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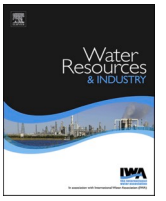

Experimental investigation of simultaneous nitrification-denitrification and phosphorus removal in pilot-scale sequencing batch moving bed biofilm reactors (SB-MBBRs)

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ABSTRACT

Sequencing batch moving bed biofilm reactors have been widely used in commercial wastewater treatment facilities for organic carbon and nitrogen removal. However, these reactors can remove low phosphorus (P) levels. Therefore, this study investigated the potential of SB-MBBRs for maximizing simultaneous nitrification-denitrification and P removal (SNDPR) potential from P-rich municipal wastewater impacted by industrial discharges. A series of experiments were carried out to investigate the effect of external volatile fatty acid (VFA) dosing, airflow rate, and temperature on SNDPR using pilot-scale SBMBBRs. Stable and robust SNDPR was achieved with an optimum acetic acid supply of 150 mg SCOD/L, at 20 ^{o}C and 2.5 L air/min. A low airflow rate (AFR) and high-temperature conditions affected P release and uptake kinetics. Efficient PHA storage, dissolved oxygen (DO) transfer (outer layer), DO diffusion limitation (inner layer) of biofilm, and conversion of NH_4-N to NO_2-N/NO_3-N enhanced SNDPR in the two pilot SB-MBBRs.

1. Introduction

Excessive amounts of P and N cause eutrophication of the aquatic environment. The N and P removal was one of the most recent targets of wastewater treatment under the European Water Framework Directive (2000/60/CE). Increasing stringent effluent discharge requirements have urged substantial global efforts to develop and implement cost-effective and environmentally friendly engineered biological nutrient removal techniques in wastewater treatment plants [1].

Complete removal of N and P has been achieved through cyclic anaerobic/aerobic/anoxic processes. Generally, activated sludge systems (ASS) have been the most widely used technology to remove P and N from municipal and industrial wastewaters. This has been accomplished by a distinct metabolic function of denitrifying P accumulating organisms (DPAOs) under an anaerobic-aerobic cyclic process using sequencing batch reactor (SBR) operations [2].

In recent years, aerobic granular sludge (AGS) has also been proposed as a compact and dense biomass for SNDPR processes [3]. It has been documented that the development of anoxic zone during the aerobic phase is limited for small granules at constant aeration (with a DO level of 2 mg/L), and anoxic zone develops during a brief period of the aeration phase for large granules [4].

On the other hand, MBBR has been widely used for nitrogen and organic carbon removal from wastewater, demonstrating high N

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and C removal efficiencies at small scale level in rural communities [5]. It has been documented that the aerobic granular sludge process shows dynamic properties. In the AGS system, it is difficult to form an anoxic zone within the granules due to the rapid and intense diffusion of DO, leading to the disintegration of the granules [4]. However, the MBBR process facilitates the long-term anoxic zone formation in the biofilm layer, minimizing the challenges encountered in the AGS systems. This condition promotes the occurrence of SNDPR in a single MBBR reactor under the influence of functional microbial communities residing at different zones of the biofilm layer [6].

In recent years, several attempts have been made to address the role of the SB-MBBR process for SNDPR treating wastewater containing low P concentrations [6–8]. A full-scale application of MBBR has not been widely investigated. Currently, a continuous MBBR biological phosphorus removal (BPR) process has been developed by HIAS, Hamar, Norway [9]. However, the diluted nature of the wastewater (scarcity of VFA), high aeration requirement (incurring high cost), compact and dense biomass growth at a lower temperature, and reduced treatment efficiency challenged the application of MBBR systems for SNDPR at full-scale wastewater treatment plants. Moreover, simultaneous enrichment of PAOs/DPAOs and slow growing nitrifying organisms in a single reactor has been complicated because of their distinct physiological characteristics [10]. This could be another possible reason why the MBBR system is not widely applied for wastewater treatment at full-scale treatment facilities.

The SB-MBBR processes have been widely used for nutrient removal. However, there is limited research output addressing the long-term effects of different operational parameters such as VFA, airflow rates, and temperature on SNDPR under high-strength P wastewater conditions in the MBBR systems. The municipal wastewater treatment facilities can receive low VFA during weekends due to lower industrial activity, affecting the reactor removal performance efficiencies. Although SNDPR have been widely investigated in wastewater treatment processes, more information is still needed to understand the kinetics and performance of SNDPR in the SB-MBBR treating P-rich municipal wastewater affected by industrial discharges.

The practical application of the SB-MBBR system has been hindered by the high aeration cost incurred, resulting in unbearable capital and operational costs for wastewater treatment in rural areas and small communities [11,12]. The application of intermittent aeration in an MBBR system has shown to be an effective strategy to achieve simultaneous nitrification and endogenous denitrification P removal resulting in better simultaneous N and P removal performance from low C/N ratio wastewater [13,14]. The effect of airflow rate on SNDPR has been investigated in a continuous flow MBBR system with 0.2–3.0 mg DO/L concentration, and the feed C: N ratio of 3.6 to treat a typical municipal wastewater with influent PO_4-P concentration of 8 mg/L. In this continuous flow intermittent aerated system, more than 80 % N and P removal efficiency has been reported [11,13].

Temperature is the other main operational parameter that affects the growth rate of microbial communities, microbial enzymatic activities, and gas transfer rate in the wastewater treatment processes [15]. A previous study has reported that specific P release reduces with decreasing temperature [16]. Low temperature has been shown to negatively affect microbial activity in SNDPR. Nitrification capacity has been significantly decreased at temperatures below 15 ^{o}C. Liu and Li [15] improved N and P removal at 15 ^{o}C in a sequencing batch reactor. Several studies have shown that room temperature operation is suitable for microorganisms responsible for SNDPR [16,17]. However, most of these studies have been carried out based on enhanced biological P removal (EBPR) in activated sludge systems. Thus, fewer studies attempt to address the effect of temperature on SNDPR and have investigated the performance of the SB-MBBR treating wastewater containing a high amount of P.

In our previous experimental study, we demonstrated lower P and N removal efficiency at 10 and 20 ^{o}C during weekends without VFA dosing even under a low nutrient level in the raw wastewater. Low VFA availability, low temperature and NO_3-N accumulation might negatively impact the SNDPR in diluted municipal wastewater impacted by industrial wastewaters [18]. Moreover, limited information exists concerning substrate utilization and the role of the aeration rate in the MBBR process for the application of SNDPR [8]. Furthermore, there is a lack of information addressing the maximum potential of SB-MBBR process for SNDPR from P-rich wastewater streams. Previously published works lack comprehensive research results presenting a practical strategy for optimizing operational conditions on SNDPR in MBBR systems to maintain long-term stable processes. Furthermore, most studies have focused on the use of synthetic wastewater and the addition of external carbon sources, mainly acetate, because of the diluted nature of the wastewater. However, there is limited research on the application of SNDPR based on MBBR for real wastewater treatment.

Therefore, present study assessed the potential of removing P from P-rich industrial discharge-influenced real municipal wastewater using two pilot-scale SB-MBBRs. The specific objectives include 1) exploring the effect of change in the amount of organic carbon dosing (in the form of VFA) during weekdays on the SB-MBBRs performances during weekends (without VFA dosing), 2) to determine the best operational conditions for SNDPR by varying VFA concentrations, airflow rates and temperatures, and 3) quantifying the maximum degree of P release, VFA uptake, P uptake, and nitrification rates under different VFA dosing, airflow rates and temperatures. The hypothesis includes 1) the internally stored polyhydroxy alkanoate (PHA) during weekdays operation would support the weekend biological P (Bio-P) performances, 2) optimized aeration strategy would improve the SNDPR in the pilot SB-MBBR process, and 3) the P release rate and P removal efficiency may not be affected with increasing temperature. The results of this study can provide valuable information to improve the SNDPR in MBBR systems treating industrial-influenced municipal wastewater containing a high fraction of P.

2. Materials and methods

2.1. The SB-MBBR setup and characteristics of the wastewater

Two pilot-scale SB-MBBRs, with a working volume of 14.7 L (including water and carrier volume), were operated in anaerobic-aerobic cyclic phases to evaluate the maximum potential of P removal under different operational conditions. The two pilot-scale

Table 1
Wastewater composition and overall reactor operational conditions.

Parameters	Units	Values
Working volume	L	14.7
HRT (each cycle)	h	8–12
Temperature	^{o}C	10–30
Carriers filling degree	%	61
Biofilm surface area	m^2	4.5
Air flow rate	L/min	1.0–2.5
N_2 flow rate	L/min	1.5
pH		7.8 ± 0.3
PO_4-P influent	mg/L	14.0–17.5
NH_4-N influent	mg/L	28.5–62.2
SCOD influent	mg/L	184.6–452.0[a]
DO level	mg/L	0.5–2.5
Mixing speed	rpm	100

rpm = Revolution per minute.
[a] including externally added VFA.

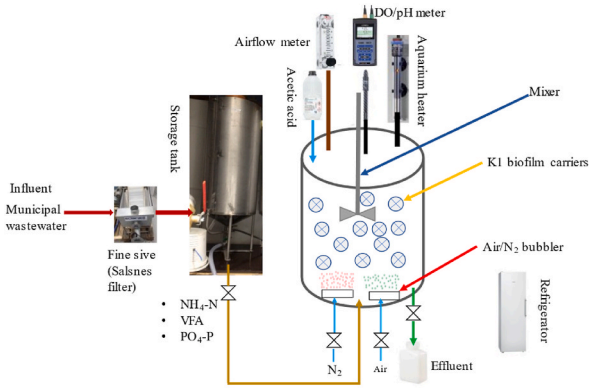

Fig. 1. Schematic presentation of sequence batch moving bed biofilm reactor.

SB-MBBRs contains the standard biofilm carriers (AnoxKaldnes™, K1) with a 61 % filling degree (biofilm carrier specific surface area of 500 m^2/m^3).

