



Nutrient recovery and microalgae biomass production from urine by membrane photobioreactor at low biomass retention times

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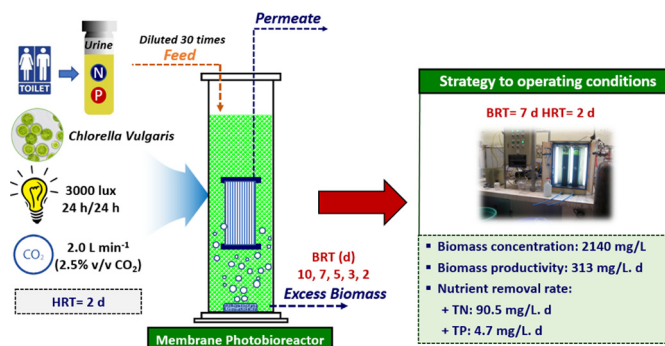
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HIGHLIGHTS

- A membrane photobioreactor was applied for nutrient capture from urine.
- Urine was indeed a potential source for microalgae biomass production.
- Biomass retention time (BRT) of 7 d was proposed for optimum operation.
- Under BRT of 2–5 d, such a drastic decrease in biomass accumulation was noticed.
- The BRT-dependent biomass accumulation governed the TN removal rate.

GRAPHICAL ABSTRACT



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ABSTRACT

Urine has been considered as an ideal nutrient source for microalgae cultivation thanks to its composition containing the high concentrations of nitrogen and phosphorus. Herein, the microalgae growth in urine was evaluated in a lab-scale membrane photobioreactor (MPBR) system. This work aimed to validate the influence of low biomass retention times (BRT) (10, 7, 5, 3, 2 d) on nutrient remediation and biomass productivity. It revealed that BRT of 7 d resulted in synergistically high biomass production (biomass productivity of 313 mg/L.d) and removal rates (TN of 90.5 mg/L.d and TP of 4.7 mg/L.d). Notably, the short BRT of 2–5 d was not sufficient to trigger actively growing microalgae and thus reduced biomass production rate. In addition, as operated at a low flux of 2 L/m².h, MPBR system required no physical cleaning for 100 days of operation. The BRT-dependent biomass concentration played a pivotal role in changing the fouling rate of MPBR; however, the fouling is reversible in the MPBR system under the low flux condition.

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1. Introduction

In recent decades, wastewater generated from human activities has caused many negative impacts on surface water resources. In developing countries, untreated municipal wastewater containing high nutrient compounds has been directly discharged to the receiving sources i.e., river, streams, canals (T.-T. Nguyen et al., 2020; T.T.D. Nguyen et al., 2020; T.T.N. Nguyen et al., 2020). This led to crucial contamination or eutrophication of the aqueous environment (Praveen et al., 2016) (T. Nguyen et al., 2019; T.T.N. Nguyen et al., 2019). One of the pollutants, urine, is a liquid waste product of the human body, which undergoes filtration of blood and is secreted via kidneys (Karak and Bhattacharyya, 2011). The composition of urine depends on feeding habits, physical activities, body sizes (Karak and Bhattacharyya, 2011). Although urine volume production is only 1.0–1.5 L/person.d and accounts only for 1% of total domestic wastewater volume, this waste source contributes approximately to 80% of nitrogen and to 50% of phosphorus load in domestic wastewater (Chatterjee et al., 2019). This fact needs to be noticed if a preferred decentralized treatment system is implemented for developing countries in which the urine source would be separated for nutrient recovery (Igos et al., 2017). For instance, source-separated urine has been utilized for liquid fertilizer for soil and plant, struvite precipitation (Karak and Bhattacharyya, 2011).

In recent decades, in order to achieve sustainability goal, microalgae cultivation is raising much attention. Therefore, several studies on microalgae grown in wastewater has gained much attention to practical application (Posadas et al., 2017; Li et al., 2019). This proposed solution favors not only nutrient recovery from wastewater but also biomass production for the value-added products such as biodiesel, bioethanol, bio-fertilizers, bio-plastic and pharmaceutical, feed supplements (Chen et al., 2015; Luo et al., 2017; Li et al., 2019). If microalgae are grown in urine, this could bring a feasible way to capture nutrients from urine (Chatterjee et al., 2019). Therefore, the use of urine for microalgae cultivation needs to be noticed. A past work has evaluated the nutrient removal and the biomass production rate of *Chlorella sorokiniana* under a series of dilution factors of 20, 10, 5, 2, which was cultured in a short-light photobioreactor (PBR) utilizing synthetic urine (Tuantet et al., 2014). Their findings indicated that a dilution factor of 2 (50% v/v urine) was found for sufficient cultivation and over 90% of total nitrogen and phosphorus removal (Tuantet et al., 2014). Another study indicated that urine at a dilution of 1:25 was a favor for cultivating microalgae with the biomass productivity of 60 mg/L.d (Jaatinen et al., 2016). The optimal dilution factor can probably be attributed to different microalgae species, light intensity, the addition of trace element, characteristics of real urine (Jaatinen et al., 2016). In practice, the pilot-scale algae-based cultivation systems are generally open pond (OP) and photobioreactor (PBR). The OP have some inherent disadvantages such water evaporation, easy contamination, requirement of large land area, and difficult control for physico-chemical conditions (Luo et al., 2017). Meanwhile, the obstacles of PBR are poor in settling ability, biomass washout, and harvesting limitation (Bilad et al., 2014). To overcome those disadvantages, a membrane photobioreactor (MPBR) was introduced as a promising technology (Luo et al., 2017).

The MPBR system is a simple combination of a conventional PBR process with a membrane module submerged in the reactor. Compared to a conventional PBR, the MPBR system enhances the light accessibility and also provides a sufficient mixing, easily accessible carbon source, an operation at lower hydraulic retention time (HRT), high loading rate, and completed retention of biomass (Luo et al., 2017). These superiorities facilitate the decrease in construction and operation costs in the MPBR system. To date, different sources (i.e., domestic, slurry wastewater, agricultural effluent, membrane bioreactor effluent) utilizing for algae cultivation were explored for evaluation of the biomass production and the nutrient recovery in the MPBR process (Gao et al., 2014; Marbelia et al., 2014; Gao et al., 2016a). It is worth noting that the

