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Influence of microalgae retention time on biomass production in membrane photobioreactor using human urine as substrate

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Abstract:

Human urine is known as the excreta with a high concentration of nitrogen and phosphorus, causing eutrophication in water bodies. In this study, human urine was used to feed microalgae (*Chlorella vulgaris*) in a membrane photobioreactor (MPBR) at various microalgae retention times (MRTs) and hydraulic retention time (HRT) of 2 days to evaluate its biomass production. The results indicate that MPBR was operated under MRT of 2 to 5 days and HRT of 2 days, which performed the optimum condition with biomass productivity from 146.43±8.52 to 151.93±15.05 mg.l^{-1}.day. Moreover, the MPBR using the urine as a nutrient source demonstrated the high performance in biomass production and strong growth of microalgae.

Keywords: **biomass production, human urine, membrane photobioreactor, microalgae, nutrient removal.**

Classification number: **3.5**

Introduction

Domestic wastewater has negatively affected the aquatic environment when human urine is discharged directly into the environment without sufficient treatment, thereby causing eutrophication. Urine contains a high concentration of nutrients (mostly nitrogen and phosphorus); it can therefore be used as a liquid fertilizer or even as a slowly soluble fertilizer (in the form of struvite - $MgNH_4PO_4.6H_2O$) [1]. Additionally, it offers a high potential to cultivate microalgae for nutrient recovery. Microalgae biomass production is a potential source of feedstock for the bio-based production of biochemicals, biofuels, fertilizer, feed for cattle, food for health, and cosmetics for humans [2]. In addition, many types of wastewaters from agricultural, industrial, synthetic, and municipal activities which have been used for microalgae cultivation coupling with wastewater treatment is regarded as a more economical and sustainable option [3, 4]. Human urine contains about 80% of the nitrogen loading in wastewater; therefore, separating urine at the source to cultivate microalgae can help to improve effluent quality, save energy consumption, and recover the investment cost of the wastewater treatment plant [1].

The cultivation of microalgae using wastewater in photobioreactors is a novel, prospective, and sustainable method to remove contaminants (mostly nutrients) from wastewater and simultaneously produce useful microalgae biomass. Significant effort has been dedicated to developing the performance and cost-effectiveness of microalgae cultivation systems. The pilot scale or commercial cultivation system are often based on open ponds technology. However, this pond technology presents many disadvantages, such as water evaporation, extensive space requirements, contamination of algal cultures, and lack of control over operating parameters [5, 6]. To overcome these issues with open pond technology, the photobioreactor (PBR) has been designed to tackle these drawbacks [4]. However, PBRs present additional challenges, such as poor settling ability, biomass washout, and harvesting limitations [7]. Therefore,

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the microalgae cultivation system has been improved by combining it with membrane separation in PBR, rendering it the membrane photobioreactor (MPBR). The advantages of MPBR relative to PBR included decoupling the hydraulic retention time (HRT) and microalgae retention time (MRT), preventing biomass washout, higher biomass production, enhanced nutrient removal efficiency, and reduced land requirement, which contributed to a decrease in construction and operation costs.

There was minimal available knowledge regarding microalgae cultivation by using human urine as a substrate incorporated with a membrane photobioreactor [2]. In several previous studies, synthetic or real urine was applied as a nutrient medium for microalgae growth [2, 8, 9]. However, ammonia production, high pH, and key-element precipitation that occurred during urea hydrolysis in concentrated urine would produce microalgae growth difficulties and render nutrient recovery ineffective [9]. In fact, Jaatinen, et al. (2016) reported that 1:25-diluted urine could be used for microalgae biomass production [8]. In addition, *Chlorella vulgaris* was known to be easy to cultivate in an inexpensive nutrient medium and exhibited a fast growth rate and a high biomass productivity [10]. At HRT of 2 days, microalgae concentration and biomass productivity of MPBR achieved 3.5-fold and 2-fold higher compared to those of PBR respectively [11]. Therefore, the first time that *Chlorella vulgaris* was grown in the MPBR system with diluted human urine as nutrients source in this study, the reactor was operated under conditions in which HRT was fixed at 2 days, and the MRT was variable. This study aims to investigate the effect of various microalgae retention times (MRTs) on algae biomass production.

