Optimized design of a stable, long term and robust attached growth mainstream partial nitritation system

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Abstract

A sustainable and cost-effective control system to achieve stable mainstream partial nitritation (PN) is essential to transition the anammox process for mainstream municipal wastewater treatment. This study identifies the optimal distinct elevated surface area loading rates (SALR), hydraulic retention times (HRTs), and airflow rates that achieve stable PN performance (i.e., optimum total ammonia nitrogen (TAN) removal kinetics and percent NO_x as nitrite) in a mainstream elevated loaded PN MBBR system. The study shows that TAN SALR, HRT, and airflow rate significantly affect TAN surface area removal rates (SARR) and percent NO_x as nitrite and, as such, identifies the optimal design parameters (TAN SALR, HRT and airflow rate) of a mainstream elevated loaded PN MBBR system. A TAN SALR of 5 g TAN/$m^{2}\cdot$d, HRT of 2h and airflow rate of 1.5 L/min are identified to provide stable PN performance with a TAN SARR of 2.30 ± 0.34 g TAN/$m^{2}\cdot$d and a percent of NO_x as nitrite of 84.8 ± 1.2%.

Keywords Anammox. Moving bed biofilm reactor. Nitrite activity suppression. Cost-effective

Introduction

Conventional nitrification and denitrification systems are well-established and widely implemented for biological nitrogen removal in many water resource recovery facilities (WRRFs) [1]. The process of nitrification includes the oxidation of ammonia to nitrite by ammonia-oxidizing bacteria (AOB) and the subsequent conversion of nitrite to nitrate by nitrite-oxidizing bacteria (NOB). In the biologically mediated denitrification process, nitrate is reduced to nitrogen gas by heterotrophic denitrifiers using an organic carbon source [2]. The organic carbon is often externally sourced and supplied to wastewater treatment trains in treatment trains with stringent effluent ammonia discharge regulations. Although conventional nitrification and denitrification processes are widely employed in treatment facilities for nitrogen removal, they are subject to significant drawbacks such as; intensive aeration demands, organic carbon requirements and increased sludge cell yields when compared to other cost-effective alternative nitrogen removal pathways [3–5]. In addition, both processes are associated with N_2O emission, a lethal greenhouse gas contributing to ozone depletion [3, 6, 7]. These practical limitations have created a need to shift towards less energy-intensive, sustainable, and cost-effective technologies to advance and upgrade WRRFs.

Partial nitritation (PN) and anaerobic ammonia oxidation (anammox), collectively referred to as PN/A, is an energy-efficient and sustainable alternative to conventional nitrification and denitrification processes [8]. The PN/A process enables nitrogen removal to be achieved with 60% less aeration, no carbon source addition, and with sludge production reduced by 80% compared to combined nitrification and denitrification processes [9–11]. PN/A is a two-step process in which the PN processes results in approximately half of the influent ammonia being oxidized to nitrite by AOB and, subsequently, the anammox process via anaerobic ammonia-oxidizing bacteria (AnAOB) oxidize the remaining ammonia to nitrogen gas in the presence of nitrite as an electron acceptor [12]. To date, the PN/A process have been successfully employed to treat elevated ammonia concentrations originating from industrial wastewaters and anaerobic digesters in sidestream municipal wastewater treatment systems [13–15]. The number of installations of PN/A industrial wastewater systems are greater than 50 facilities and the sidestream treatment systems in Europe, Asia and North America currently exceeds 200 full-scale facilities [4, 16, 17].

The direct implementation of the PN/A process to mainstream municipal wastewater treatment is of great interest to further progress efforts of WRRFs to achieve net zero energy or positive energy production within wastewater facilities [18]. To date, studies have established the possibility of mainstream PN/A process through experimental evidence [18–22]. However, it remains difficult to accumulate nitrite and maintain the ideal NH_4^+ :NO_2^- metabolic ratio in mainstream wastewaters due to the challenge of sustaining the suppression of NOB populations or their activity [17, 23]. NOB are detrimental to the PN/A process, as NOB activity readily oxidizes nitrite and hence prevents the AnAOB from performing the subsequent anammox process; thus stymying the entire PN/A process [23–26]. In regards to achieving effective NOB population or activity suppression, the moving bed biofilm reactor (MBBR), a biofilm-based technology, has been studied to achieve stable PN for subsequent anammox operations across a range of operational control strategies [27–30]. MBBR systems have been considered advantageous for PN as the biofilm housing structure of the system may enable the AOB biomass to be preferentially retained within the system biofilms due to the availability of both ammonia and dissolved oxygen (DO) in the bulk liquid phase. While outcompeting the NOB population for the shared substrate of DO within the biofilm structure. Hence, the NOB population is susceptible within PN MBBR systems to DO-limiting conditions that can suppress their population size or activity [25].