performance of an MPBR process is influenced by vital factors such as characteristics of cultivation sources (C:N, N:P ratios of wastewater), operating conditions, environmental factors (pH, DO, CO₂ influent concentration) (Luo et al., 2017; Vo et al., 2018; Vo et al., 2019). For the design and operating parameters, in reality, hydraulic retention time (HRT) and biomass retention time (BRT) are considered as two pivotal parameters to govern the successful operation of the MPBR system. It is important to note that decoupling of HRT and BRT in the MPBR system favored producing higher biomass concentration and improving nutrient removal (Marbelia et al., 2014). For instance, biomass productivity in the MPBR was 3.8–9.1-fold times higher than that in the PBR (Bilad et al., 2014; Gao et al., 2014). A higher biomass concentration retaining in MPBR facilitated the decrease in HRT and the enhancement of nutrient removal (Luo et al., 2017). Not only does the MPBR system exhibit a smaller footprint, but it also helps to avoid wash-out of microalgae biomass (Marbelia et al., 2014; Bilad et al., 2014; Gao et al., 2014; Luo et al., 2017). The impact of HRTs (2.0, 2.5, 3.3, and 5.0 days) on the performance of MPBR was investigated systematically under typical conditions: *Chlorella vulgaris* used, fully retaining the biomass (e.g., BRT = ∞) (Marbelia et al., 2014). Their works indicated that the HRT of 2 days was an optimal condition for the increase in biomass productivity (60 mg/L.d) but still be acceptable for nutrient removal from the MBR permeate (Marbelia et al., 2014). On decreasing the HRT from 24 h to 8 h, the biomass productivity increased from 65.5 to 72.7 mg/L.d when treated sewage was used as a nutrient source (Honda et al., 2017). The findings indicated the short HRT facilitated maximizing biomass productivity, but this operation did not favor sufficient nutrient removal (Xu et al., 2015; Gao et al., 2016a, 2016b; Honda et al., 2017). This fact highlights a trade-off between biomass productivity and nutrient removal efficiency if only controlling the HRT parameter in the MPBR. Apart from HRT, the BRT is also a crucial parameter that needs to be noticed in the MPBR. This parameter helps to control microbial growth rate in the reactor and the amount of biomass wasted from the MPBR system, which therefore affects the biomass concentration, microalgae productivity, and nutrient removal (Luo et al., 2017). Under the operation of a short HRT (6 h) and BRT (5 days), the MPBR facilitated to promote the biomass productivity (131.7 mg/L.d) but this was not concomitantly achieved with high nitrogen removal (Xu et al., 2015). This outcome was also found for much longer BRT operation of 18 days and a HRT of 24 h in another study (Honda et al., 2012). Another hand, the BRT of 10 days was suggested as a proper condition to attain sufficient nutrient removal from secondary effluent (Xu et al., 2015). These findings suggested nitrogen uptake by biomass was influenced not only by BRT-dependent biomass concentration but also nutrient loading rate governed by HRT. As reported an MPBR operated at extended BRT allows to retain a high biomass concentration in the reactor and to minimize waste biomass (Luo et al., 2017). Nevertheless, this operation is not beneficial for nitrogen uptake by microalgae (Xu et al., 2014). A prolonged BRT-induced high biomass can probably cause shelf-shading or mutual shading of microalgae. This operation induced dark respiration of microalgae which led to the significant production of algal-derived organic matter (AOM); thus accelerating the fouling degree in MPBR (Luo et al., 2017).

Given the research gaps indicated above, it is essential to investigate for a low BRT and high HRT conditions in MPBR. These proposed conditions appear to attain sufficient both algal biomass production and nutrient removal from wastewater. This investigation is a critical need if fresh urine with high strength nutrient concentration is utilized for microalgae cultivation. While urine is a potential source for microalgae cultivation thanks to rich nutrients (e.g., TN of 3480 ± 130 mg/L and TP of 190 ± 52 mg/L) (Chatterjee et al., 2019), little attention is directed towards the MPBR-based microalgae grown in urine. Therefore, in the current study, *Chlorella vulgaris* was grown with urine using the MPBR system. For operating conditions, a prolonged HRT of 2 days was retained while a series of low BRTs (10; 7; 5; 3; 2 days) was alternated for the evaluation of the MPBR performance. Not only does the current

study evaluate the effect of the low BRTs (10; 7; 5; 3; 2 days) on the nutrient recovery and microalgae biomass production from real urine but it proposes a proper BRT for operating a MPBR system.

2. Materials and methods

2.1. Microalgae strain and urine

The microalgae used in the current work was *Chlorella vulgaris*, which performed a sufficient nutrient uptake via biomass (T.-T. Nguyen et al., 2020; T.T.D. Nguyen et al., 2020; T.T.N. Nguyen et al., 2020). This strain was provided by the Research Institute of Aquaculture II, Vietnam. *Chlorella vulgaris* was pre-cultivated in a sterilized Bold's Basal Medium (BBM) and cultured in a bubble column photobioreactor (PBR) under typical conditions: the light intensity of 100 $\mu\text{mol}/\text{m}^2\cdot\text{s}$, room temperature, air aeration of 2 L/min, which has been described elsewhere (Nguyen et al., 2016a; Vo et al., 2018). The pre-cultured algae cells during the log growth phase were taken and centrifuged at 3600 rpm for 10 min to remove supernatant, followed by washed with DI water and used for the experiment of BRTs with initiating the dry microalgae concentration of 50 mg/L.

Fresh urine was collected from the male toilet in Ho Chi Minh City University of Technology, Vietnam. The use of real urine in our current study was received certain permission from volunteers (male students in our university). We put a plastic box in the urinal basin with a note that "The collected urine is used for the scientific research experiment". To minimize the effect of urea hydrolysis, the fresh urine was immediately stored at 4 °C in a refrigerator. The fresh urine was characterized with the composition as follows (average \pm standard deviation, n = 5): total nitrogen (TN) of 5015 \pm 209 mg/L, $\text{NH}_4^+\text{-N}$ of 2258 \pm 43 mg/L, $\text{NO}_3^-\text{-N}$ of 7 \pm 3 mg/L, $\text{NO}_2^-\text{-N}$ of 1 \pm 0.5 mg/L, total phosphorus (TP) of 347 \pm 2 mg/L. prior to experiments, the urine was diluted 30 times with tap water to obtain the appropriate concentration of nutrients (N, P), which allowed to attain a favorable feeding culture of *Chlorella vulgaris* in a photobioreactor (Jaatinen et al., 2016). A detailed composition of used urine was presented in Table 1.

