilm. The oxygen diffusion gradient across the biofilm creates aerobic zones near the surface for nitrification and anoxic zones deeper within the matrix for denitrification, even when bulk DO is not entirely depleted. Similar mechanisms have been reported in other MBBR studies (McQuarrie & Boltz, 2011; Wang et al., 2020).

The intermittent aeration also caused DO levels to drop significantly during OFF periods, facilitating effective denitrification. This observation aligns with Verlicchi et al.

(2010), who emphasized the importance of DO fluctuations in promoting nitrogen transformation in biofilm systems. The optimal OLR (0.03 kg COD/m³·d) in Phase III further supported denitrification by ensuring sufficient organic carbon (Henze et al., 2006). The results are consistent with other MBBR studies of hospital wastewater treatment, which report TN removal efficiencies of 80-90% under optimized aeration regimes (Sindhi & Shah, 2015).

To further explain the influence of DO levels, we conducted a correlation analysis showing that TN removal improves with DO up to 6.0-6.5 mg/L, beyond which performance plateaus (~88%). This plateau likely results from oxygen saturation in the biofilm, which disrupts anoxic zones needed for denitrification. High DO levels inhibit denitrifying bacteria by eliminating anoxic microenvironments (Henze et al., 2006; Wang et al., 2020).

These findings confirm that intermittent aeration enhances nitrogen removal while maintaining high COD removal. DO control and phase timing are thus critical for optimizing SND. Maintaining DO within 4.5-6.0 mg/L is recommended for maximizing TN removal while limiting energy use. Real-time DO monitoring in full-scale systems can improve efficiency and operational sustainability. It should be noted that while average DO values were monitored across each phase (Figure 2), high-resolution DO profiling during individual ON/OFF cycles was not performed. Such data would provide more precise insights into short-term oxygen dynamics and better validate SND mechanisms. Future research should address this through intra-cycle DO monitoring. One limitation of this study is the absence of microbial community analysis, which limits insight into the specific microbial populations responsible for pollutant removal. Incorporating microbial profiling methods such as 16S rRNA gene sequencing in future studies would provide a deeper understanding of biofilm structure and functional dynamics in MBBR systems.

4. Conclusions

This study demonstrates that intermittent aeration in an MBBR system significantly improves TN removal while maintaining high organic matter degradation efficiency. The optimized 40:20-min aeration cycle achieved approximately 87% TN removal, with effluent TN concentrations consistently below 5 mg/L, meeting stringent hospital wastewater discharge standards. In Thailand, the regulatory standard for TN in hospital wastewater effluent is 35 mg/L, indicating that the Phase III results (TN ≈ 5 mg/L) significantly exceed compliance requirements and confirm the system's suitability for practical implementation.

This aeration strategy offers several operational advantages. It can be integrated into full-scale WWTPs with minimal infrastructure modifications, providing a cost-effective and scalable solution. By reducing oxygen input during non-aerated phases, the system lowers energy consumption while enhancing SND performance. Additionally, the observed low sludge production decreases maintenance and disposal requirements, further contributing to operational sustainability.

Future applications should focus on pilot-scale implementations to assess long-term performance under varying operational conditions. The integration of automated aeration control and real-time nutrient monitoring could further enhance system responsiveness to fluctuations in wastewater characteristics. Overall, this approach supports energy-efficient, resilient, and environmentally sustainable wastewater treatment in hospital settings.

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6. Authors' Contributions

Paranee Sriromreun interpreted the results and prepared the first draft of the manuscript. Yanika Lerkmahalikit performed the statistical analyses and assisted in data interpretation. Suchira Thongson supported the experimental operation and contributed to manuscript revision. Suthida Theepharaksapan conceived and supervised the study, coordinated the experiments, and finalized the manuscript for submission

ORCID
Suthida Theepharaksapan https://orcid.org/0000-0002-4351-4445

7. Conflicts of Interest

The authors declare no conflicts of interest

8. AI Declaration

To improve clarity and readability, the authors used AI-assisted technologies during the writing process. However, the authors retained full responsibility for the final publication, ensuring that the content was thoroughly reviewed and refined.

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