Raw municipal wastewater was used for all experimental conditions. The raw municipal wastewater composition was manipulated by adding PO_4-P and acetic acid to mimic industrial influenced wastewater. As a result, the influent PO_4-P concentration (14.0–17.5 mg/L) was manipulated by adding $NaH_2PO_4.2H_2O$. The SCOD concentrations varied between 92.2 and 154.4 mg/L (on average) during the four months operational period. These values include the externally dosed VFA in the form of acetate to mimic the industrial influence. The influent NH_4-N concentrations varied between 28.5 and 62.2 mg/L without any adjustment. Table 1 summarizes the influent wastewater characteristics and operational conditions. The storage of municipal wastewater and reactor feeding strategies have been described in a previous study [18]. Briefly, the SB-MBBRs were operated with 2–4 h and 6–8 h anaerobic aerobic cyclic processes, respectively, which included 3 min filling and 3 min emptying time per cycle.

A refrigerator (10 ^{o}C) and an aquarium heater (20 and 30 ^{o}C) were used to maintain the temperature of the reactors in the desired range. For creating favorable conditions for PHA storage by PAOs/DPAOs, nitrogen gas was purged at the beginning of the anaerobic phase (DO < 0.02 mg/L). During the aerobic conditions, compressed air was purged to make the airflow rates in the reactors in the desired range (1.0, 1.5, 2.5 L air/min). Both to ensure the movement of the biofilm carriers and to avoid settling of the detached biomass (suspended solids), the systems were mechanically mixed using a custom-made mixer for the entire cycle period. However, when the water level drops in the reactor (about 75 %) while the reactor is draining, the mixer stops completely. The custom-made microcontroller was used to control the cyclic sequence of the system. It was equipped with a display and data storage unit, which was powered by a Raspberry platform. A simplified diagram of the SB-MBBR is shown in Fig. 1.

2.2. Experimental setup

Two pilot-scale SB-MBBRs were used for the study. These reactors had been in operation for over a year at 10 and 20 ^{o}C. To ensure consistent performance, the reactors were run at 20 ^{o}C for 22 days before the optimization experiments began. The experiments evaluated the effects of different operational conditions at varying levels of VFA dosing (as SCOD concentrations in Experiment 1), airflow rates (Experiment 2), and operating temperatures (Experiment 3). The experiment was conducted for a period of 3–4 weeks to

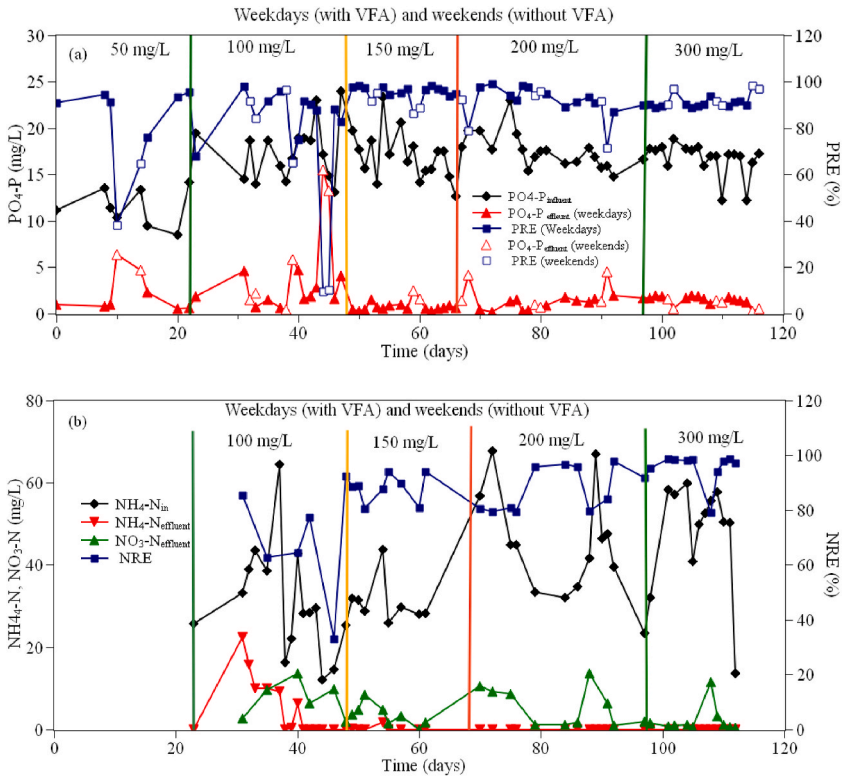

Fig. 2. Effect of different VFA concentrations (100, 150, 200 and 300 mg SCOD/L) on P removal during weekdays (with VFA) and weekends (without VFA) and N removal (b) at 20 ^{o}C and 2.5 L air/min.

examine the effect of each operational parameter. After changing the operational parameters, kinetic studies were conducted 5–7 days later. Only one parameter was varied at a time, while all the other parameters except hydraulic retention times (HRT) were kept constant.

In the first experiment, the removal of P during weekends was evaluated by increasing the dosing of VFA during weekdays. The concentration of externally dosed VFA was varied between 100 and 300 mg SCOD/L, which resulted in a concentration of 184.6–452 mg/L, including the SCOD present in the raw wastewater (Table 1).

An anaerobic HRT of 2–4 h was chosen to minimize the transfer of SCOD from the anaerobic to the aerobic phase. The aerobic HRT varied between 6 and 8 h, resulting in an overall HRT between 8 – 12 h as shown in Table 1. This experiment was conducted at a temperature of 20 °C and an airflow rate of 2.5 L/min. To monitor the long-term performance of the reactors, grab samples were collected 3–5 times per week. These samples were analyzed for PO_4-P, SCOD, NH_4-N, NO_2-N, and NO_3-N. For kinetic studies, samples were collected every 15 min for the first hour at the beginning of the anaerobic phase and every 30 min for the first 2 h of the aerobic phase.

In the second experiment, the impact of airflow rates on the potential of P and N removal performance at 20 °C was evaluated. Airflow rates of 1.0, 1.5, and 2.5 L/min were used, resulting in DO concentrations of 0.5, 3.4, and 4.6 mg/L, respectively (Table 1). The experiment was conducted with varying SCOD concentrations ranging from 106.3 to 217.3 mg/L, which included 150 mg/L of externally added acetic acid.

Finally, this study evaluated the pilot-scale SB-MBBR P removal potential at different temperatures (10, 20 and 30 ^{o}C, Exp 3) with sufficient substrate availability (150 mg SCOD/L dosing). The airflow rate was maintained at 2.5 L/min, resulting in 4.6 mg DO/L.

2.3. Sample analysis

2.3.1. Chemical analysis

Two pilot-scale SB-MBBRs operational conditions were studied, and the systems' development was monitored for each experimental condition. Samples were collected for the entire cycle (8–12 h) for kinetic studies. All samples were immediately filtered through 0.45 μm filters. The concentration of SCOD, PO_4-P, NH_4-N, NO_2-N, and NO_3-N were measured using Dr. Lange cuvette with HACH LANGE GmbH (DR 1900, China) spectrophotometer. All analyses were performed immediately during the cycle test. The temperature, pH, and DO of the wastewater samples were measured using a portable combined pH- and DO-meter (WTW, Multi 36030 IDS).

Table 2
Summary of reactors experimental operational conditions, influent and effluent wastewater characteristics for N and P removal (n > 10, Mean ± std).

Parameters	[a]VFA (mg SCOD/L)				Air flow rate (L/min)			T (°C)		
	100	150	200	300	1.0	1.5	2.5	10	20	30
[b]SCOD: P ratio	10.8 ± 8.6	15.7 ± 11.5	20.9 ± 25.3	27.4 ± 39.1	20.9 ± 39.3	17.8 ± 20.9	15.5 ± 29.9	19.9 ± 30.7	15.9 ± 13.0	21.2 ± 38.9
SCOD: N	12.5 ± 1.0	11.3 ± 0.4	7.5 ± 0.1	8.4 ± 0.5	6.3 ± 0.5	7.7 ± 0.2	7.1 ± 0.2	6.5 ± 0.8	7.5 ± 0.6	5.6 ± 0.1
HRT (h)	8	8	10	12	8	8	8	8	8	8
Duration (days)	30	20	27	18	16	15	14	15	17	14
DO level (mg/L)	4.6 ± 1.2	4.6 ± 0.7	4.6 ± 1.6	4.6 ± 1.4	0.5 ± 0.2	3.5 ± 0.7	4.6 ± 1.4	4.6 ± 1.0	4.6 ± 0.7	4.6 ± 1.2
pH	7.8 ± 0.3	7.8 ± 0.3	7.8 ± 0.3	7.8 ± 0.3	7.9 ± 0.3	7.9 ± 0.2	8.0 ± 0.2	7.8 ± 0.3	7.8 ± 0.3	7.8 ± 0.3
Influent NH_4-N (mg/L)	32.6 ± 9.8	30.9 ± 6.3	38.7 ± 13.5	53.5 ± 6.7	54.5 ± 9.1	37.6 ± 5.1	34.4 ± 6.9	54.6 ± 10.2	37.2 ± 5.1	53.8 ± 8.9
Effluent NH_4-N (mg/L)	3.0 ± 0.5	UMR	UMR	UMR	42.9 ± 19.5	0.1 ± 0.1	UMR	37.2 ± 12.9	UMR	6.8 ± 7.0
Effluent NO_3-N (mg/L)	8.9 ± 2.3	4.0 ± 2.3	4.9 ± 4.2	2.3 ± 3.6	0.6 ± 0.3	3.7 ± 2.1	4.0 ± 2.3	0.8 ± 0.7	3.7 ± 2.3	1.8 ± 0.3
Effluent NO_2-N	0.3 ± 0.5	0.02 ± 0.01	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.2	0.03 ± 0.02	0.02 ± 0.01	0.3 ± 0.3	0.05 ± 3.5	2.5 ± 1.5
NH_4-N removal (%)	64.6 ± 5.0	88.9 ± 4.2	89.3 ± 7.6	95.7 ± 6.3	27.5 ± 17.3	86.2 ± 12.0	88.8 ± 4.2	25.7 ± 10.5	89.0 ± 8.8	85.5 ± 7.2
Influent PO_4-P (mg/L)	16.5 ± 3.7	17.5 ± 2.8	17.3 ± 1.9	17.0 ± 1.7	16.8 ± 0.9	14.5 ± 0.9	16.8 ± 1.0	16.3 ± 1.5	16.2 ± 2.2	16.1 ± 1.0
Effluent PO_4-P (mg/L)	2.4 ± 1.6	0.6 ± 0.2	1.1 ± 0.6	1.4 ± 0.5	12.2 ± 1.6	0.5 ± 0.3	0.4 ± 0.1	0.3 ± 0.3	0.5 ± 0.1	0.4 ± 0.1
PO_4-P removal (%)	86.2 ± 8.0	96.9 ± 1.1	93.6 ± 3.7	91.8 ± 3.5	23.8 ± 9.1	96.5 ± 1.9	97.3 ± 0.9	98.0 ± 1.8	99.6 ± 1.2	96.3 ± 0.1
Effluent SCOD	21.6 ± 3.7	28.6 ± 5.0	27.7 ± 8.9	26.4 ± 5.9	45.6 ± 9.4	27.0 ± 1.5	28.6 ± 5.0	39.0 ± 5.9	28.7 ± 4.3	38.8 ± 7.8