2.2. Lab-scale system and operating conditions

The photobioreactor was installed inside a thick wood box (5 mm) to maintain a constant temperature and to prevent natural light. Three lamps with a total light intensity of 3000 lx were set up inside the box to provide continuous illumination (24 h/24 h). Two identical photobioreactors with a diameter of 100 mm and a height of 600 mm were employed for microalgae cultivation to obtain duplicate experiments. Diluted urine (30 times) from the feed tank was pumped into the photobioreactor for microalgae cultivation. A submerged microfiltration (MF) membrane installed in feed tank as a pretreatment step to avoid suspended solids and bacterial contamination coming to the MPBR. An electric floater was installed inside the photobioreactor to assure the working volume of 4 L. A hollow fiber membrane (MF) module (width \times height = 95 mm \times 320 mm, Mitsubishi, Japan) made of polyvinylidene fluoride (PVDF) was submerged into the photobioreactor for separating algal biomass and treated wastewater. The membrane has a pore size of 0.4 μm and a working surface area of 0.05 m^2 . Air mixture flow into the photobioreactor was provided via an air pump and a pure

carbon dioxide tank. The aeration system for the reactor consisted of a 20 mm diameter air diffuser which was located at the bottom of the column which could provide sufficient carbon source for microalgae photosynthesis, but also achieve mixing condition. Three rotameters of an air pump, CO_2 gas, and gas mixture were employed to adjust the carbon dioxide/air mixture at 2 L/min flow rate possessing 2.5% (v/v) of CO_2 . Two suction pumps were installed to retain an operating flux of 2 $\text{L}/\text{m}^2\cdot\text{h}$. A detailed schematic diagram of the experimental lab-scale MPBR is presented in Fig. 1.

The membrane photobioreactor (MPBR) was automatically operated using timers, solenoid valves to maintain a fixed hydraulic retention time (HRT) of 2 days. An operation of infinity BRT was initiated to favor acclimatization for microalgae growth. No biomass was withdrawn from the photobioreactor during this acclimatization period. A discharged valve was installed at the bottom of photobioreactor. During the operation, it was used to control BRTs (10, 7, 5, 3, 2 days) by withdrawing the biomass volume of 0.4, 0.6, 0.8, 1.3, 2.0 L respectively. The pH of diluted urine was in the range of 8.3–8.6 before pumping into MPBRs. Each membrane permeate was intermittently withdrawn by a suction pump in an 8-min on/2-min off cycle. The digital pressure gauge was installed to record the daily change in the transmembrane pressure (TMP) as an indication of membrane fouling. As denoted in Table 2, the MPBR system was operated in turn with BRTs: 10 d (day 13th–80th), 7 d (day 81st–101st), 5 d (day 102nd–183rd), 3 d (day 184th–236th), and 2 d (day 237th–272nd).

2.3. Microalgae biomass analyses

Cell density was determined each day using a hemocytometer (Germany) under a microscope (Eclipse E50i; Nikon, Tokyo, Japan). In detail, each 50 μL sample of microalgae was put in the counting chamber using a micropipette. For a detailed counting chamber, it consists of one large square containing 16 mediums. Each medium has 25 small squares. The small square with the size of depth is 1/10 (mm), which possessed an area of 0.025 mm^2 . The formula to calculate the cell density after counting is $A/(a \times b \times c)$.

Where A is total cells in total counted mediums (cell/ml), a is the size of the depth of a small square (0.1 mm), b is the area of a medium ($b = 0.0025 \times 16$), c: number of media counted.

When the cell density from the above method was obtained, calculating the dry biomass concentration was done using the formula for the standard curve equation from our previous works (Nguyen et al., 2016a; Vo et al., 2018). Also, biomass productivity was determined using Eq. (1) (Gao et al., 2018; T.-T. Nguyen et al., 2020; T.T.D. Nguyen et al., 2020; T.T.N. Nguyen et al., 2020).

$$\text{Biomass productivity } (\beta, \text{mg/L}\cdot\text{d}) : \beta = \frac{X}{\text{BRT}} \quad (1)$$

where X is dry biomass concentration in membrane photobioreactor (mg/L), BRT is biomass retention time (d).

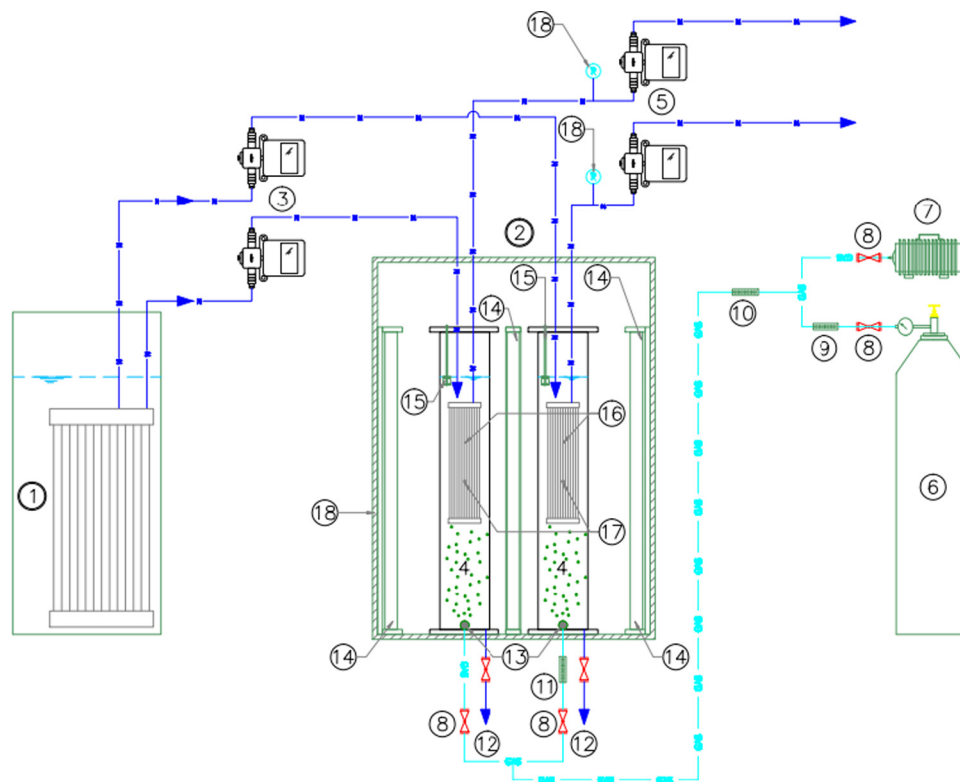
2.4. Analytical parameters

Prior to analyses, a 200 mL sample was filtered using a filter with a pore size of 0.45 μm (Fisher Whatman puradisc-25 mm). Such

Table 1
Composition of urine with 30 dilution times.