Several studies on mainstream PN MBBR systems rely on employing various operational control strategies to establish oxygen-limiting conditions to selectively inhibit NOB populations or NOB activity to achieve stable PN [31]. Kowalski et al. [30] demonstrated the potential for a stable PN process with NOB activity suppression using a combination of DO/total ammonia nitrogen (TAN) ratio control and NOB inhibition using free ammonia (FA). Other studies have explored intermittent system aeration under low DO concentration and continuous system aeration conditions under low DO setpoints between 0.15-0.22 mg O_2/L to selectively inhibit NOB populations or activity [18, 32]. In contrast, rather than maintaining low DO concentrations in the bulk solution, NOB suppression has also been reported at high DO concentrations greater than 4 mg O_2/L within systems that exhibit thin biofilms and alter the feed stream between mainstream wastewaters at 15ºC and synthetic reject water at 30ºC. The synthetic reject waters elevated FA concentrations caused the inhibition of NOB activity in these systems [29]. In addition, a recent study has employed ammonia-based aeration control to selectively suppress NOB populations or activity [33]. Thus, it is evident that various operational control strategies in an MBBR system can be used to achieve PN. However, these control strategies and are operationally intensive and do not demonstrate long-term significant NOB population and/or activity suppression, which has directly limited the application of mainstream PN/A MBBR systems [24, 27].

Recently, Schopf et al. [34] have demonstrated the feasibility of using a passive and low operational intensity design strategy to achieve stable, long term and robust PN in an MBBR system. In particular, Schopf et al. [34] employed elevated TAN loading rates as a design strategy to achieve PN in an MBBR system fed with TAN concentrations of 125 mg TAN/L which are higher than traditional mainstream municipal concentrations. This design strategy (elevated TAN loading rate) has the potential to provide the necessary design for a high-rate, small footprint, and low operational intensity system for stable, long term and robust mainstream PN. However, no studies in the current literature have evaluated the potential of elevated TAN loading rate as a PN design and control strategy under mainstream conditions. Therefore, this study aims to optimize the design of the elevated loading rate PN MBBR system to remove TAN from mainstream municipal wastewater. In particular, the study determines the effects of distinct TAN surface area loading rates (SALR), hydraulic retention times (HRTs), and airflow rates on TAN removal kinetics and nitrite accumulation as percent NO_x and isolates the optimal design of a mainstream elevated loaded PN MBBR system.

Materials and methods

Experimental setup

Three parallel 2L MBBR reactors, PN_1, PN_2 and $PN_{3,}$ with identical dimensions, volumes, and fill fractions of 9.5% were operated in this study (Fig. S1). The reactors were filled with high-density polyethylene AnoxK^{TM}5 carriers (AnoxKaldnes, Lund, Sweden) with a protected biofilm surface area of 800 m^2/m^3. Synthetic wastewater was fed to the reactors with a peristaltic pump, and the reactors were continuously aerated from the base using an air pump. The air was dispersed by an air diffuser stone connected to a regulator to allow adequate control and provide continuous uniform mixing and DO to the reactors. All reactors were operated at ambient temperature, and no external pH or temperature control was applied.

Reactor inoculation and start-up

The AnoxK^{TM}5 carriers were seeded carriers harvested from a single bench scale PN MBBR system operated at elevated TAN loading rates. Before this study, carriers were seeded from a biological oxygen demand (BOD) removal municipal integrated film-activated sludge (IFAS) wastewater treatment system located in Hawkesbury, Ontario, Canada [34]. Prior to the optimization of the PN MBBR system and isolation of optimal design parameters (TAN SALR, HRT, and airflow rate) in this study, the harvested carriers were distributed into three identical reactors, PN_1, PN_2 and $PN_{3,}$ all designed to run in parallel under the same operational conditions: Influent TAN of 41.1 ± 1.2 mg TAN/L, TAN SALR of 7 g $TAN/m^{2\cdot}d$, HRT of 2h, DO concentration of 6.5 ± 0.2 mg O_2/L, pH of 7.5 ± 0.1, temperature of 19.8 ± 0.2°C. The initial phase of the study was performed until each of the reactors, PN_1, $PN_{2,}$ and PN_3 demonstrated TAN surface area removal rates (SARR) of greater than 2.93 g $TAN/m^{2\cdot}d$ and percent of total oxidized TAN as nitrite (NO_x as nitrite) of greater than 80%, which indicated stable PN was achieved. These conditions in the initial phase were maintained for a minimum of six weeks, during which time the three reactors were tested to validate steady-state operation, with steady-state operation defined as ± 10% fluctuation in TAN removal rate and percent NO_x as nitrite.

Reactor operation

To identify the loading conditions that achieve optimal TAN removal kinetics and percent NO_x as nitrite of the mainstream elevated loaded PN MBBR system, TAN SALR values were applied to reactor PN_1 at values of 4, 5, 6, and 7 g TAN/$m^{2}\cdot d$. The corresponding applied target influent TAN concentrations to reactor PN_1 were 25, 30, 40, and 45 mg TAN/L, which are within the conventional limits of mainstream municipal TAN concentrations. Preliminary experiments were operated over a period of four months, with each condition being run for two weeks at a time to provide the necessary HRT and airflow rate ranges across distinct TAN SALR of 4, 5, 6, 6.5 and 7 g TAN/$m^{2}\cdot d$ to begin the study (Fig. S2, S3).

The preliminary findings informed the use of an HRT of 2h and an airflow rate of 1.5 L/min for the initial SALR optimization experiments (Table 1). At each investigated distinct TAN SALR, the PN_1 reactor was operated for a minimum period of seven weeks. Once steady-state was established, a minimum of three triplicated data points were obtained to quantify the kinetics at each of the applied TAN SALRs of 4, 5, 6, and 7 g TAN/$m^{2}\cdot d$. The optimal design SALR established in this phase was used to revalidate and isolate other critical design parameters of HRT and airflow rate in this study.