Materials and methods

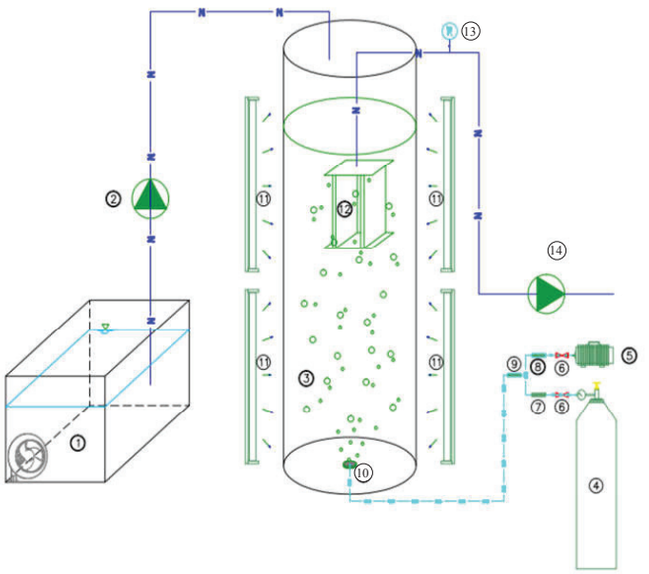
Membrane photobioreactor structure

The MPBR system was installed in a wooden box with a thickness of 10 mm to prevent temperature change. It was then continuously illuminated with four 18 W white fluorescent lamps (11), and the intensity of the lighting was 4.4 kLux. MPBR (3) was made from transparent acrylic and designed with an internal diameter of 100 mm and 1200 mm in height; the working volume was 8 l. A hollow fiber membrane module (12), which was made from polyvinylidene fluoride (PVDF) (Mitsubishi, Japan) and had a pore size of 0.4 µm with a membrane area of 0.035 m^2; it was submerged in the reactor.

Operating conditions of the MPBR system

The flow rates of CO_2 (4) and air (5) mixture, which were 0.1 l/min and 4.0 l/min respectively, were injected into the MPBR via a 20 mm-diameter air diffuser installed at the bottom of the reactor.

The diluted human urine (30 times) was pumped from the feed tank (1) into the MPBR by an automatic feed pump (2). The permeate was intermittently withdrawn in a cycle (8 min of operation and 2 min idle) by a suction pump. A digital pressure gauge (13) was installed on a pipe connected with a permeate pump (Fig. 1).



1: feed tank; 2: feed pump; 3: photobioeactor; 4: compressed CO_2 cylinder; 5: air blower; 6: valve; 7-9: rotameters; 10: air distributor; 11: fluorescent lamp; 12: membrane module; 13: digital pressure gauge; 14: permeate pump.

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

Microalgae retention time (MRT, day) was calculated by the following expression [11]:

$$MRT = \frac{V}{F_{retentate}}$$

where V was volume of reactor (l), and $F_{retentate}$ was daily volume of wasted retentate (l/day).

To determine the optimum MRT, MPBR was operated in four phases at MRTs changing from 5 days (during operation period from day 18 to day 113), to 3 days (between day 114 and day 175), to 2 days (between day 176 and day 190) and 1.5 days (from day 191 to day 218) and the discharged biomass amounts were 1.6, 2.67, 4.0, and 5.3 l/day, respectively. However, the reactor was operated in during the start-up time (from day 0 to day 17) to achieve a sufficiently high initial microalgae concentration. While MRT was changed in turn, HRT was controlled at 2 days for all operated MRTs. HRT (day) was defined by the following expression [11]:

$$HRT = \frac{V}{F_{in}}$$

where F_{in} was influent flowrate (l/day).

Feed wastewater characteristics and microalgae strain

Chlorella vulgaris was used in this study provided by The Research Institute for Aquaculture No. 2, Ho Chi Minh city, Vietnam with initial dry weight of 36 mg/l.

Fresh human urine was collected from male toilet in Ho Chi Minh city University of Technology and stored at 4°C in a refrigerator to reduce the effect of urea hydrolysis before use. Then urine was diluted 30 times with tap water and contained in feed tank. The diluted urine contained PO_4^{3-}P of 4-8 mg/l, total phosphorus (TP) of 8-15 mg/l, NH_4^+-N of 6-12 mg/l and total Kjeldahl nitrogen (TKN) of 180-350 mg/l.

Analysis

Daily, 200-ml samples were taken from influent and permeate for analysis. In addition, 50-ml samples of mixed liquor suspended solids (MLSS) were taken from middle of MPBR to measure biomass concentration [10]. MLSS was measured using a Whatman glass fiber filter membrane and then drying biomass after filtering until a constant weight was reached at 105°C [12]. The water quality parameters including TKN, TP, nitrite, nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), and biomass concentration were analysed, following the Standard Method for The Examination of Wastewater [12]. pH was measured using a pH meter (HANA, USA).

Biomass productivity (P, mg.l^{-1}.day) was calculated based on the following expression [11]:

$$P = X_{MPBR} \times \frac{D}{\nu} = X_{MPBR} \times \frac{1}{HRT} \times \frac{HRT}{MRT} = \frac{X_{MPBR}}{MRT}$$

where, X_{MPBR} was biomass concentration in MPBR (mg/l), D was dilution rate (day^{-1}), and υ was dilution factor.