Urine composition (mg/L, except pH)	BRT = 10 d	BRT = 7 d	BRT = 5 d	BRT = 3 d	BRT = 2 d
pH	8.3 \pm 0.5	8.6 \pm 0.2	8.3 \pm 0.3	8.3 \pm 0.2	8.6 \pm 0.2
TN	217 \pm 110	181 \pm 90	195 \pm 45	171 \pm 35	178 \pm 14
$\text{NO}_3^-\text{-N}$	0.2 \pm 0.1	0.2 \pm 0.1	0.4 \pm 0.1	0.4 \pm 0.1	0.4 \pm 0.1
$\text{NO}_2^-\text{-N}$	0.4 \pm 0.1	0.1 \pm 0.03	0.02 \pm 0.01	0.02 \pm 0.01	0.06 \pm 0.02
TP	12.0 \pm 3.8	9.4 \pm 3.4	10.7 \pm 4.7	9.9 \pm 3.9	11.3 \pm 3.5

Remark: BRT: Biomass retention time (n = 68, 21, 82, 53, and 36 for BRT of 10, 7, 5, 3, 2 d).



1-Feed tank; 2-Membrane photobioreactor; 3-Feed pump; 4-Photobioreactor; 5-Permeate pump. 6: Compressed CO₂ cylinder; 7-Air blower; 8-Valve; 9;10;11-Rotameters; 12-Discharge valve; 13-Air diffuser; 14-Fluorescent lamp 15-Electric floater; 16-Submerged membrane module; 17-Sampling valves;18-Wooden box; 19-Digital pressure

Fig. 1. Schematic diagram of the lab-scale membrane photobioreactor system.

parameters of total Kjeldahl nitrogen (TKN), total phosphorus (TP), nitrite (NO₂⁻-N) and nitrate (NO₃⁻-N) were analyzed based on the standard method (APHA, 1992). The pH was measured directly by using the pH meter HANA. Transmembrane pressure (TMP) was recorded daily by a digital pressure gauge.

2.5. Statistical analysis

Results were showed as the average value \pm standard deviation. Parametric one-way analysis of variance (ANOVA) was used to examine significant differences among groups of BRT conditions using the IBM SPSS statistics software 20. $p < 0.05$ indicated significance at 95% confidence.

3. Results and discussion

3.1. Microalgae growth and biomass production under various biomass retention time

As presented in Fig. 2, microalgae growth in urine diluted 30 times was evaluated under various biomass retention times when the MPBR

Table 2
Operating conditions of the membrane photobioreactor.

HRT (day)	BRT (day)	Operating time	Operation day (d)
2	Infinity	Day 0–2th	12
	10	Day 13th–80th	86
	7	Day 81st–101st	21
	5	Day 102nd–183rd	82
	3	Day 184th–236th	53
	2	Day 237th–272nd	36

system was employed for cultivation. For the acclimatized stage with $BRT = \infty$ (i.e., without withdrawing microalgae biomass volume), it was found that after 12 d the biomass concentration significantly increased from 50 mg/L to 2120 mg/L. No lag phase was observed in the microalgae growth curves in the first 10 d, suggesting that *Chlorella vulgaris* can probably adapt well in the urea medium of urine. The lack of lag phase is attributed to urine possessing a high concentration of organic carbon and nutrient (Chatterjee et al., 2019; Tuantet et al., 2019). As reported, a control of BRT is essential for governing the successful operation of the MPBR system because this factor affects the biomass concentration and microalgae productivity, and nutrient removal (Luo et al., 2017). Therefore, a BRT of 10 d was initiated on day 14th and it was evaluated during 68 operation days. It is noted that under this condition there was a high fluctuation of nutrient feed loading rate (i.e., 109 ± 55 mg N/L.d) and this was acceptable with the use of real urine. This fact led to the unstable growth of microalgae with a biomass concentration of 2020 ± 412 mg/L. For BRT of 10 d, microalgae biomass reached a peak concentration of 3100 mg/L. The high cell density-induced light limitation might be another reason for the fluctuation in microalgae growth (Praveen et al., 2019). The findings indicated that the microalgae could grow proficiently in the diluted urine.. It is important to note that the microalgae biomass concentration obtained in this study was significantly higher compared to that of the past studies (e.g., 730 mg/L) in which a photobioreactor and a urine dilution of 20 times was employed for cultivation (Chatterjee et al., 2019). These results highlighted that the MPBR outperformed the PBR in terms of retaining the high microalgae biomass concentration when urine was utilized as a substrate. This fact also reinforced that the membrane module submerged in the photobioreactor assisted to prevent the algal wash-out and thereby facilitate a higher biomass concentration

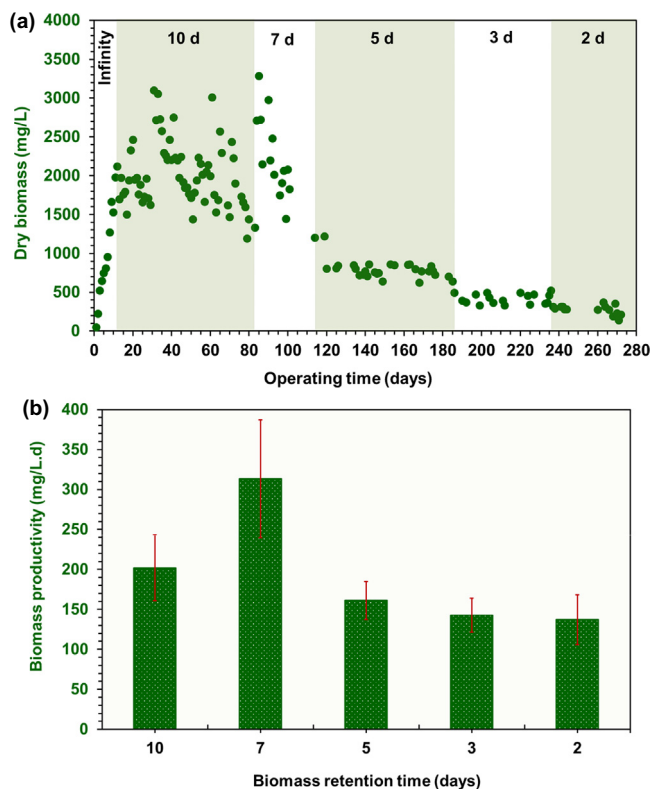


Fig. 2. Biomass production in membrane photobioreactor under different biomass retention times (10, 7, 5, 3, 2 d): Dry biomass (a), biomass productivity (b). Data in panel a is the average value obtained from duplicate experiments.