Table 1 Operational TAN SALR, HRT, and airflow rate conditions, average ± 95% confidence interval

Reactor	Distinct effect	Influent TAN concentration (mg TAN/L)	SALR (g TAN/$m^{2}\cdot d$)	HRT (h)	Airflow rate (L/min)	DO (mg O_2/L)	Temperature °C	pH
PN_1	TAN SALR	24.7 ± 1.7	3.9 ± 0.1	2.0 ± 0.3	1.5 ± 0.1	6.9 ± 0.1	20.2 ± 0.2	7.7 ± 0.2
		32.8 ± 2.3	4.8 ± 0.2					
		40.3 ± 1.8	6.3 ± 0.1					
		44.6 ± 1.2	7.0 ± 0.2					
PN_2	HRT	25.4 ± 0.1	5.2 ± 0.4	1.5 ± 0.3	1.5 ± 0.1	7.0 ± 0.1	19.5 ± 0.2	7.8 ± 0.3
		30.6 ± 0.3	5.0 ± 0.2	2.0 ± 0.3				
		35.8 ± 0.5	5.2 ± 0.3	2.2 ± 0.2				
		40.9 ± 0.2	5.1 ± 0.4	2.5 ± 1.2				
PN_3	Airflow rate	31.5 ± 0.2	5.2 ± 1.2	2.0 ± 0.2	1.0 ± 0.2	6.7 ± 0.1	19.8 ± 0.1	7.8 ± 0.2
		-			1.5 ± 1.2	6.8 ± 0.3		
		-			2.0 ± 0.2	6.8 ± 0.2		
		-			4.0 ± 0.6	6.9 ± 0.3		

To verify the optimal HRT that results in optimum performance of the mainstream elevated loaded PN MBBR system based on identified optimal TAN elevated SALR, reactor PN_2 was operated at varying distinct HRTs of 1.6, 2, 2.2, and 2.5h. The applied target influent TAN concentrations to reactor PN_2 were 25, 30, 35, and 40 mg TAN/L, corresponding to identified optimal TAN SALR of 5 g $TAN/m^{2}\cdot d$. To isolate the optimal HRT, each distinct HRT was applied to the reactor at a given time (Table 1). Each HRT investigated was maintained for a minimum of seven weeks to validate steady-state performance; once steady state is achieved, triplicate samples were collected across three days and three data points. The identified optimal design HRT and TAN SALR in this phase were used to validate the optimum airflow rate.

To confirm the optimal airflow rate that demonstrates optimum PN performance, reactor PN_3 was operated at varying distinct airflow rates of 1, 1.5, 2 and 4 L/min. The reactor was operated at a constant TAN SALR and HRT (Table 1). At each studied airflow rate, PN_3 was operated for a minimum of seven weeks. Once steady-state was reached, three triplicated data points across three days were obtained.

Each design (combination of TAN SALR, HRT, and airflow rate) for a total of twenty-two weeks at each condition was repeated a minimum of three times to enable the study to progress through all the various conditions and verify the repeatability of the generated results. The optimal design of TAN SALR of 5 g $TAN/m^{2}\cdot d$, HRT of 2h, and an airflow rate of 1.5 L/min were further operated for an additional 60 days until steady-state was reached, and samples were collected in triplicate and analyzed.

Wastewater feed

The synthetic wastewater was prepared based on the recipe by Delatolla et al. [35]. Synthetic wastewater simulating post-carbon removal municipal wastewater treatment was used in this study. The specific composition of synthetic wastewater at a TAN SALR of 4 g $TAN/m^{2}\cdot d$ is as follows (per L of synthetic wastewater): 0.12 g $(NH_4^+)_2SO_4$ (corresponding in a concentration of approximately 25 mg NH_4^+-N/L), 0.325 g $NaHCO_3$, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.02 g $CaCl_2 \cdot 2H_2O$, 0.05 g KH_2PO_4, and 0.003 g $FeSO_4 \cdot 7H_2O$. Trace nutrients (per L of synthetic wastewater): $MnCl_2 \cdot 4H_2O$: 0.10 mg, $Na_2MoO_4 \cdot 2H_2O$: 0.03 mg, $CuSO_4 \cdot 5H_2O$: 0.10 mg, $CoCl_2 \cdot 6H_2O$: 0.001 mg, $ZnSO_4 \cdot 7H_2O$: 0.03 mg. The carbon source composition (per L of synthetic wastewater): glucose 4.86 mg, sodium acetate 2.59 mg and peptone 4.86 mg resulting in a sCOD concentration of 25 mg sCOD/L mimicking post-carbon effluent [34]. At TAN SALRs of 5, 6 and 7 g TAN/$m^{2}\cdot$d, the wastewater composition for all ingredients was adjusted and augmented proportionally.

Analytical methods

Wastewater influent and effluent samples of each reactor were collected at a minimum of three times per week. Standard methods were used to quantify TAN (Nessler-4500C-NH_3), nitrite (4500B- NO_2^-) and nitrate (4500A- NO_3^-), using a DR 5000 spectrophotometer (HACH, Loveland, CO, USA). The sCOD, total suspended solids (TSS), and volatile suspended solids (VSS) were measured using standards methods [36]; HACH 8000, 2540 D-TSS, and 2540 E-VSS, respectively. The DO and temperature were measured using a HACH Flexi HQ30d DO probe meter (HACH, Loveland, CO, USA), and airflow rate was controlled and measured using a Dwyer VFA -24 Visi-Float acrylic airflow meter (DWYER, Michigan City, IN, USA). The pH was measured using a SympHony VWR pH probe (VWR, Canada, Ontario).