[a] External VFA dosing.
[b] influent, n = number of samples.

2.3.2. Biomass analysis

Biofilm carriers were sampled weekly to determine total attached biomass presented as g TSS/L or g TSS/m^2 following the described procedure [51]. Biofilm carriers were sampled weekly to determine the total attached biomass, presented as grams of total suspended solids per liter (g TSS/L) or per square meter (g TSS/m^2), using the described procedure [19]. The surplus sludge (detached biomass) and treated wastewater were drained and discharged. The sludge was collected for experiments or analysis whenever necessary.

3. Results and discussions

3.1. Overall performance of the SB-MBBR Bio-P reactor

The first experiment aimed to determine if increasing VFA supply on weekdays would stabilize Bio-P performance during weekends with no external VFA supply. The trial revealed that gradually increasing the supply of VFA during weekdays led to higher P removal efficiency (86–97 %), which then plateaued after a 150 mg SCOD/L dosage (see Fig. 2 a and Table 2). As a result, the SB-MBBR system demonstrated stable operations and exhibited significant improvement in P removal efficiency during weekends with an average efficiency of 85.9 %–95.6 %, except for the extreme values on day 44/45, Fig. 2 a). This means that the PHA stored on weekdays was sufficient to maintain efficient phosphorus removal on weekends when VFA was not dosed. The study findings imply that the absence of VFA in municipal wastewater on weekends may not affect the reactor's performance, provided that the biomass accumulates sufficient PHA during weekdays. This supports the initial hypothesis. However, during days 44 and 45, extreme rainfall and snow melting events caused the pilot-scale SB-MBBR performance to deteriorate on weekends (see Fig. 2 a). Table 2 presents a summary of the experimental conditions and results of this study. The N removal efficiency (NRE) was found to be 64.6–95.7 % on average, as shown in Fig. 2 b. Higher values of NRE were observed when the supply of VFA was equal to or greater than 150 mg SCOD/L.

The deep layer of the biofilm contains DPAOs that can use NO_2^-/NO_3^- for anoxic P uptake and denitrification [6]. During this study, it was noticed that the removal of P persisted even in the presence of O_2, NO_2-N and NO_3-N. The kinetic study showed a decrease in the levels of NO_2-N, NO_3-N, and PO_4-P in the bulk solution, which suggested SNDPR occurred under aerobic conditions. This research also revealed that all SCOD was consumed under anaerobic conditions (without nitrate). Therefore, it can be conclude that both DPAOs and regular PAOs contributed to the removal of P under aerobic conditions. It is well-known that many ordinary heterotrophs can perform denitrification using NO_2^-/NO_3^- as electron acceptors under anaerobic conditions. However, in the present study, the SCOD was completely consumed during anaerobic conditions, which resulted in a minimum or negligible SCOD amount left for other heterotrophic processes to occur during aerobic conditions. This might further restrain the performance of the traditional denitrifiers in the anoxic layer. Therefore, the contribution of regular denitrifiers in the anoxic layer of the biofilm can be neglected. Overall, this study

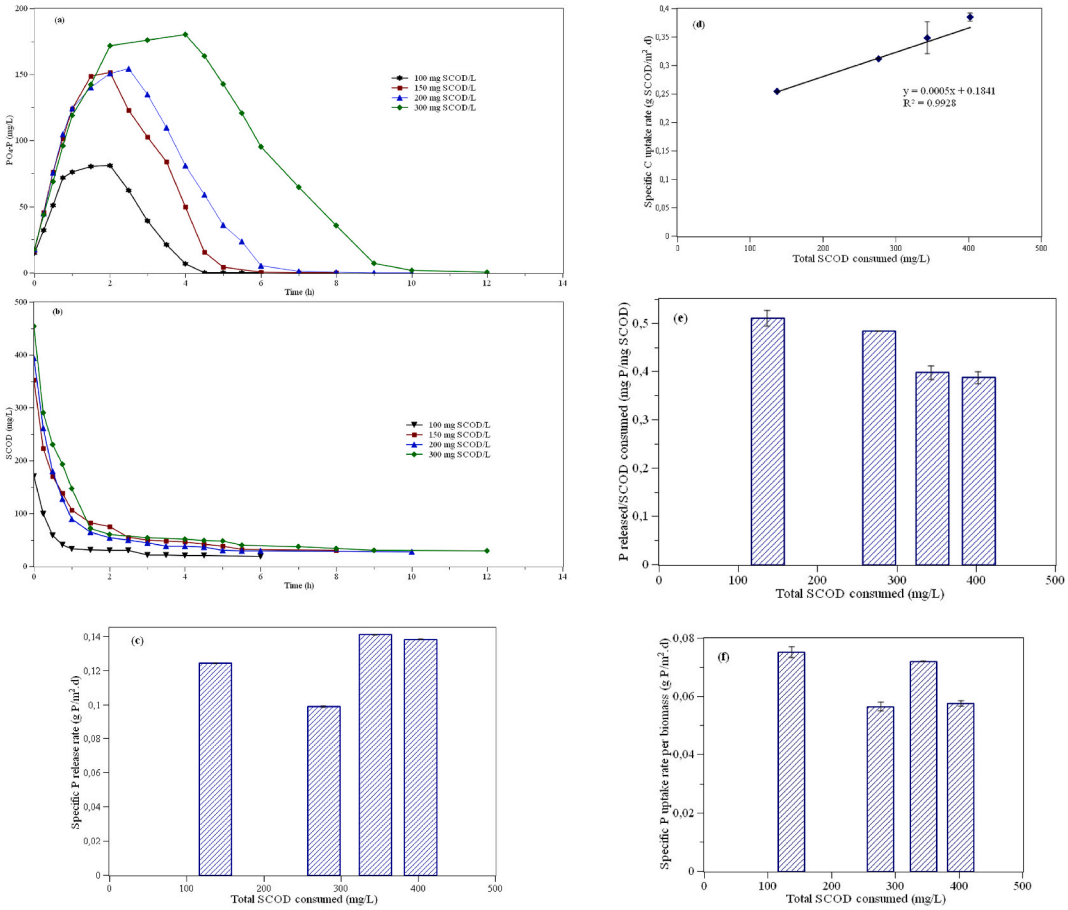

Fig. 3. PO_4-P release and uptake (a) and SCOD consumption (b) profile at different external VFA dosing in a typical cycle, the ratio of specific P release rate to biomass (c), the ratio of SCOD uptake to biomass (d), the ratio of net P release to net SCOD consumed (e) and the ratio of specific P uptake to biomass (f).

showed that the uptake of P continued in the presence of O_2, NO_2^- and NO_3^- indicating no inhibition of the P uptake with concurrent presence of these electron acceptors. However, previous study revealed that DPAOs utilize NO_3^- preferentially over NO_2^- for anoxic P uptake [20].

The long-term effect of airflow rates (1.0, 1.5, 2.5 L/min) and temperatures (10, 20, 30 ^oC) was examined with VFA dosing of 150 mg SCOD/L. The findings indicated that decreasing the airflow rates gradually (from 2.5 to 1.0 L air/min) led to a significant reduction in the removal efficiencies of P (23.8 %) and N (27.5 %), see Table 2. The possible explanation for this could be reduced nitrification activities (due to very low DO level in the water phase), lower anoxic P uptake (due to low NO_2^-/NO_3^- availability), leading to lower N removal efficiency. It would be interesting to investigate the impact of gradually increasing the airflow rate on the SNDPR in the pilot SB-MBBR system.

The P removal efficiency was not inhibited for the chosen operational temperatures (10, 20, 30 ^oC). However, the amount of phosphorus released was lower at 10 and 30 °C compared to that at 20 °C, suggesting that there may be a shift in biomass microbial composition due to prolonged high-temperature (30 ^oC) operations. Besides, the NRE was highly affected as the temperature decreased from 20 to 10 ^oC (Table 2), possibly suggesting the temperature sensitivity of ammonia-oxidizing bacteria and DPAOs.

The finding of this study suggests that previously stored PHA (with sufficient VFA dosing during weekdays) improved the SB-MBBR performance during weekends, except in the extreme events such as rain or snow melting. This indicates that the PHA left from the weekdays' operations was enough to support the weekend Bio-P performances. This observation explains the reason for the lower P removal efficiency observed in the previous study [18]. Ensuring reliable, robust, and reproducible evaluation of SNDPR requires long-term observation of all experiments executed under pilot-scale SB-MBBR operational conditions.