(Honda et al., 2012; Gao et al., 2014). As the BRT was shortened to 7 d, the microalgae biomass still retained a high concentration of 2194 ± 530 mg/L. These results indicated there was no significant difference in biomass concentration as operated the BRT between 10 and 7 d (One-way Anova, $p > 0.05$). However, a lower biomass concentration of 806 ± 129 mg/L was found at a lower BRT of 5 d and these concentrations remained stably during 63 operation days (from day 120th to 183th). The biomass concentration continued to decrease to 428 ± 79 mg/L and 275 ± 59 mg/L with decreasing to the BRT of 3 and 2 d, respectively. Such a drastic decrease in biomass accumulation in MPBRs has been demonstrated earlier when lowering BRTs (Xu et al., 2015). The reason laid in the withdrawal of a large amount of biomass (20–50% volume) corresponding to BRTs (5–2 d), which might be difficult to compensate in a shorter period. However, compared to prolonged BRT, it can probably accept that a lower BRT facilitates to shorter time attaining a stable biomass concentration in the MPBR system. This result is consistent with the findings obtained by a past study (Praveen et al., 2019).

To propose the proper BRT conditions for biomass production, biomass productivity was calculated and compared (Fig. 2-b). The results showed that the high biomass productivity was 202 ± 41 and $313 \pm$

74 mg/L.d for the BRT of 10 and 7 d, respectively. As above-mentioned the results, although there was no difference in biomass concentration between these BRTs ($p > 0.05$), an operation at a shorter BRT (7 d) resulted in superior biomass production. Prolonged BRT of 10 d posed lower biomass productivity and this fact can be probably attributed to lower food to microalgae ratio (Table 3) and high-density cell-induced limited light supply. As reported the light density supply has a significant effect on the microalgae growth and this factor is limited due to high-density microalgae cell at extended BRT (Luo et al., 2017). This led to shelf-shading of algae which induced the problem of respiration in the dark (Luo et al., 2017). However, this outcome was not expected for an operation at a shorter BRT of 5, 3, 2 d, which posed the lower biomass productivity of 161 ± 24 , 143 ± 21 , 137 ± 31 mg/L.d, in that order. Given biomass productivity between conditions had no significant difference based on statistical analysis ($p > 0.05$). This implied that as biomass was continuously removed from the MPBR with the abundant withdrawal of 20–50% volume, a short BRT of 2–5 d was not sufficient to trigger actively growing microalgae. Our finding suggested the BRT of 7 d is critical operation to gain concomitantly high biomass production rate and biomass concentration. Given an optimal condition, a biomass removed from the MPBR was readily compensate without influencing the biomass accumulation. Overall, biomass productivity at BRTs obtained in this study exhibited a remarkably higher rate compared to other works (Gao et al., 2014; Marbelia et al., 2014). Their study showed biomass productivities were 39.3 and 60 mg/L.d when treated sewage was employed as the cultivated substrate. These findings suggested that urine is undoubtedly effective substrate source for microalgae biomass production.

3.2. Effect of biomass retention time on nutrient removal of membrane photobioreactor

As reported, real urine possesses two nitrogen features (i.e., urea and ammonium). Urea might be hydrolyzed to ammonium (NH_4^+-N) and this form is a favored nutrient source for the growth of *Chlorella vulgaris*. Since fresh urine was collected directly from the male student toilet with a frequency of every couple of days, urea hydrolysis-induced change in total nitrogen concentration was inevitable. This fact led to a high fluctuation in TN feed concentration. As denoted in Fig. 3, under the BRTs of 10, 7, 5, 3 d, the feed TN concentration (mg/L) was 217 ± 110 , 181 ± 90 , 195 ± 45 and 171 ± 35 , respectively. It was found that for the BRTs of 10 and 7 d there was a remarkable difference between feed and permeate TN concentration, indicating sufficient nitrogen removal. These results were consistent with high biomass concentration (2020, 2194 mg/L) and biomass productivity (202, 313 mg/L.d) obtained in those BRTs. When urine was utilized as a nutrient medium for microalgae cultivation, the nitrogen elimination was governed by such main ways: microalgae assimilation, ammonia volatilization, and struvite precipitation (Gao et al., 2016a). In this study, the feed pH was remained around 8.3. Thus, the influences of ammonia volatilization and struvite precipitation were negligible. It has been demonstrated that *Chlorella vulgaris* was highly capable of nutrient uptake and the dominant mechanism of TN elimination is assimilation to microalgae cells (Tuantet et al., 2014; Kim et al., 2015; Najm et al., 2017;

Table 3

Microalgae biomass concentration, nutrient loading rate, ratio of nitrogen to microalgae, and nutrient uptake rate under different biomass retention times.

BRT (d)	Microalgae biomass (mg/L)	Nutrients loading rate		Ratio of nitrogen to microalgae (1/d) ^a	Average nutrients uptake rate	
		TN (mg N/L.d)	TP (mg P/L.d)		TN (mg N/g biomass.d)	TP (mg P/g biomass.d)
10	2020 ± 412	108.5 ± 55.0	6.0 ± 1.9	0.05	39.4	0.9
7	2194 ± 530	90.5 ± 45.0	4.7 ± 1.7	0.04	31.9	1.1
5	806 ± 129	97.5 ± 22.5	5.4 ± 2.4	0.12	38.5	2.8
3	416 ± 79	85.5 ± 17.5	5.0 ± 2.0	0.21	40.9	3.0
2	275 ± 59	89.0 ± 7.0	5.7 ± 1.8	0.32	56.4	6.6

Remarks: BRT: Biomass retention time (n = 86, 21, 82, 53, and 36 for BRT of 10, 7, 5, 3, 2 d).

^a This ratio in the algae process is similar to F/M ratio in activated sludge process.