Statistical analysis

The student *t*-test was used to validate statistical significance, with a *p*-value of less than 0.05 being considered significant. The correlation between investigated design parameters was determined using a Spearman's rank correlation test with p-value less than 0.05 indicating significance. Error bars in the figures indicate 95% confidence intervals.

Results and discussion

Optimal TAN SALR

The findings from the preliminary study informed the operation of the reactor PN_1 at a consistent HRT of 2.00 ± 0.31h, airflow rate of 1.50 ± 0.07 L/min, DO concentration of 6.90 ± 0.13 mg O_2/L, pH of 7.72 ± 0.11 and temperature of 20.2 ± 0.2°C; to identify the TAN SALR 4, 5, 6 and 7 g TAN/ $m^{2\cdot}$d that achieve optimal PN performance in the mainstream elevated loaded PN MBBR system (Fig. 1). The evaluated TAN SALR 4, 5, and 6 g TAN/$m^{2\cdot}$d applied in this study and their respective TAN SARR demonstrate a first-order reaction with respect to the bulk liquid TAN concentration (linear relation between TAN SALR values of 4 and 5 g TAN/$m^{2\cdot}$d and TAN SARR of 1.71 ± 0.07 and 2.21 ± 0.06 g TAN/$m^{2\cdot}$d) that transitions to a mixed-order reaction (transition between first-order and zero-order reaction at an TAN SALR of 5 g TAN/$m^{2\cdot}$d and TAN SARR 2.21 ± 0.06 g TAN/$m^{2\cdot}$d) to a zero-order reaction (linear slope of approximately zero-order, between TAN SALR of 5 to 7 g TAN/$m^{2\cdot}$d and TAN SARR 2.21 ± 0.06 to 2.44 ± 0.23 g TAN/$m^{2\cdot}$d) (Fig. 1a). The TAN SARR, 1.71 ± 0.07 g TAN/$m^{2\cdot}$d of the system loaded at TAN SALR of 4 g TAN/$m^{2\cdot}$d is statistically significantly lower than the TAN SARR of 2.21 ± 0.06 g TAN/$m^{2\cdot}$d measured at a TAN SALR of 5 g TAN/$m^{2\cdot}$d. This lower TAN SARR at TAN SALR of 4 g TAN/$m^{2\cdot}$d, indicates that the kinetics were likely operated at first order with respect to the bulk liquid TAN concentration, as the system is operating at TAN substrate concentration mass transfer rate limited condition. Meanwhile, the values of TAN SARRs, of 2.21 ± 0.06, 2.23 ± 0.08 and 2.44 ± 0.23 g TAN/$m^{2\cdot}$d measured at TAN SALRs of 5, 6 and 7 g TAN/$m^{2\cdot}$d respectively, shows no statistically significant difference. The lack of distinction between the kinetics at these varying TAN SALRs of 5, 6 and 7 g TAN/$m^{2\cdot}$d indicates that the reactions are zero-order, as the reaction rate no longer increases in relation to TAN SALR values. Hence, the systems transition from TAN substrate concentration mass transfer rate limited while exhibiting first order reaction relation to a DO concentration mass transfer rate limited zero order reaction as TAN SARR becomes independent of TAN SALR and hence TAN concentration [37].

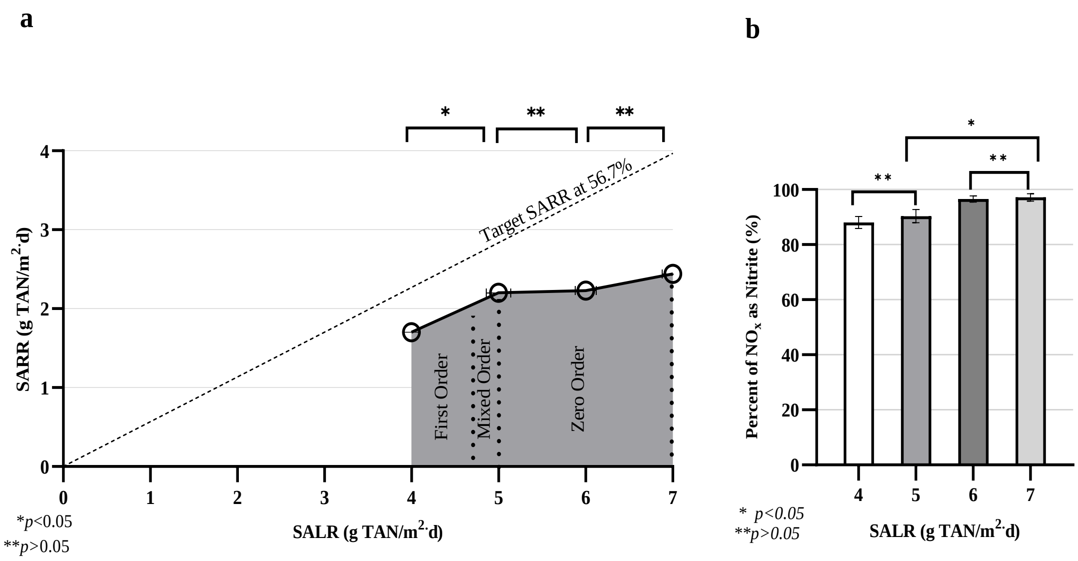

Fig.1 PN MBBR performance: **a** TAN SARR across TAN SALR, with 56.7% removal (equal to metabolic NH_4^+/NO_2^- ratio) represented with a diagonal dashed line (average ± 95% confidence interval) and estimated kinetics indicated with the dark grey shaded area and vertical dotted lines; **b** Percent NO_x as nitrite across TAN SALR (average ± 95% confidence interval)