3.2. Kinetics of P release, uptake, and N removal

3.2.1. Effect of organic carbon concentration on denitrifying phosphate removal

The biomass of the pilot-scale SB-MBBRs was evaluated for its ability to uptake VFAs, release P, uptake P, nitrification, and

Table 3
Total biomass and biomass P content at different concentration of VFA dosing.

VFA (mg SCOD/L)	Influent SCOD/P ratio (mg/mg)	[b]PRE (%)	[c]NRE (%)	Total attached biomass (g TSS/m^2)	P content (mg P/mg TSS)	P release/SCOD uptake (mg/mg)
100[a]	11.5 ± 1.0	99.1 ± 0.1	83.9 ± 2.4	12.6 ± 2.3	0.07 ± 0.01	0.51 ± 0.02
150[a]	19.5 ± 1.6	99.0 ± 0.3	95.4 ± 2.1	17.4 ± 2.7	0.1 ± 0.001	0.48
200[a]	21.4 ± 0.9	99.7 ± 0.4	94.9 ± 1.4	19.4 ± 0.6	0.08 ± 0.01	0.40 ± 0.01
300[a]	25.4 ± 0.1	99.0 ± 0.1	98.4 ± 0.5	18.0 ± 0.8	0.08 ± 0.02	0.39 ± 0.01

[a] External acetic acid dosing only.
[b] PRE: P removal efficiency.
[c] NRE: nitrogen removal efficiency.

denitrification. The results showed that higher dosages of VFA led to a significant release of P, indicating increased storage of PHA by PAOs/DPAOs under anaerobic conditions. As a result, the biomass showed excellent P removal (almost 100 %) characteristics, with a high P release to SCOD uptake ratio. Fig. 3 a and b demonstrate the typical profile of P release, P uptake, and SCOD consumption.

The specific rates of PO_4-P release, SCOD consumption and P uptake were calculated using linear regression considering 1 h anaerobic HRT (Fig. 3 a and b). During the initial anaerobic stage, SCOD was rapidly consumed for all VFA levels examined, followed by a gradual decline during the final anaerobic stage (Fig. 3 b). The specific SCOD uptake rate per biomass (0.25–0.38 g SCOD/m^2.d) increased linearly ($R^2 = 0.9928$) with increasing VFA dosing (Fig. 3 d). The findings of this study were in line with the research that has been conducted by Ref. [21], which showed that the rate of SCOD uptake increased as the VFA dosing increased. Anaerobic retention time of 3 h and 4 h were considered for 200 and 300 mg SCOD/L dosing, respectively, to ensure complete SCOD uptake (Fig. 3 b). However, the biomass consumed all the SCOD within 2 h anaerobic retention for all VFA levels assessed in this study.

The release of P is closely linked to the consumption of SCOD. It is evident that a higher dosage of VFA at the beginning of the anaerobic stage results in a proportional increase in P release. This demonstrates the typical conversion of VFA to PHA in the biofilm during anaerobic phase. In this study, the maximum P release obtained (162.4 mg PO_4-P/L for 300 mg SCOD/L) was mainly due to extended anaerobic retention time (4 h), which could be due to the consumption of fermentation product causing secondary P release. A previous study based on SB-MBBR has demonstrated that a high release of P is advantageous in achieving a high net P uptake [6]. The study found that the release rates of P from biomass ranged from 0.10 to 0.14 g P/m^2.d (Fig. 3 c). The trend for the relationship between specific P release rate and total SCOD consumed was almost similar for dosing of 200 and 300 mg SCOD/L (Fig. 3 c). This trend suggests that P release may already have reached maximum capacity after a sufficient lapse of anaerobic HRT.

The anaerobic net PO_4-P release per SCOD consumed was 0.200 and 0.15 mmol P/mmol C for 100–150 and 200–300 mg SCOD/L dosing, respectively (Fig. 3 e). The ratios were found to be lower than the reported range (0.32–0.60 mmol P/mmol C) of PAO enriched cultures of the ASS [22,23]. In the current study, specific PO_4-P uptake rates were also determined directly from the cycle test performed under different VFA dosing at 20 ^oC and 2.5 L air/min. The specific uptake rates values were determined from the slope of the regression line considering 2 h aerobic HRT data points. The specific P uptake rate per biomass was slightly higher at lower VFA levels (100 mg SCOD/L) dosing (0.078 g P/m^2.d) as shown in Fig. 3 f. During the cycle test, complete P uptake was observed for all VFA levels examined (Fig. 3 a) and N removal efficiency ranged between 83.9 and 98.4 % (Table 3). Overall, significant anaerobic P release, anoxic/aerobic P uptake, and N removal were observed. This biomass behavior is consistent with the conventional ASS system as reported by Ref. [21,24]. The study provides a guiding overview for applying SNDPR using the SB-MBBR process that targets optimization of substrate utilization and reduce air usage, resulting in energy savings.

The total attached biomass increased from 12.6 to 19.4 g TSS/m^2 with increasing external VFA dosing of up to 200 mg SCOD/L. However, it began to decrease (18.0 g TSS/m2) at VFA dosing of 300 mg SCOD/L as the biomass started to shed off (Table 3). Slightly high biomass P content (0.1 mg total P/mg TSS or 10 %) was measured at VFA dosing of 150 mg SCOD/L (Table 3). A previous study has shown that the biomass contained 5 % P for the biofilm system operated with 300 mg COD/L at 15–25 ^oC [25], which is lower than the one obtained in the current study (7.0–10.0 %). The P content of the current study was also comparable with a previous study that reported 7.5 % in MBBR process [26]. Overall, the efficiency of the SB-MBBR process performance largely depends on the quality of the biomass developed than the biomass concentration [26].

It was crucial to ensure sufficient VFA availability during the anaerobic stage to induce P release and achieve efficient removal of P. In this study, the influent SCOD/P ratios were varied between 11.5 and 27.4 mg SCOD/mg P during the optimization experiment execution for long-term operations. The results of the kinetic study indicated complete P removal for all VFA dosing levels tested, with influent SCOD/P ratios between 11.5 and 25.4 mg/mg (Table 3). Variable influent SCOD/P ratios were reported in the literature. For instance, a good P removal was achieved with a SCOD/P ratio of 7 and 10 using acetic acid as a sole organic carbon source [27]. According to Oehmen et al. [22], effective P removal can be achieved with propionic acid as the sole organic carbon source in an anaerobic sequencing batch reactor at a COD/P ratio of 15 in ASS system. The proportion of GAOs and PAOs in EBPR systems is influenced by the SCOD/P ratio of the influent. A previous study found that the dominance of glycogen accumulating organisms (GAOs) in biological systems is associated with P limitation [27].

Based on the long-term operation, it was found that external dosing of VFA (150 mg SCOD/L) and an influent SCOD:P ratio of 15.7

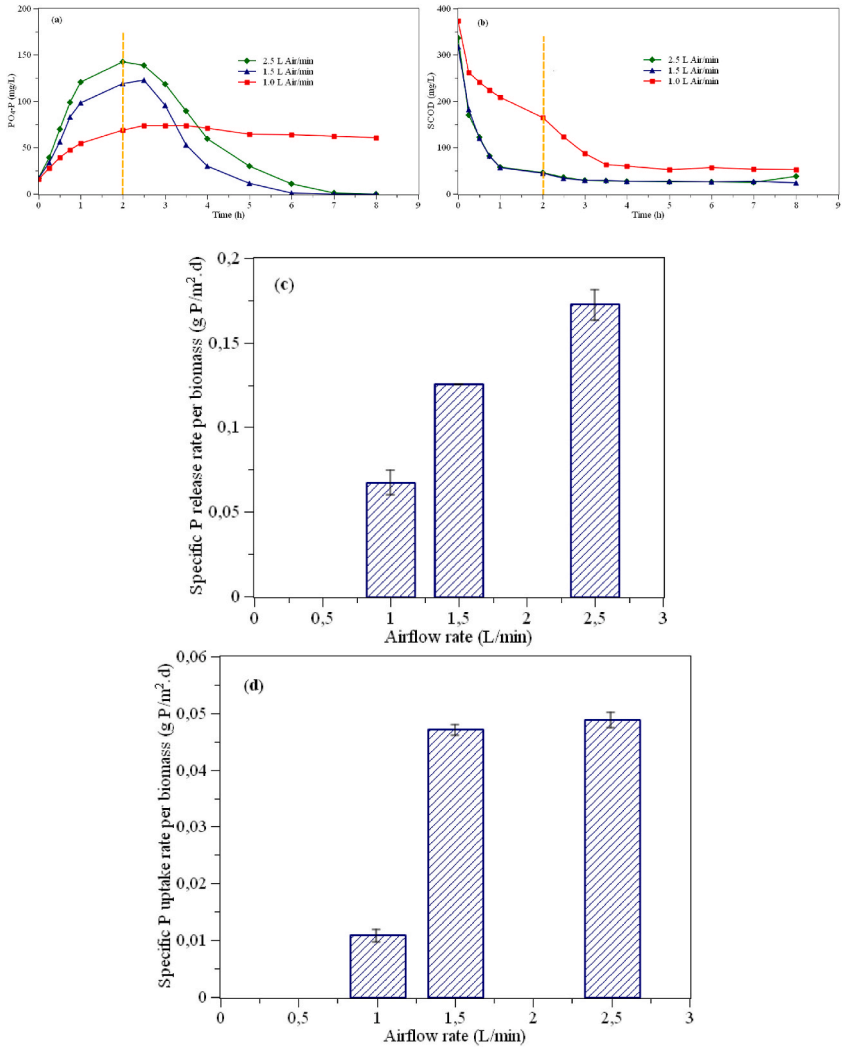

Fig. 4. PO_4-P release and uptake (a) and SCOD consumption (b), specific P release rate per biomass (c) and specific P uptake rate per biomass (d) at different airflow rates.