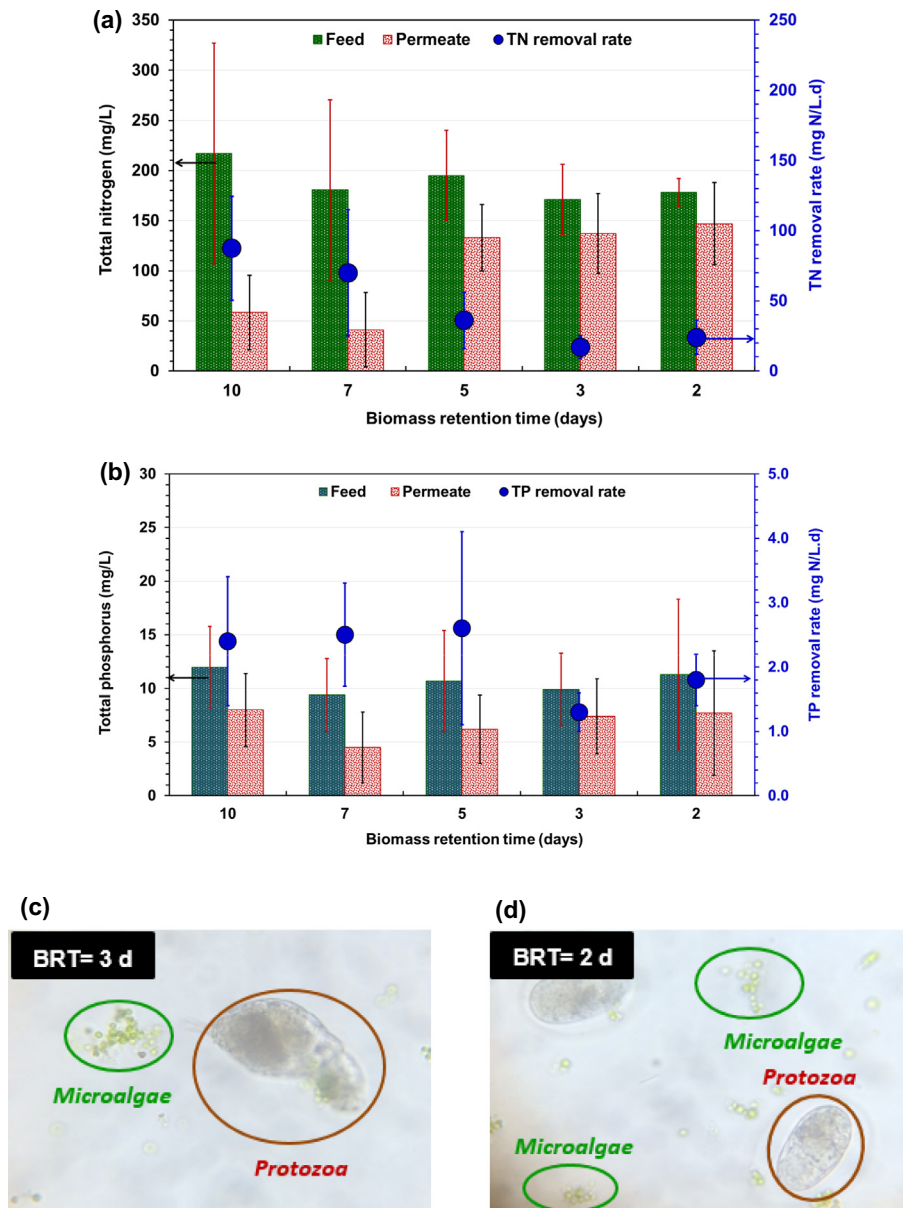


Fig. 3. Nutrient recovery of membrane photobioreactor under various biomass retention times: Total nitrogen (a), total phosphorus (b). Presence of *protozoa, rotifer* at the BRTs: 3 days (c) and 2 days (d), observed by microscope under 100 X magnification.

T.-T. Nguyen et al., 2020; T.T.D. Nguyen et al., 2020; T.T.N. Nguyen et al., 2020). This fact appeared to suggest that a significant nitrogen elimination was attained due to abundant assimilation of microalgae.

Under the BRTs of 7–10 d, the MPBR system exhibited a high TN removal rate of 87 ± 37 and 70 ± 45 mg/L.d, respectively. It was found that there was no significant difference in TN removal rate between these conditions ($p > 0.05$), which posed an average permeate concentration of 41–58 mg/L. By ways of contrast, a lower removal rate was found at a shorter BRT of 5, 3 and 2 d (36 ± 20 , 17 ± 8 , and 24 ± 12 mg N/L.d in that order). For these conditions, the lower biomass concentrations (275–806 mg/L) induced a low removal efficiency of TN that resulted in a high permeate concentration of 133–147 mg/L. These findings highlighted that the BRT-dependent biomass accumulation governed the TN removal rate in the MPBR system. It has been confirmed that retaining higher biomass concentration in the MPBR can lead to the more rapid removal of nitrogen (Åkerström et al., 2014). Table 3 shows that shorter BRTs of 5, 3, 2 d exhibited a higher nitrogen uptake of 38.5, 40.9, 56.4 mg N/g biomass.d compared to the other BRTs (10, 7 d). This implied that a high ratio of nutrient (N) to algae biomass

concentration (0.21–0.32 1/d), which is similar to F/M ratio in the activated sludge process, facilitated to trigger of more nitrogen uptake of microalgae. The presence of *protozoa and rotifers* was observed in these BRTs (Fig. 3-c, d). As demonstrated the presence of protozoa increases the nitrification rate, probably because of the ability of protozoa to influence bacterial growth (Madoni, 2011). As the results, the NO_2^- -N concentration was noticed in the permeate as follows: 5 d (47 ± 21 mg/L), 3 d (54 ± 25 mg/L), 2 d (44 ± 21 mg/L). These results highlighted that the nitrification process also occurred in these conditions. As reported *Chlorella vulgaris* did not favor uptake of the NO_2^- -N form and this led to its high concentration in the permeate (Markou et al., 2014; T.-T. Nguyen et al., 2020; T.T.D. Nguyen et al., 2020; T.T.N. Nguyen et al., 2020). This was in agreement with the findings reported from a past study, which suggested NH_4^+ -N and NO_3^- -N was priority source for assimilation of microalgae cells (Markou et al., 2014). The findings combined with the nitrification occurrence posed an insufficient TN elimination under the BRTs of 3, 2 d. It was found that given these conditions the NO_3^- -N concentration in permeate was 40 ± 16 and 53 ± 18 mg/L, respectively. Another point to support a low TN

removal rate was low biomass productivity. As the above-mentioned results, if biomass was continuously removed from the MPBR with the abundant withdrawal of 20–50% volume, a short BRT of 2–5 d was not sufficient to trigger actively growing microalgae and thus not enhanced biomass production rate even through obtaining significant higher nitrogen uptake rate. As a final comment, it has been demonstrated that the nutrients uptake and biomass production rate had no clear correlation under the changing BRTs, which has been reported from the previous works (Luo et al., 2017; Praveen et al., 2019). It can be concluded that the BRT can be considered as an indirect factor influencing nutrients removal in the MPBR (Luo et al., 2017; Praveen et al., 2019).