The dashed line in Fig. 1a indicates 56.7% TAN oxidation, which approximates the ideal TAN oxidation efficiency required for anammox process based on the theoretical NH_4^+:NO_2^- stoichiometric ratio of 1:1.32 [38]. An increase in TAN oxidation efficiency from 32.5 ± 0.9 to 45.7 ± 0.6% is shown between the TAN SALRs of 4 and 5 g $TAN/m^2{\cdot}d$. While the TAN oxidation efficiency did not statistically differ between TAN SALRs of 6 and 7 g $TAN/m^2{\cdot}d$; between the TAN SALRs of 5 and 7 g $TAN/m^2{\cdot}d$, the TAN oxidation efficiency decreases from 45.7 ± 0.6 to 33.7 ± 0.2%. The trend of elevated loading rate resulting in the reduction of TAN oxidation efficiency as the kinetics transitions from first order to mixed order and zero-order with respect to bulk TAN concentration is expected in a biofilm technology [39]. The observed trend in TAN oxidation efficiency with elevated loading rate, although identical to previous studies by Schopf et al. [34], at similar elevated TAN SALR. The TAN oxidation efficiency is not comparable to the values Schopf et al. [34] reported, as the operation conditions, such as the influent TAN concentration and airflow rate, were different. Furthermore, the reduction of TAN oxidation efficiency with increasing TAN

SALRs of 5 to 7 g TAN/m^{2}·d is possibly an indication that the system remained DO concentration mass transfer limited while performing stable PN as the TAN SALR increases above 5 g TAN/m^{2}·d.

The percent NO_x as nitrite did not statistically significantly vary between TAN SALR of 4 and 5 g TAN/m^{2}·d with average values of 88.1 ± 2.1 and 90.3 ± 2.4%, respectively (Fig. 1b). Comparing TAN SALR of 5 to 7 g TAN/m^{2}·d, the percent NO_x as nitrite shows a statistically significant but slight increase ($p=0.009$) to an average value of 94.8 ± 1.4%. Previous studies have inferred that operating at an elevated TAN SALR can potentially suppress the NOB populations or activity due to the preferential uptake of oxygen by the AOB populations, resulting in high nitrite accumulation in the effluent [40]. Moreover, the strong positive correlation ($R_s=0.8621$) between the elevated TAN SALR and percent NO_x as nitrite in this study is probably an indication that elevated TAN SALR can be an effective and efficient design strategy for achieving PN in an MBBR system under mainstream municipal conditions. Although under sidestream conditions, elevated TAN SALR as a PN design strategy was reported ineffective at maintaining adequate NOB populations and/or activity suppression [41]. Further investigation of the microbial communities structure in the biofilm across the elevated TAN SALRs under the conventional TAN concentration is required to identify the mechanism of PN and to identify whether the high percent NO_x as nitrite measured occurs due to NOB activity suppression and/or NOB community suppression in the microbiome.

Overall assessment of the elevated loaded PN MBBR system shows that the design TAN SALR of 5 g TAN/m^{2}·d demonstrates stable performance with a TAN SARR of 2.21 ± 0.06 g TAN/m^{2}·d and percent NO_x as nitrite of 90.3 ± 2.4%. The performance of the elevated loaded mainstream PN MBBR system is comparable to those reported in previous, less stable or unstable mainstream PN MBBR systems that required elevated operation intensity to maintain PN performance [28–30, 42]. PN was achieved in these studies with a combination of DO/TAN ratio control and FA inhibition [28, 30] and a combination of alternate feeding between mainstream and sidestream with limited biofilm thickness [29], control strategies of which are operationally intensive. Whereas elevated TAN SALR, a simple and passive design strategy, used in this study, has been herein shown to achieve robust and stable PN performance without operational intervention. This is possible by designing the system as an elevated TAN SALR, hence likely providing multiple morphological benefits to the biofilms that impact the embedded AOB and NOB communities, as shown in Schopf et al.[34, 43]. Ultimately the elevated loading design strategy results in a stable performance of the mainstream PN system. Therefore, it is believed that elevated TAN SALR is a promising design approach to provide high and stable effluent nitrite for the subsequent anammox treatment under mainstream municipal conditions.