± 11.5 mg/mg (as shown in Table 2) was sufficient to ensure efficient and stable operation of the SB-MBBR at 8 h HRT, a temperature of 20 °C and an air supply of 2.5 L/min (with dissolved oxygen maintained at 4.6 mg/L). This study demonstrated efficient SNDPR in the pilot SB-MBBR process, likely achieved by the capability of PAOs/DPAOs to store VFA as PHA in anaerobic conditions and efficient nitrification in aerobic conditions. It was challenging to directly estimate the relative proportion of regular PAOs and DPAOs in the biofilm based on the current experimental results. Hence, conducting separate experiments was recommended to evaluate aerobic and anoxic P uptake of the biomass enriched with DPAOs. Additionally, another experimental study is necessary to determine whether the enriched DPAOs used both NO_2^- and NO_3^- or depended on one of them to carry out SNDPR. The pilot-scale SB-MBBR did not require conditions such as sludge recirculation and separate anoxic reactor installation. The strategy of using SB-MBBR for denitrification and P removal shows promise in minimizing energy cost and organic carbon utilization, improving nitrification efficiency, and preventing biomass washout [6,28].

3.2.2. *Effect of airflow rates on P removal*

In experiment 2, the impact of different aeration rates (1.0, 1.5, and 2.5 L air/min) on the ability of the biomass developed in pilot-scale SB-MBBRs to release P, take up SCOD, and remove P and N was investigated. The long-term operation of the SB-MBBR showed that reducing airflow rates from 2.5 to 1.0 L air/min (0.5 mg DO/L concentration) hindered the SNDPR activity and resulted in lower P (23.8 %) and N (27.5 %) removal efficiencies (Table 2).

During both anaerobic and aerobic conditions, the bulk liquid was mechanically mixed at 100 rpm to evenly distribute the biofilm carriers throughout the reactors. However, achieving the desired nutrient removal efficiency was hindered by limitations in oxygen transfer and substrate transport in the bulk liquid phase. The study found that a low airflow rate of 1.0 L/min consistently created a

Table 4
P release and uptake at different airflow rates (at 150 mg/L VFA as externally dosed SCOD).

Air flowrate (L/min)	Influent SCOD/P (mg/mg)	**NRE (%)**	**PRE (%)**	Total attached biomass (g TSS/m2)	P content (mg P/mg TSS)	P release/SCOD uptake (mg/mg)
1.0	23.4 ± 1.2	3.7 ± 1.3	–	12.6 ± 2.3	0.06 ± 0.01	0.24 ± 0.02
1.5	19.6 ± 1.1	96.2 ± 0.5	99.5 ± 0.4	16.2 ± 0.8	0.08 ± 0.02	0.40 ± 0.003
2.5	20.2 ± 0.8	97.0 ± 0.5	99.6 ± 0.4	15.4 ± 2.7	0.1 ± 0.01	0.44 ± 0.01

stagnant zone that hindered achieving the desired DO concentration in the bulk liquid. The conditions in the system were unfavorable, resulting in low nitrification rates and complete anaerobic/anoxic conditions. In MBBR systems, a DO level of more than 2 mg/L is often maintained to facilitate simultaneous nitrification and denitrification (SND) by ensuring sufficient oxygen diffusion through the aerobic biofilm layer, thereby sustaining the nitrification process [7].

The study found that increasing DO concentration from 0.5 to 4.6 mg/L resulted in more than double the net P released, ranging from 46.1 to 130.1 mg P/L on average. This result is in line with a previous study that has shown increasing the DO amount from 4 to 6 mg/L doubles the P content in the biofilm, indicating high DO concentration favors the P uptake and Poly-P storage in the biofilm [29]. However, there should be a balancing mechanism during the biofilm development stages because the high P content in the biofilm prolongs the P removal capacity [29].

The kinetic study demonstrated that all the SCOD was consumed completely for the airflow rates of 1.5 and 2.5 L/min. However, more than 50 % of the SCOD was introduced to the aerobic phase at 1.0 L/min airflow rate (0.5 mg DO/L) see Fig. 4 b, which in turn affected nitrification and P uptake. To avoid aerobic heterotrophic growth, all the SCOD should be consumed by the desired micro-organisms (PAOs/DPAOs) under anaerobic conditions [6]. A low aeration rate could affect the Bio-P activity, restricting the PHA formation because it hinders the intrinsic biofilm from being exposed to alternating anaerobic-aerobic conditions [21]. Consequently, a lower maximum P release (46.1 ± 9.1 mg/L) was observed at 1.0 L air/min (Table 4, Fig. 4 a). The low anaerobic P release could be linked to the limited Poly-P cleavage in the cell, suggesting insufficient PHA storage by the biomass [30]. The specific P release rate increased with increasing airflow rate (0.07–0.17 g PO_4-P/m^2.d, Fig. 4 c).

The net P released: SCOD consumed ratios (0.24–0.44 mg P/mg SCOD) increased with increasing airflow rate (Table 4). The P release to SCOD consumed ratio could show the biochemical transformation pathway of PAOs [23]. Generally, the amount of P released depends on the amount of Poly-P and PHA contained in the biomass. The PHA storage in turn depends on the amount of VFA and P contained in the raw wastewater.

Relatively lower total attached biomass (12.6 g TSS/m^2) and P content (0.06 mg total P/mg TSS) were observed at 1.0 L air/min (Table 4). The observed biomass P content may indicate that the PAO metabolism might be temporarily inactive; however, the Bio-P activity was restored quickly when the operational airflow rate was adjusted to normal conditions (2.5 L air/min).

The benefit of aeration should not be limited to facilitating an even distribution of biofilm carriers and providing sufficient DO levels, but it should also enhance diffusion (without affecting the anoxic processes in the biofilm layer), mass transfer, and substrate utilization (P uptake, PHA, and ammonia oxidation). This promotes Poly-P formation in the biofilm during aerobic conditions [21].

In the long-term operation, it was speculated that the biomass might be highly enriched with PAOs/DPAOs, facilitating excellent P removal (>96 %) and complete ammonium oxidation with more than 86 % N removal efficiency at 1.5 and 2.5 L air/min (Table 4). The results confirm the second hypothesis; achieving better SNDPR efficiency with increasing airflow rate due to efficient nitrification processes.

A slow specific P uptake rate per biomass (0.01 g P/m^2.d, Fig. 4 d) and negative P uptake (Fig. 4 a) were observed at a low airflow rate. It was speculated that the NO_2^- produced during the nitrification process was reduced to N_2 gas by DPAOs instead of oxidizing to NO_3-N (low accumulation). This approach reduces the extensive use of oxygen. This study was in agreement with the study of Lin et al. [31] that evaluated a single-stage denitrifying P removal biofilter utilizing intracellular carbon source for advanced nutrient removal and P recovery, achieving 74.81 % denitrification and 91.15 % P removal [31].

An incomplete anaerobic SCOD uptake leads to a breakthrough of SCOD to the aerobic phase, enhancing heterotrophic growth. This occurrence may limit PHA oxidation and Poly-P restoration in aerobic conditions. The lower P (23.8 %) and N (27.5 %) removal were not only due to low airflow rate (1.0 L air/min, 0.5 mg DO/L) but also due to a limited anaerobic PHA storage in the biomass and lower nitrification under aerobic conditions. In addition, the incomplete coupling of nitrification-denitrification and P removal organisms might have resulted in poor N and P removal from the bulk wastewater in the current study. The finding of this research implied that SNDPR at a low airflow rate was not feasible in the pilot-scale SB-MBBRs. Therefore, a minimum of 1.5 L airflow rate was required to achieve efficient SNDPR. Generally, insufficient aeration slows the nitrification process, hindering the accessibility of NO_2^-/NO_3^- for anoxic P uptake and denitrification processes in biological systems. It would be interesting to further investigate how biomass responds in performing SNDPR at a higher airflow rate.

3.2.3. Effect of temperature on P and N removal

A significant increase in SNDPR efficiency was observed at 20 ^oC under steady-state operations, enhancing nitrification, anoxic/aerobic P uptake, and denitrification processes in the SB-MBBRs. This was achieved through DPAOs and regular PAO removing P and N that utilize NO_2^-/NO_3^- and O_2, respectively, as electron acceptors. The system demonstrated higher P (more than 99 %) and N removal

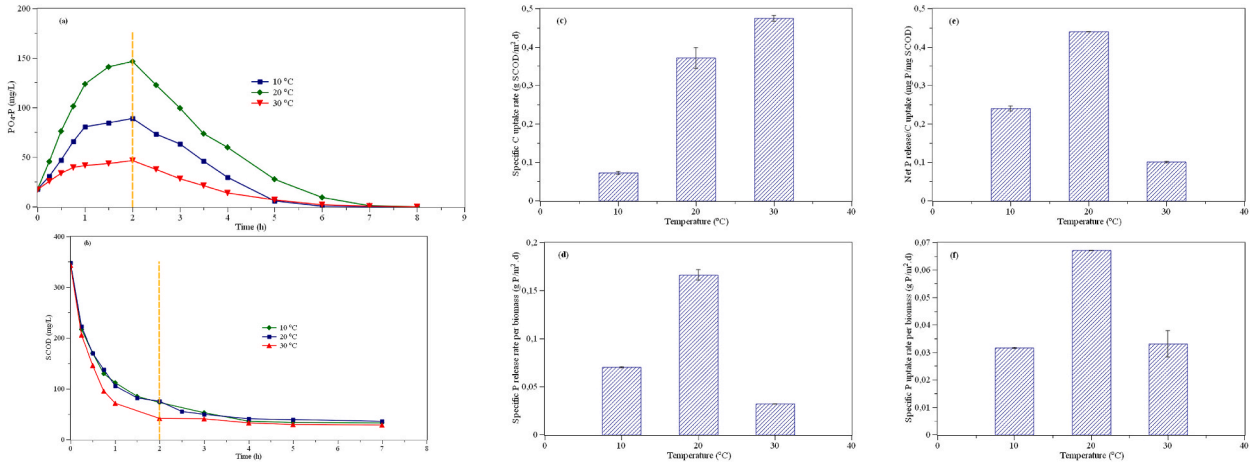

Fig. 5. PO_4-P release and uptake (a) and SCOD consumption (b) profiles, specific SCOD uptake per biomass (c), specific P release rate per biomass ratio (d), ratio of P release rate to SCOD uptake rate (e), ratio of P uptake rate to biomass (f) at 10, 20 and 30 °C (150 mg SCOD/L and 2.5 L/min airflow rate) at different temperatures.