As shown in Fig. 3-b, TP removal rates at BRTs of 10, 7, 5 d had no significant difference ($p > 0.05$), attaining 2.4 ± 1.0 , 2.5 ± 0.8 , and 2.6 ± 1.5 mg P/L.d respectively. These outcomes were significantly higher compared to the others i.e., 3, 2 d. It was clear that the phosphorus removal depended on both the assimilation of the microalgae cell and pH change-induced precipitation (Gao et al., 2016a; T.-T. Nguyen et al., 2020; T.T.D. Nguyen et al., 2020; T.T.N. Nguyen et al., 2020). Under pH around 8.2, the phosphate precipitation is very minor. This fact thus suggested that TP remediation was mainly governed by microalgae uptake. It means that a higher biomass productivity facilitated to higher TP removal rate. This fact accepted that a lower TP removal rate was found at the shorter BRTs of 3, 2 d (1.3 ± 0.3 , 1.8 ± 0.4 mg P/L.d in that order). The highest TP removal efficiency of 52.1% was for the BRT of 7 d. This outcome was comparable with a past work in which their findings showed a TP removal efficiency in the cultivation of a PBR-based diluted urine 75 times. Under controlling the HRT of 1 d and the BRT of 21 d, the MPBR system exhibited a TP removal rate of 0.36 mg P/L.d as a secondary wastewater effluent (i.e., a low phosphorus concentration 0.80 mg P/L) was utilized for cultivation (Gao et al., 2018). This value was significantly lower compared to our work (TP removal rate of 1.3–2.6 mg P/L.d). A higher nutrient loading rate and shorter BRTs in our study might support the distinction. These findings implied that the phosphorus removal was strongly impacted by the change in BRTs. Overall findings suggested that under the BRT of 7 days with high nutrient loading urine-based culture, MPBR concomitantly attained high biomass concentration and nutrients removal rate. Our findings suggested that if the relatively short BRT (below 10 days) was chosen for operation, it is considered that the contact time between microalgae and nutrients (HRT) would play a vitally important role in complete nutrients elimination.

3.3. Fouling propensity of membrane photobioreactor

Fouling is strictly influenced by the operational flux (T.-T. Nguyen et al., 2020; T.T.D. Nguyen et al., 2020; T.T.N. Nguyen et al., 2020). If the operational flux exceeds critical/threshold values, the fouling will become serious (T. Nguyen et al., 2019; T.T.N. Nguyen et al., 2019). As reported the ranged fluxes of 1.5–16.8 L/m².h were investigated for the fouling behavior of the membrane photobioreactor operation (Luo et al., 2017). It was found that TMP exceeds 30 kPa after 68 d when a flux of 16.8 L/m².h was operated (Low et al., 2016). In this work, we proposed a constant low flux of 2.0 L/m².h and this aimed to minimize the fouling impact and thus to gain a sustainable operation (Nguyen et al., 2016b). As expected, the negative impact of fouling was minimal as the TMP reached only 30 kPa after a prolonged 100 days. Under operating at the BRTs of 10 and 7 d, the fouling rate was 0.62 kPa/d. The fouling level was minimized as operating at lower BRTs of 5, 3, 2 (0.42, 0.36, 0.22 kPa/d in that order). The high biomass concentration (2020–2194 mg/L) was retained in the former BRTs (10 and 7 d) and this fact resulted in boosting the fouling rate. These findings highlighted that the BRT-dependent biomass concentration played a pivotal role in the change in the fouling rate of membrane photobioreactor. As indicated by Fig. 4, it is important to note that the fouling caused by the deposition of microalgae cells could be easily removed by a simple physical cleaning using tap water, indicated the fouling of the MPBR was

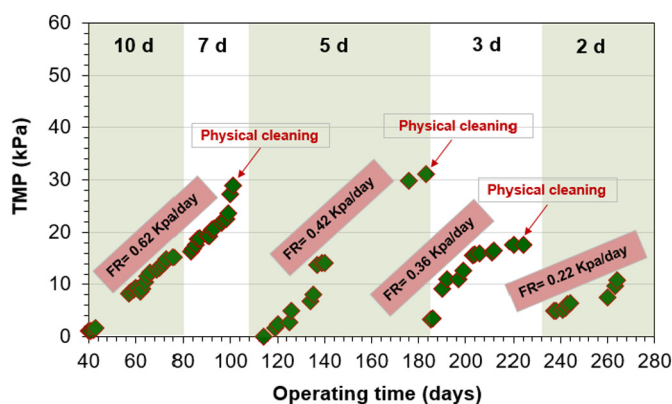


Fig. 4. TMP change and the fouling rate (FR) during 280 operation days of the membrane photobioreactor system. The physical cleaning using tap water was conducted as TMP reached over 20 kPa. This action aimed to remove the cake layer from the membrane surface.

reversible. Therefore, membrane fouling was not an issue in this MPBR operation. However, it has been considered that MPBR operation at the low flux of 2.0 L/m² h was not an economical benefit as this operation entails a high membrane cost (Luo et al., 2017). For example, for the commercialization of aerobic MBR in wastewater treatment, a chosen flux of 15 L/m² h is generally for operation in reality (Pollice et al., 2008). Overall findings suggested that an operation at the higher flux (>15 L/m² h) combined with various BRTs need to be further explored for compromising between performance (i.e., biomass production and nutrient removal) and fouling control.

3.4. Implication of this work

For our MPBR system, a chosen surface/volume ratio of 39.25 m²/m³ was significantly lower compared to the other PBR systems (80–100 m²/m³) (Marbelia et al., 2014). It means that the MPBR entails less construction area and this fact thus will bring economic benefits. The maximum biomass concentration was 2140 mg/L and this outcome was a lower value of 3568 mg/L obtained by a past work (Vo et al., 2018). The fact is attributed to the use of real urine possessing an N:P ratio of 19:1 which was relatively higher than optimal ratios for *Chlorella vulgaris* growth (i.e., 16:1 (Gao et al., 2016a) or 15:1 (Vo et al., 2018)). It is clear that the N:P ratio is a vital factor influencing microalgae growth (Vo et al., 2018). In practice, some industrial wastewaters possess the low N:P ratios (i.e., 0.5:1.0 of aquaculture, 2.5–3.8:1 of a brewery, and 8:1 of the shrimp farming) (Muylaert et al., 2017). Therefore, the reuse of industrial wastewaters in combination with urine (separated from domestic wastewater) might be a sound alternative to adjust the N:P ratio to the ideal ratio (15:1) if the enhanced biomass production is considered as a priority mission.

For the design and operating parameters, in reality, hydraulic retention time (HRT) and biomass retention time (BRT) are two pivotal parameters governing the successful operation of the MPBR system. A summary of nutrient removal rate, biomass concentration/productivity were presented in comparison with the previous studies (Table 4). These performance factors were expressed under different BRTs, HRTs, N:P ratios, and nutrient loading rates. As denoted in Table 4, our work showed 1.2–4.8 times higher biomass concentration compared to the other studies (443–1784 mg/L) as the membrane photobioreactor was operated under the BRT of 7 d. Meanwhile, for the use of secondary effluent with low-strength nutrients (i.e., 6.7 mg N/L.d and 0.4 mg P/L.d), a prolonged BRT of 35 d was applied to attain a high biomass concentration of 1784 mg/L (Gao et al., 2016b). This extension of BRT facilitated the compensation in biomass yield in the MBPR system. Another key point to support the distinction in biomass concentration was due to the higher nutrient loading rate (i.e., 90.5 mg N/L.d and 4.7 mg P/L.d)

Table 4
Biomass production and nutrient removal rate of this study in comparison with the previous works.