Optimal HRT

The PN MBBR, PN_2 was operated at distinct HRTs of 1.6, 2, 2.2, and 2.5h, at now identified optimal average TAN SALR of 5.22 ± 0.93 g $TAN/m^{2·}d$, temperature maintained at 19.5 ± 0.2°C, airflow rate at 1.50 ± 0.14 L/min, DO at 7 ± 0.12 mg O_2/L, and pH 7.81 ± 0.24 to verify optimal HRT that maintains optimum TAN SARR and percent NO_x as nitrite (Fig. 2). An HRT of 1.6h shows the statistically lowest TAN SARR of 1.57 ± 0.11 g $TAN/m^{2·}d$ and TAN oxidation efficiency of 27.5 ± 0.1% compared to other HRTs of 2, 2.2, and 2.5h investigated in this study (Fig. 2a). The low TAN SARR and TAN oxidation efficiency are likely due to the decrease in AOB populations and/or activity at this short HRT of 1.6h, specifically compared to the typical HRTs of 4h [34] and 12h [28] that have been reported in PN MBBR systems. Another possible contributing factor to this could be that the oxygen transfer efficiency into the biofilm has been lowered under an elevated TAN SALR of 5.26 ± 0.4 g $TAN/m^{2·}d$ at short HRT of 1.6h, inhibiting effective oxygen utilization by the AOB populations [20, 44].

On the other hand, with the increase in HRT from 1.6 to 2h, the TAN SARR and TAN oxidation efficiency statistically significantly increases to 2.39 ± 0.05 g $TAN/m^{2·}d$ and 45.1 ± 0.5%. Meanwhile, a further shift in HRT to 2.2 and 2.5h shows unstable performance, which is indicated by the large variations in TAN SARR (Fig. 2a) and TAN oxidation efficiency measurements of 41.4 ± 6.1 % and 40.2 ± 7.4%. Comparing the TAN SARR and TAN oxidation efficiency measured values at all investigated HRTs, the HRT of 2h, is observed to be optimal and likely supports a high, stable AOB population or activity in the PN MBBR system [19]. Moreover, in a biofilm-based system such as the

MBBR technology, a relatively short HRT of 2h is understood to have a lesser impact on active retained AOB biomass in biofilm systems compared to a suspended growth system, which likely explains the stable performance of the PN MBBR system at a short HRT of 2h [45–47]. In addition, it has been previously suggested that maintaining an appropriate HRT intensifies the microbial interactions within the biofilms [19]. An example includes the competition between AOB and NOB for available shared electron acceptors (DO), particularly under DO limiting conditions achieved through operating at an elevated TAN SALR of 5.01 ± 0.22 g TAN/m^2·d. Therefore, at an HRT of 2h, AOB possibly has a higher competitive advantage for DO than NOB, which causes the DO to be preferentially utilized for TAN oxidation in the PN reactor [48–51].

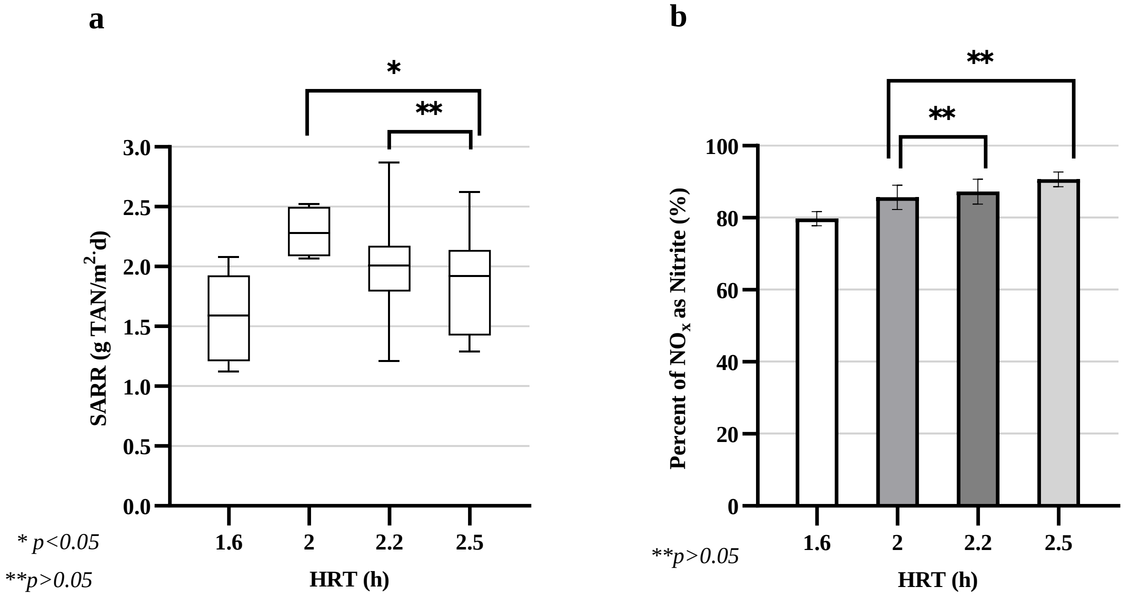

Fig. 2 PN MBBR performance*:* **a** SARR across HRTs, **b** Percent NO_x as nitrite across HRTs, average ± 95% confidence interval

With an increase in HRT from 1.6 to 2h, the percent NO_x as nitrite demonstrates a statistically significant but slight increase (*p = 0.03*) from 79.8 ± 1.8 to 85 .7 ± 3.0% (Fig. 2b). At HRTs of 2, 2.2 and 2.5h, percent NO_x as nitrite did not statistical significantly (*p>0.05*) vary from each other and demonstrates an average percent NO_x as nitrite of 86.6 ± 1.8%. The statistical similarity and moderate correlation ($R_s = 0.4632, p = 0.08$) between increasing HRT and percent NO_x as nitrite may indicate that operating the MBBR system beyond HRT of 2h does not likely support increased NOB populations or activity suppression [32]; or the PN MBBR system has reached its limit of NOB suppression achieving an average percent NO_x as nitrite of 88.4 ± 1.3%. In addition, there is the possibility that a longer HRT could promote faster oxidation of nitrite to nitrate due to increased oxygen transfer resulting from the system operating under reduced TAN SALR, causing deeper oxygen penetration into the biofilm. Finally, it is important to note that a short HRT of 2h is beneficial with respect to the sizing of the system, and hence the capital cost and energy savings due to the aeration of a smaller reactor and an overall smaller land footprint [46]. Therefore, the stable TAN SARR of 2.39 ± 0.05 g TAN/$m^{2\cdot}$d and high percent NO_x as nitrite of 85 .7± 3.0% at an HRT of 2h shows that a PN MBBR system can be operated within a small tank volume to achieve stable PN performance.