Table 5
P release and uptake at different operating temperatures (at 150 mg/L VFA as externally dosed SCOD, 2.5 L/min airflow rate).

T (°C)	Influent SCOD/P (mg/L)	NRE (%)	PRE (%)	Max PO_4-P release (mg/L)	Total attached biomass (g TSS/m2)	P content (mg P/mg TSS)	PO_4-P release/SCOD uptake (mg/mg)
10	20.0 ± 0.7	25.2 ± 6.2	99.4 ± 0.5	67.8 ± 5.0	22.1 ± 2.4	0.08 ± 0.002	0.24 ± 0.01
20	19.9 ± 1.0	97.2 ± 0.4	99.0 ± 0.5	130.8 ± 2.7	15.9 ± 2.7	0.10 ± 0.001	0.44
30	20.3 ± 0.5	95.2 ± 2.4	99.2 ± 0.04	28.9 ± 0.6	13.1 ± 0.3	0.06 ± 0.03	0.10 ± 0.002

(89 %) efficiencies, indicating efficient cooperation of nitrification-denitrification and P removal organisms at 20 °C. It has been documented that the anoxic denitrifying P removal ability of DPAOs would be advantageous for designing the SNDPR system, which curtails plant operational costs [32].

This study also investigated the biomass P release, SCOD, and P uptake potential at different temperatures (see Fig. 5 a and b). The relationship between biomass SCOD uptake and temperature revealed that specific SCOD uptake rates increased with increasing temperature (Fig. 5 c). Maximum net P release and specific P release rate per biomass was observed at 20 °C (see Table 4 and Fig. 5 d), indicating the P release strongly depends on the temperature.

In the current study, an increase in temperature from 20 °C to 30 °C resulted in a substantial reduction in anaerobic P release, demonstrating lower net PO_4-P release/SCOD uptake ratio (0.1 mg P/mg SCOD) in the attached biomass (see Fig. 5 e). This result is in line with the previous study that has reported intracellular P content decreases with increasing temperature [17].

A previous study has shown that higher proportions of PAOs would be advantageous to maximize the amount of P released per acetate consumed. However, higher proportions of GAOs would lower the P release to acetate consumed ratio, resulting in less P transformation for the same amount of acetate consumed [33]. High temperature could result in deterioration of Bio-P activity over an extended operational period since temperature governs the glycolytic pathway, leading to microbial metabolic shifts [34]. Additionally, the specific P release per biomass was decreased as the temperature decreases to 10 °C (Fig. 5 d).

No SCOD breakthrough to aerobic conditions occurred for all temperatures investigated in this study. In the present study, the SCOD uptake occurred at a slower specific rate at 10 °C (Fig. 5 c). At 10 °C operation, the Bio-P activity could continue until the biomass falling is restricted due to EPS production [18]. Maximum specific P release rate per biomass (Fig. 5 d), net P release/SCOD consumed ratio (Fig. 5 e), and specific P uptake per biomass (Fig. 5 f) was observed at 20 °C.

The net P release to SCOD uptake ratio at 20 °C (0.42 mol P/mol SCOD) observed in the current study was comparable to the previous laboratory-scale experimental study based on ASS (0.41–0.51 mol P/mol C) [33].

Although the kinetics of the anaerobic and aerobic processes were affected in the pilot-scale SB-MBBR, high levels of P uptake were observed both at 10 °C (99.4 %) and 30 °C (95.2 % P). This observation could be supported by the observed biomass P content (0.06–0.10 mg P/mg TSS, Table 5), which was not drastically affected by the gradual change in temperature.

The total attached biomass concentrations were varied between 13.1 and 22.1 g TSS/m^2, and decreased with increasing temperature (Table 5). The results were comparable to the previous study that demonstrated higher attached biomass concentrations at a lower temperature, resulting in lower P removal efficiency [18].

Several researchers have documented contradicting results regarding EBPR under different operating temperatures. Previous

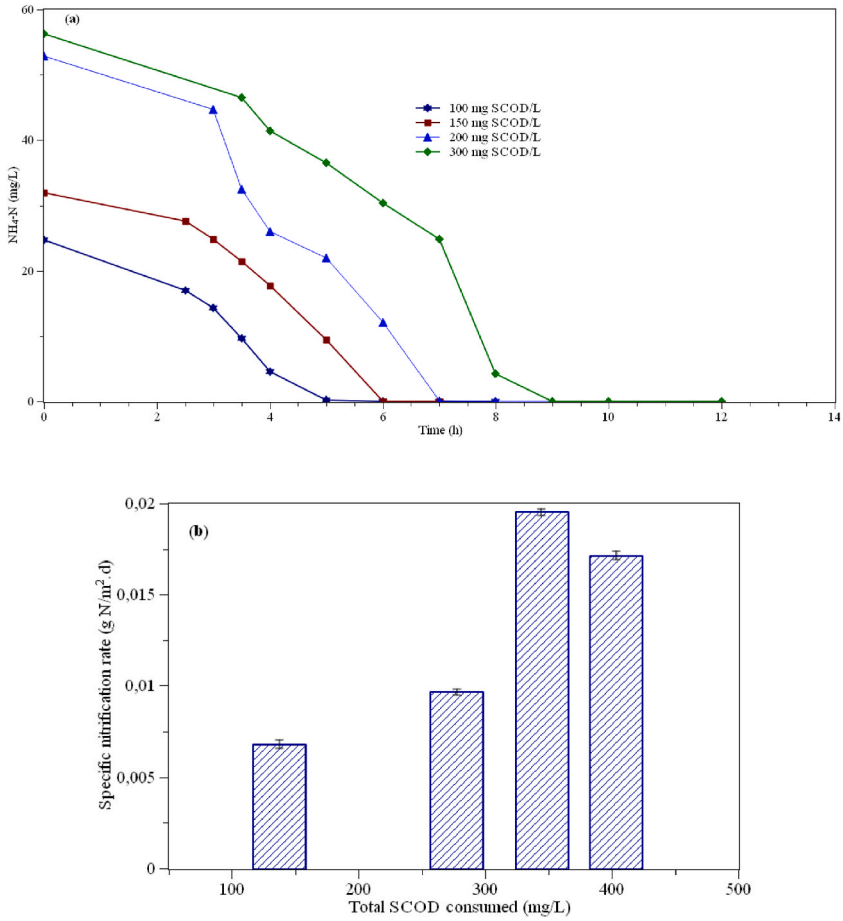

Fig. 6. A typical kinetic study illustrating the nitrification results at different VFA dosing (a) and the relationship between nitrification rate and SCOD consumed (b) at 2.5 L air/min and 20 ^{o}C.

studies have demonstrated that temperatures higher than 20 ^{o}C deteriorate P removal efficiency by lowering the desired microbial population and substrate affinity of PAOs in biomass [35,36]. Other researchers have reported that increasing the temperature to 30 ^{o}C results in the disappearance of PAOs and GAO, leading to the dominance of heterotrophic organisms [3,36]. According to Lopez-Vazquez et al. [37], GAOs have a clear advantage over PAOs for substrate uptake at temperatures above 20 ^{o}C [37]. Other researchers have reported that both GAOs and PAOs showed a similar maximum acetate uptake rate at a temperature lower than 20 ^{o}C, leading these organisms to substrate competition [38].

In the current study, a significant decrease in biomass P release and the low PO_4-P/SCOD ratio was observed at 30 ^{o}C (Table 5). The possible explanation for such a change in the reactor performance may be due to the proliferation of other microbial communities like GAOs at elevated temperatures. This may eventually lead to a stressful environment for PAOs/DPAOs and fail the reactor performance over the extended operational period. Since the biomass P content was still high (0.06 mg P/mg TSS), it was possible to restore the system rapidly by reducing the temperature to normal operational conditions (20 ^{o}C).

3.3. Simultaneous nitrification and denitrification under different operational conditions

3.3.1. Nitrogen removal performance at different initial VFA dosing

The N removal efficiencies ranged between 64.6 % and 95.7 % for the influent SCOD: N ratios between 7.5 and 12.5 (Table 2) in the current study. A previous report demonstrated efficient SNDPR and COD removal at C:N ratio of 5, resulting in over 89 % of NH_4-N oxidation [8]. Iannacone et al. [13] reported more than 80 % total inorganic nitrogen and PO_4-P removal efficiencies at 3.6 C:N ratio [13].

Complete nitrification (100 %) occurred for all levels of VFA examined (at 20 ^{o}C and 2.5 L air/min) in the present study. The NRE increased with increasing VFA dosing (64.6–95.7 %), and the highest NRE (95.7 %) was observed at 300 mg SCOD/L dosing. Low SNDPR observed at low VFA dosing (100 mg SCOD/L) could be explained by higher NO_3-N accumulation (8.9 mg/L, Table 2).