Wastewater	Microalgae	Operating conditions			Nutrients loading		N:P ratio	Nutrients removal rate		Microalgae growth		Reference
		BRT (d)	HRT (d)	BRT/HRT ratio	TN (mg N/L.d)	TP (mg P/L.d)		TN (mg N/L.d) (%)	TP (mg P/L.d) (%)	Biomass concentration (mg/L)	Biomass productivity (mg/L.d)	
Urine (1:30 dilution)	<i>Chlorella vulgaris</i>	7	2	3.5	90.5	4.7	19.3	70.0 (77.3)	2.50 (53.2)	2140	313	This work
Treated sewage	<i>Chlorella</i>	4.5	2	2.3	14.4	1.8	8.0	11.5 (79.9)	1.4 (77.8)	–	69	(González-Camejo et al., 2020)
Synthetic wastewater	<i>Chlorella Vulgaris</i>	10	2	5.0	11.1	1.1	10.1	4.6 (41.4)	0.80 (72.7)	590	60	(Marbelia et al., 2014)
Treated wastewater	<i>Chlorella Vulgaris</i>	18	2	9.0	8.4	0.6	14.0	4.1 (48.8)	0.40 (66.7)	878	49	(Gao et al., 2018)
Domestic secondary effluent	<i>Chlorella Vulgaris</i>	35	2	17.5	6.7	0.4	16.8	5.8 (86.6)	0.30 (75.0)	1724	51	(Gao et al., 2016b)
Treated wastewater	<i>Chlorella Vulgaris</i>	12	1	12.0	15.0	0.3	50	10.5 (70.0)	0.29 (96.7)	314	26	(Honda et al., 2017)
Treated wastewater	<i>Chlorella Vulgaris</i>	18	1	18.0	7.5	0.2	37.5	6.9 (92.0)	0.1 (50.0)	923	48	(Honda et al., 2012)

Remarks: TN = total nitrogen; TP = total phosphorus; “–” indicated irrelevant information; The values in bracket presented the removal efficiency. A membrane photobioreactor was employed for all works.

employed in our work. It is highlighted that the low BRT operation (i.e., largely withdrawn of biomass volume) favored with the condition of high nutrient loading rate so as to gain the stably desirable biomass production and harvesting.

On comparing the results obtained by other works, this action was to propose the guided operating conditions for the MPBR system. Under culture medium with a low nutrient loading rate (i.e., treated wastewater), for the HRT of 2 d, it was generally accepted that higher biomass concentration attained at the prolonged BRT (18 and 35 d). By ways of contrast, higher biomass productivity and nutrient removal rate was favored for an operation at lower BRT (4.5 d). As reported nutrient elimination is mainly due to the assimilation into the microalgae biomass (Judd et al., 2015; T.-T. Nguyen et al., 2020; T.T.D. Nguyen et al., 2020; T.T.N. Nguyen et al., 2020). The findings indicated that if the culture medium with a low nutrient loading rate was adopted, short BRT conditions would be proper for the MPBR operation in the priority of wastewater treatment. However, this operating condition (i.e., BRT of 5 d) could induce a drastic decrease in biomass concentration which was an obstacle for biomass harvesting (Xu et al., 2015). In our work, operating at a short BRT of 7 d resulted in sufficient biomass yield (2140 mg/L) and biomass productivity (313 mg/L.d), which posed remarkably higher than the previous studies. In practice, since the inherent climate conditions were in a tropical country like Vietnam, this favors microalgae cultivation by using urine as a cultured medium, and the products from microalgae might be helpful for oil production (Rafie et al., 2014).

As the results indicated in Table 4, the influence of BRT and HRT factors on the microalgae growth and nutrient removal is obvious. Therefore, the ranged values of HRTs (1, 2 d) and BRTs (10, 12, 18 and 35 d) were chosen to define BRT/SRT ratio. This ratio was suggested as a pivotal parameter governing successfully biomass production and nutrient removal (Xu et al., 2015). Under the HRT of 1 d, as increased the BRT/HRT ratios from 12 to 18, it favored a higher increase in biomass concentration and nutrient removal efficiency. This outcome was also consistent with the higher HRT operation (2 d) as the BRT/HRT was increased from 5 to 17.5. Their findings highlighted that if a low concentration of nutrients in treated wastewater is employed, controlling a higher BRT/HRT ratio would be more advantageous. The ratio of 18 might be sound like a guide for the MPBR operation. This guideline is not consistent with our findings (a BRT/HRT ratio of 3.5) and this discrepancy is attributed to a considerable difference in nutrient loading rate. The findings implied that choosing the optimal BRT, HRT, and BRT/HRT ratio is strictly dependent on the strength level of wastewater types employed for microalgae cultivation. Overall findings pinpointed that the use of urine in combination with the MPBR system brings

promising outcomes on biomass production in microalgae cultivation. The final point was that an optimal operation with governing BRT/HRT ratios need to be explored further studies. As our outcomes, it is suggested a relatively short BRT of 7 d and higher HRT of 2 d could be a reference value to ensure synergistic algal biomass production and nutrient removal in the fed wastewater like urine.

4. Conclusions

This work demonstrated the certain influence of low biomass retention time on both biomass productivity and nutrient removal rate in MPBR system. The BRT of 7 d was a critical operation to gain concomitantly high biomass concentration and biomass production rate. It was highlighted that the BRT-dependent biomass accumulation governed the TN removal rate in MPBR system, proposed the proper BRTs of 7 d. When operating a relatively short BRT of 7 d and an extended HRT (> 2 d) is implemented, the environmental impact was minimized by the effective nutrients capture from the fed wastewater like urine.

CRedit authorship contribution statement

Thanh-Tin Nguyen, Thi-Thuy-Duong Nguyen: Investigation, Software, writing original draft.

Hong-Hai Nguyen, Kim-Quy Nguyen, Bao-Trong Dang: Data curation, Conceptualization, Methodology.

Xuan-Thanh Bui, Huu Hao Ngo, Julien Némery, Ky-Phuong-Ha Huynh: Funding, Supervision, Writing - Reviewing and Editing.

Xuan-Thanh Bui, Takahiro Fujioka, Cong-Hung Duong, Sunita Varjani: Editing, Revise the final MS.

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.

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