Optimal airflow rate

The PN MBBR reactor PN_3 based on the optimal isolated TAN SALR and HRT, was operated at distinct varying airflow rates of 1, 1.5, 2, and 4 L/min, measured TAN SALR at 5.21 ± 1.21 g TAN/$m^{2\cdot}$d, HRT 2.02 ± 0.23h, temperature 19.8 ± 0.1°C, and pH 7.81 ± 0.12 to validate the optimum airflow rate of the mainstream elevated loaded PN MBBR system (Fig. 3). As the airflow rate increases from 1 to 1.5 and to 2 L/min, the average TAN SARR & TAN oxidation efficiency were 1.95 ± 0.21 g TAN/$m^{2\cdot}$d & 37.4 ± 0.2%, 2.32 ± 0.06 g TAN/$m^{2\cdot}$d & 43.6 ± 0.6% , and 2.18 ± 0.11 g TAN/$m^{2\cdot}$d & 40.2 ± 0.2%, respectively (Fig. 3a). As the steady incremental airflow rate of 0.5 L/min did not show an observable statistically significant difference, the airflow rate was increased further to 4 L/min. With the change in airflow rate from 2 to 4 L/min, the TAN SARR significantly increases to 3.19 ± 0.17 g TAN/$m^{2\cdot}$d, corresponding to a TAN oxidation efficiency of 63.8 ± 0.2%. Furthermore, the increase in airflow rate strongly correlates to an increase in TAN SARR and TAN oxidation efficiency ($R_s=0.9532$), highlighting the sensitivity of nitrifying communities to the change in DO concentrations of the bulk liquid [52, 53].

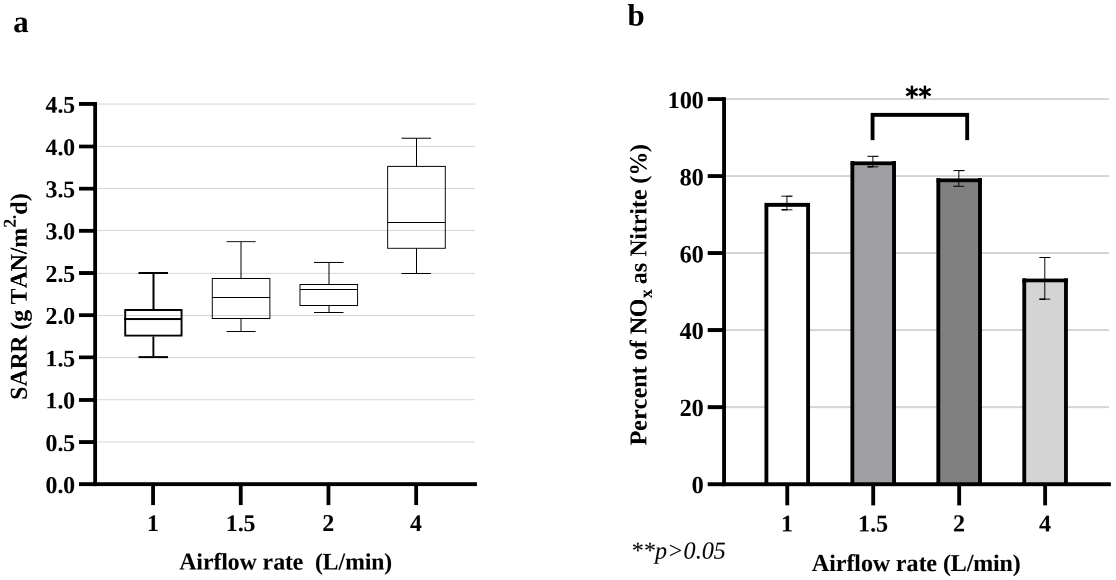

Fig. 3 PN MBBR performance: **a** SARR across airflow rates, **b** percent NO_x as nitrite across airflow rates, average ± 95% confidence interval

The percent NO_x as nitrite shows a statistically significant increase from 73.1 ± 1.3 to 84.8 ± 1.4%, with a change in airflow rate from 1 to 1.5 L/min. However, as the airflow rate increases from 1.5 to 2 and 4 L/min, the percent NO_x as nitrite decreases from 84.8 ± 1.4 to 79.5 ± 1.9 and 53.5 ± 5.0% (Fig. 3b). On the other hand, the bulk DO concentration in the reactor across the airflow rates of 1.5, 2 and 4 L/min did not statistically differ from each other, and demonstrates an average DO concentration of 6.90 ± 0.14 mg O_2/L. It is expected that with the increase in airflow rate above 1.5 L/min, the system is gradually transitioning from PN to complete nitrification. Moreover, with an increased airflow rate, the mass transfer of oxygen from the bulk solution to the biofilm surface improves, subsequently promoting deeper DO penetration through the biofilm to embedded biomass [54, 55]. Therefore, it is possible that the microbial community has shifted, and a significant quantity of NOB cells has accumulated in the system with the shift in airflow rate from 2 to 4 L/min, initiating greater oxidation of nitrite to nitrate (-49.5 ± 1.4%).