Fig. 6 a shows the typical cycle test on the ammonia oxidation during the aerobic stage. The influent NH_4-N variations show summer and winter operations. The kinetic study showed a maximum specific nitrification rate of 0.02 × 10^{-3} g N/m^2.d at 200 mg

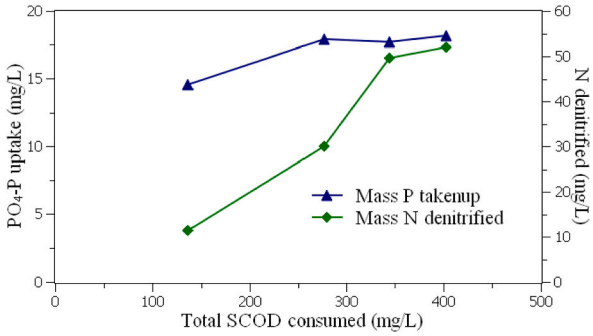

Fig. 7. P and N removal at different total SCOD consumption.

SCOD/L dosing (Fig. 6 b). The biomass-specific nitrification rate decreased slightly with the addition of 300 mg SCOD/L, possibly due to the acetic acid sensitivity of nitrifying organisms. A previous study has shown that the biomass nitrification rate decreases with increasing VFA concentration [39]. It has been reported that the nitrification rate of biofilters decreases with increasing organic carbon addition [40]. Low levels of NO_2-N (0.02–1.2 mg/L) and NO_3-N (0.7–3.2 mg/L) were measured at the end of the aerobic phase, which might indicate the denitrification potential of DPAOs with increasing SCOD concentrations.

Efficient N and P removal was accomplished through the SNDPR process, which was likely driven by the presence of an anoxic zone in the deeper biofilm layer as discussed earlier. Nitrate and nitrite were not detected during anaerobic conditions, restricting the growth of ordinary denitrifiers. The DPAOs uptake P under anoxic conditions utilizing NO_2^-/NO_3^- produced in the outer aerobic layer of the biofilm. A previous study has shown that SNDPR depends on the formation of the anoxic zone in the deeper part of the biofilm layer that is triggered by oxygen mass transfer limitation [6,9,41,42].

Periodic exposure of PAOs to NO_2^-/NO_3^- could promote the selective growth of DPAOs, enhancing anoxic P uptake and reduction of NO_2^-/NO_3^- to N_2 gas in a single SB-MBBR Bio-P system. The current study showed better P and N removal efficiencies than the study conducted by Lin et al. [31] where they performed denitrification (74.81 %) and P (91.15 %) removal using a single-stage biofilter system, which was operated under anoxic/anaerobic mode with the addition of nitrite and nitrate as an electron acceptor [31].

The SNDPR was achieved successfully in the SB-MBBR Bio-P system. The results showed that P uptake increased with increasing SCOD concentration up to 150 mg SCOD/L dosing and then leveled off with the further increase in SCOD dosing. However, denitrification increased rapidly for 100–200 mg SCOD/L dosing, but it was not affected by a further increase in SCOD concentration (Fig. 7). The results suggest that increasing SCOD was more advantageous for denitrification than P removal. The ratio of P removed to SCOD consumed decreased with increasing SCOD. However, the N removal to SCOD consumed ratio increased with increasing SCOD dosing. Generally, the finding of this study showed that SNDPR requires sufficient VFA availability in the influent wastewater. However, the previous study has shown that it is challenging to achieve SNDPR under low VFA concentrations [18].

3.3.2. Nitrogen removal performance at different airflow rate

This study investigated the influence of different airflow rates on SNDPR in the SB-MBBR system. Efficient nitrogen removal was observed at 1.5 L air/min (3.6 mg DO/L), and 2.5 L air/min (4.6 mg DO/L, Table 2), which was likely due to efficient nitrification on the top aerobic layer and minimum amount of DO diffusion to the deep biofilm layer, facilitating denitrification by DPAOs. On the other hand, a low DO level (0.5 mg/L) repressed the nitrification and DPAO performances in the SB-MBBR system. A previous study reported 60 % total P removal at a low DO level (1.0 mg/L) and C/N ratio of 4.2 using MBBR system [7]. The lower P removal efficiency reported by Iannacone et al. [7] could be due to the lower carrier filling degree (40 %) considered, which is lower than the present study (61 %).

The airflow rate plays a crucial role in improving the system performance concerning SNDPR in MBBR processes. However, the system performance strongly depends on the strength of the wastewater. It has been documented that substrates transport through the biofilm are governed by diffusion processes, creating anaerobic-aerobic-anoxic zones in the biofilm carriers. The substrate concentration gradient depends on biofilm thickness and density and the concentration of the essential substrate in the liquid phase [7]. These characteristics of the biofilm system make the MBBR process a more attractive technology for SNDPR compared to the conventional ASS due to the low density and size of the flocs in ASS [43].

Optimization of aeration rates would enhance nitrification-denitrification and P removal efficiencies in SB-MBBR. The existence of active biomass on the top layer and a deep anoxic biofilm layer resulted in efficient P and N removal in the SB-MBBR. This efficient SNDPR was observed when the SB-MBBR was aerated sufficiently (2.5 L air/min, 4.6 mg DO/L) and provided with sufficient external VFA (150 mg SCOD/L) at 20 ^{o}C. The cycle study showed complete nitrification (100 %) at airflow rates of 1.5 L/min (3.6 mg DO/L) and 2.5 L air/min (4.6 mg DO/L, Fig. 8 a). However, the nitrification process was adversely affected at an airflow rate of 1.0 L/min (0.5 mg DO/L). The specific nitrification rate ($3.5 \times 10^{-4} - 2.2 \times 10^{-2}$ g N/m^2.d) increased with increasing DO level (0.5–4.6 mg/L, see Fig. 8 b). The dependency of nitrification on DO concentrations showed linear relationship ($R^2 = 0.9992$ see Fig. 8 b). This result was inconsistent with a previous study that reported specific nitrification rates between 1.0 and 1.2 g N/m^2.d for full-scale MBBR treatment plants [44]. These differences could be explained by full-scale MBBR (previous study) versus pilot-scale SB-MBBR (current study) operations, recirculation-based continuous flow (previous study) versus fill-empty-based sequence batch operations (current study),

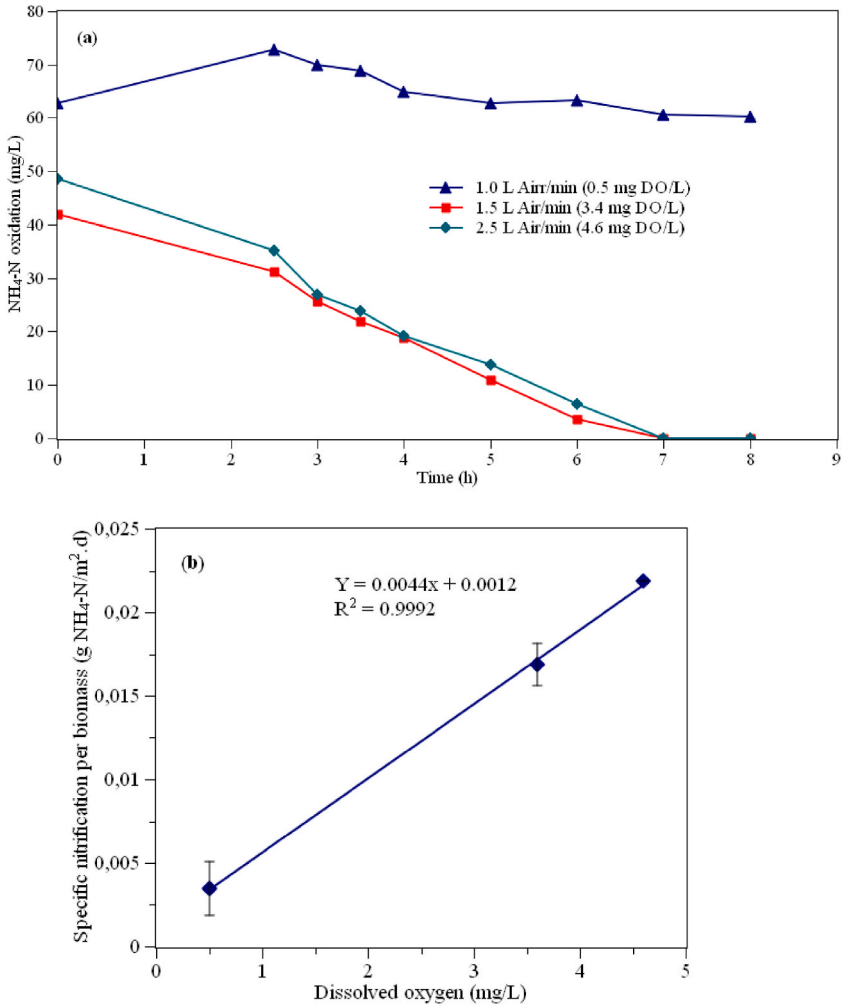

Fig. 8. A typical kinetic profile demonstrating the effect of airflow rate (DO concentrations) on NH_4-N oxidation (a) and the relationship between specific nitrification rate and dissolved oxygen concentrations (b).

the variation in the influent NH_4-N concentration, the selected operational temperature, and DO concentrations applied in the two MBBR systems. These researchers also highlighted that higher DO concentration can enhance nitrification rates [44]. Overall, the system showed stable performances for the long-term operation at airflow rates of 1.5 and 2.5 L/min and 3.6 and 4.6 mg DO/L, resulting in NRE of 86.2 and 88.8 %, respectively (Table 2). As a result, it can be concluded that higher DO concentration could enhance nitrification rates in the MBBR system.

No nitrite and nitrate accumulation were observed in the system compared to the previous study operated with diluted wastewater, and high DO levels [18]. This is because the NO_2^-/NO_3^- produced in the outer layer of the biofilm was reduced to N_2 gas in the anoxic layer of the biofilm (denitrification and anoxic P uptake occurs). However, SNDPR was highly impaired (with no net P uptake) at an airflow rate of 1.0 L/min (0.5 mg DO/L). This ultimately leads to deteriorated SNDPR performance, indicating suppression of nitrifiers and PAOs/DPAOs performances. The result was consistent with a previous study, where a low level of aeration resulted in a low DO level in the reactor, hindering nitrifiers' performance [45].