Generally, it is believed that NOB populations are more sensitive to DO than AOB as they have a higher oxygen half-saturation coefficient (K_o) than AOB [56]. Hence, high DO concentrations or oxygen-enriched conditions can provide NOB with a competitive advantage over AOB and possibly promote their proliferation or activity in the system. While Chen et al. [19], attributed unchanged bulk DO concentration with increased airflow rate to likely increase in utilization by AOB populations, there was still a possible NOB role, as *Nitrospira* genera were detected in the reactors with an approximately relative abundance of 1.9% [32]. Therefore, although the bulk liquid DO concentration remained unchanged in this study, the increase in oxygen supply with an increased airflow rate greater than 1.5 L/min possibly results in a greater oxygen uptake rate of the reactor, thus, accelerating the activity and/or population of NOB.

This study hence identifies the optimal design TAN SALR, HRT, and airflow rates of 5 g TAN/$m^{2}\cdot$d, 2h, and 1.5 L/min, respectively, for the mainstream elevated loaded PN MBBR system. The overall performance of the optimized low-operational intensive PN MBBR system operated at a temperature of 19.8 ± 0.3ºC, DO of 6.96 ± 0.42 mg O_2/L, and pH of 7.68 ± 0.16 are shown in Table 2. This study shows that stable PN is feasible using an elevated TAN SALR as a design strategy under mainstream conditions. Elevated TAN SALR as a passive and simple design strategy provides a stable PN system with no additional operational control measures. Successful implementation of elevated TAN SALR, a design strategy could progress current efforts to identify low operational intensity and effective PN control strategies at mainstream municipal wastewaters where NOB populations and/or activity suppression have been reported with multiple and complex control strategies resulting in high operational intensive systems [57]. Therefore, this result provides insight into the design of a promising robust, reliable, and low operational intensity PN MBBR system for treatment at mainstream municipal wastewaters.

Table 1 Performance of the mainstream elevated loaded PN MBBR system (average ± 95% confidence interval)

	Influent	Effluent
TAN concentration (mg TAN/L)	32.5 ± 2.21	18.1 ± 0.28
Nitrite concentration (mg NO_2^--N/L)	0.23 ± 0.12	10.9 ± 0.34
Nitrate concentration (mg NO_3^--N /L)	B/LOQ	1.41 ± 0.42
COD (mg/L)	27.6 ± 1.80	18.7 ± 0.81
Alkalinity (mg/L $CaCO_3$)	311.3 ± 13.8	213.5 ± 7.53

B/LOQ-Below the limit of quantification

Conclusion

This study identifies optimal TAN SALR, HRT, and airflow rates that achieves optimum TAN removal kinetics and percent NO_x as nitrite for stable PN performance of the mainstream elevated loaded PN MBBR system. The increase in TAN SALR significantly affects the TAN SARR and percent NO_x as nitrite of the mainstream elevated loaded PN MBBR system. The optimal TAN SALR of 5 g TAN/$m^{2}\cdot$d results in a TAN SARR of 2.21 ± 0.06 g TAN/$m^{2}\cdot$d and percent NO_x as nitrite of 90.3 ± 2.3%. The change in HRT from 1.6 to 2h is shown to affect SARR and percent NO_x as nitrite significantly. At the same time, a further increase in HRT from 2.2 to 2.5h demonstrated an unstable TAN SARR with no significant observable change in percent NO_x as nitrite of the elevated loaded PN MBBR system. The optimal and short HRT of 2h shows a stable PN performance with an average TAN SARR of 2.39 ± 0.05 g TAN/$m^{2}\cdot$d and a percent of NO_x as nitrite of 85.7 ± 3.0%. With an increase in airflow rate from 1 to 4 L/min, the TAN SARR increases from 1.95 ± 0.21 to 3.19 ± 0.17 g TAN/$m^{2}\cdot$d while the percent of NO_x as nitrite decreases from 84.8 ± 1.4 to 53.5 ± 5.0%, as the systems gradually transitions from PN to complete nitrification. The MBBR system demonstrates stable PN at an airflow rate of 1.5 L/min with TAN SARR of 2.30 ± 0.34 g TAN/$m^{2}\cdot$d and a percent of NO_x as nitrite of 84.8 ± 1.2%. The optimal isolated design parameters, TAN SALR of 5 g TAN/$m^{2}\cdot$d, HRT 2h and airflow rates of 1.5 L/min demonstrate a stable PN performance with TAN SARR of 2.30 ± 0.34 g TAN/$m^{2}\cdot$d, and a percent of NO_x as nitrite of 84.8 ± 1.2%. The results herein present the optimal design parameters of the mainstream elevated loaded PN MBBR system. The study provides a possible direct design pathway for implementing a low operational intensity PN control strategy that would provide stable effluent quality, small footprint, robust and high rate PN at mainstream municipal wastewaters.

Conflict of interest

The authors declare that they have no conflicts of interest.

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