Despite the nitrification inhibition, the NRE reached 27.5 % (0.5 mg DO/L) over the long-term operation (Table 2). In this case, the N removal observed might be due to the presence of heterotrophic bacteria instead of the DPAOs, which is explained by the observed negative P removal efficiency. A previous study has shown that the DPAOs uptake VFA and store it as PHA under anaerobic conditions and they metabolize this internally stored PHA (like the ordinary PAOs) for SNDPR [30,46]. Generally, the DO level, the NO_2^- and NO_3^- pathways, and the optimized use of the organic carbon were keys for the successful SNDPR via DPAOs. The current results show that biomass enriched may be DPAO dominated. However, further investigation was required to confirm the P uptake process (anoxic or aerobic) and which microbial community played a significant role in denitrification processes.

3.3.3. Nitrogen removal performance at different temperatures

Temperatures of 10, 20, and 30 ^oC were employed to evaluate SNDPR. The experiment was conducted at an airflow rate of 2.5 L/

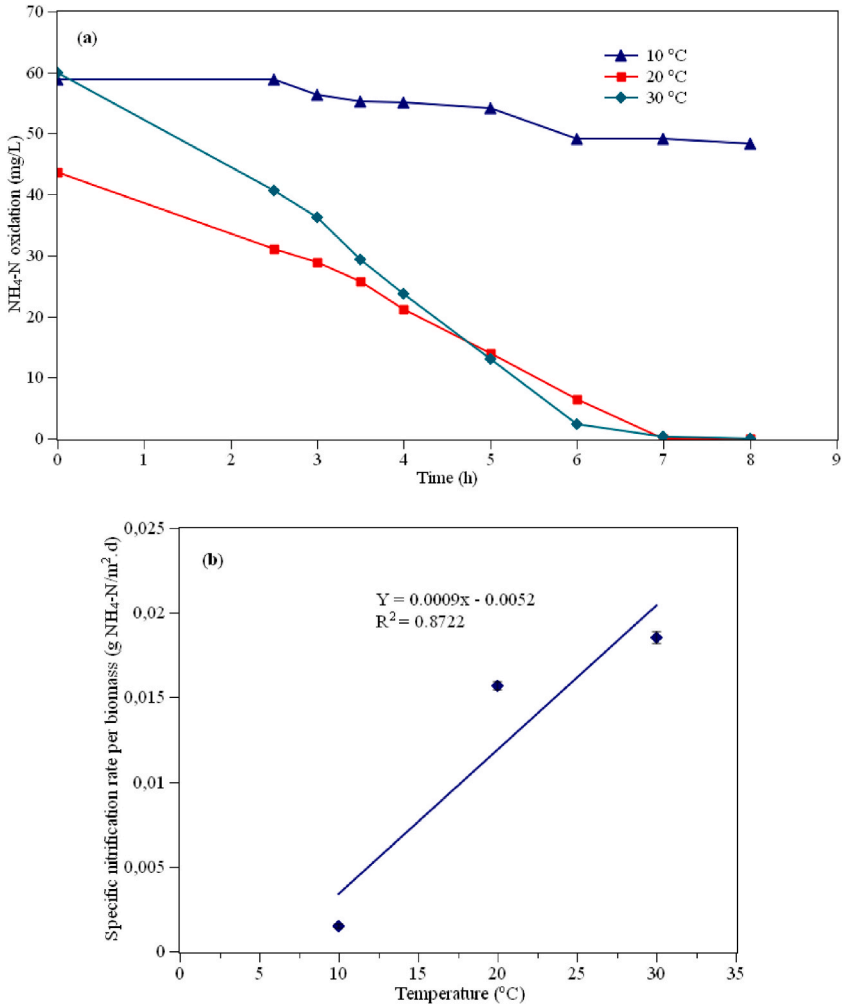

Fig. 9. A typical kinetic study on **a**mmonia conversion at different temperatures (a) and the relationship between specific nitrification rate and temperature (b).

min (4.6 mg DO/L) and a VFA concentration of 150 mg SCOD/L. The findings showed that ammonium oxidation was affected at 10 °C, resulting in 34.5 % nitrification efficiency. Fig. 9 a presents the NH_4-N oxidation profile in a typical cycle test. The results were in line with a previous study that demonstrated N removal from wastewater was severely affected at 10 °C [47]. The specific nitrification rates were estimated by the regression line, considering the first 2 h aerobic HRT. The specific nitrification rate per biomass increased with increased temperature ($1.5 \times 10^{-3} - 1.9 \times 10^{-2}$ g N/m^2.d), indicating a positive correlation for studied temperature ranges (R^2 = 0.8722, Fig. 9 b), which was consistent with the literature [48]. The specific nitrification rate obtained in this study was incomparable with a previous study that reported a volumetric nitrification rate between 300 and 400 g N/m^3.d in the MBBR operated at 10 °C [49]. The current study agrees with a previous study that reported specific ammonia oxidation rates decreased by 1.5 times when the temperature decreased from 25 to 15 °C [50]. However, other researchers have shown that the nitrification rates are not affected in the temperature range between 7 and 18 °C, provided that the reactors are operated at a DO level of 5.0 mg/L [49].

The highest NRE was 97.2 % and 85 % at 20 and 30 °C, respectively, but the lowest value was obtained at 10 °C (25.2 %) see Tables 2 and 5 The findings confirm the third hypothesis that stated the specific nitrification, P release, and P uptake rates were affected at 10 and 30 °C; however, the overall PRE was not affected.

3.4. Comparison of N and P removal efficiencies at different operational conditions

An integrated P and N removal in the biofilm processes occurs provided that the desired microbial communities stored sufficient PHA and are exposed to NO_2^-/NO_3^-. In the present study, N and P removal efficiencies increased with increasing VFA dosing as shown in Fig. 10 a. Similarly, high N and P removal efficiencies was observed at airflow rates of 1.5 and 2.5 L air/min. However, both N and P removal were highly affected at airflow rate of 1.0 L/min (Fig. 10 b). It is well known that nitrification is highly dependent on DO level in the solution. The SB-MBBR system was not well aerated at a lower airflow rate, which restricted the biofilm carrier movement.

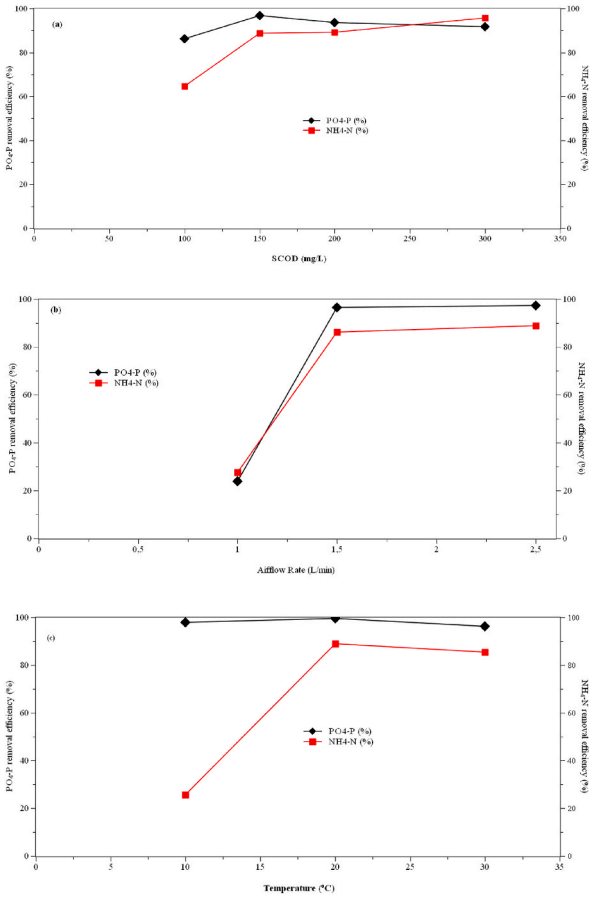

Fig. 10. N and P removal efficiency at different VFA dosing (a), airflow rate (b) and temperature (c).

Therefore, attaining the desired DO level becomes challenging, hindering the conversion of NH_4-N into NO_2-N and NO_3-N. Similarly, nitrification was affected at lower temperatures, resulting in a lower N removal efficiency. However, P removal efficiency was not affected at the three temperatures (Fig. 10 c). Overall, operating the SB-MBBR reactor at room temperature with reduced airflow rate and having enough VFA and P available resulted in a stable SNDPR performance.

4. Conclusions

The present study showed that the SB-MBBRs system was of great interest for the SNDPR from P-rich industrial influenced municipal wastewater, without the need for pre- or post-denitrification processes or sludge recirculation. The following conclusion were drawn from the present study:

- The present study successfully demonstrated stable and efficient SNDPR on weekends (85.9–95.6 % PRE) when the SB-MBBR receives sufficient VFA supply during weekdays operations.
- Reliable and efficient SNDPR (86–97 %) has been recorded with an optimum airflow rate of 2.5 L air/min, 150 mg SCOD/L dosing and temperature of 20 ^{o}C.
- Reduced airflow rate (1.0 L/min) was shown to affect the SNDPR process the most, demonstrating low P release and removal.
- Increasing temperature from 20 to 30 ^{o}C affected the P release kinetics, demonstrating a substantial reduction in P release.
- The results of this study could serve as a guiding document for the wastewater treatment plant operators, seeking to manipulate the SNDPR processes, improve the design of the SNDPR reactors and apply appropriate substrate management strategies.
- It would be interesting to study how the SNDPR process is affected by multi-factor operational conditions.
- PHA and microbial community analysis are required to verify the results of this study.

CRediT authorship contribution statement

A.B. Fanta: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **S. Sægrov:** Visualization, Project administration. **K. Azrague:** Visualization. **S.W. Østerhus:**

Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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