The nutrients loading (mg.l^{-1}.day) and food/microorganism (F/M) ratio of MPBR were calculated using the following equation [13]:

$$Nutrients\ loading = \frac{C_{inf} \times Q}{V}$$

$$\frac{F}{M} = \frac{Q \times C_{inf}}{V \times X_{MPBR}}$$

where, C_{inf} was the concentration (mg/l) of TN (or TP) in the influent.

Microalgae cell density was determined every day by counting method following Fuchs-Rosenthal and Burker method with hemocytometer (Germany). After counting the microalgae cell via light microscope, cell density is calculated by the following formula:

$$Cell\ density = \frac{number\ of\ cell}{ml} = \frac{number\ of\ cell\ on\ a\ large\ square}{volume\ of\ a\ large\ square \times dilution\ rate}$$

Results and discussion

Figure 2 demonstrates that the variation of *Chlorella vulgaris* biomass concentration in MPBR operated at different MRTs during the entire cultivation period of 218 days. At the start-up period, biomass concentration achieved 615 mg/l at day 9. Based on the observed results, there was no lag phase in the first 18 days (start-up period), which reflected the results of Gao, et al. [13]. This proved that *Chlorella vulgaris* adapted effectively to human urine as a feeding substrate.

At MRT of 5 days, biomass concentration was maintained in the range of 540-860 mg/l. This high concentration of microalgae was achieved through the effect of the submerged membrane in MPBR, which allowed the reactor to operate under a longer MRT but a shorter HRT [4]. However, at the initial time

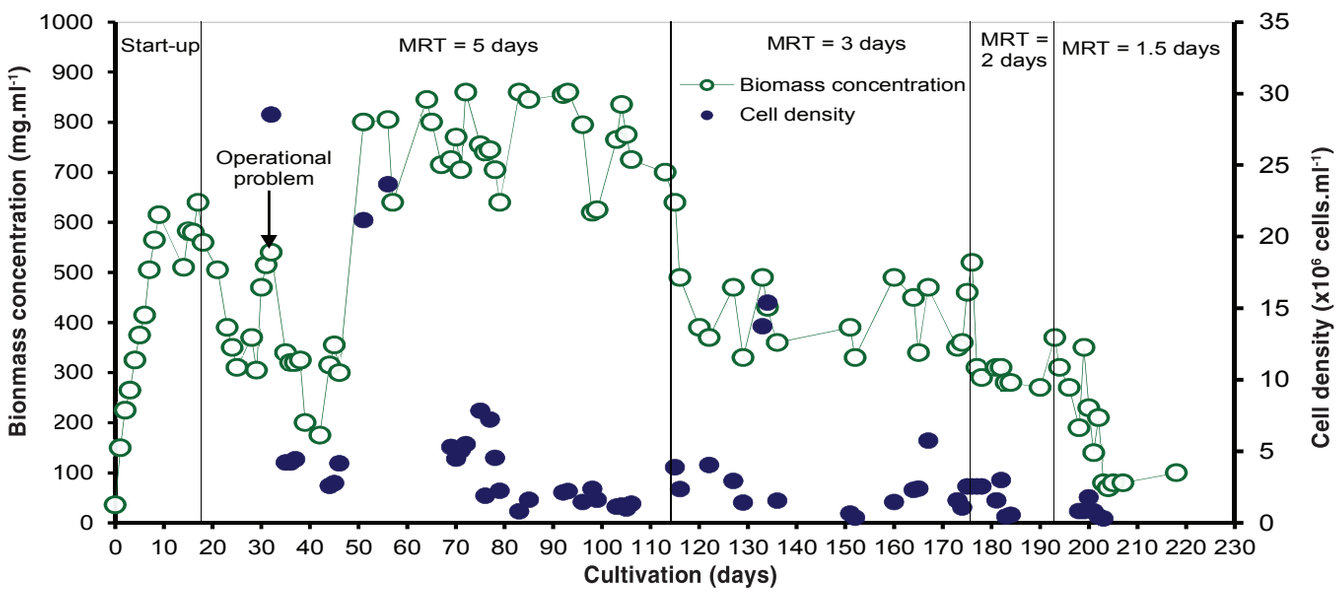


Fig. 2. Microalgal growth curve and cell density of *Chlorella vulgaris* at different MRTs.

of this MRT, biomass concentration was reduced from 560 mg/l on day 18 to 305 mg/l on day 29 due to the operational problem (clogging of the electrical floater) of the system. Biomass concentration was then continuously increased to 540 mg/l on day 32. Similarly, on day 32, a biomass washout incident again occurred due to the previously described operational problem. Therefore, biomass concentration was again gradually reduced to 175 mg/l on day 42. From day 46, biomass concentration was restored and achieved a steady state (800 mg/l) from day 51 onwards. At the steady state of 5-day MRT, the average biomass productivity was 151.93±15.05 mg.l^{-1}.day (Fig. 3).

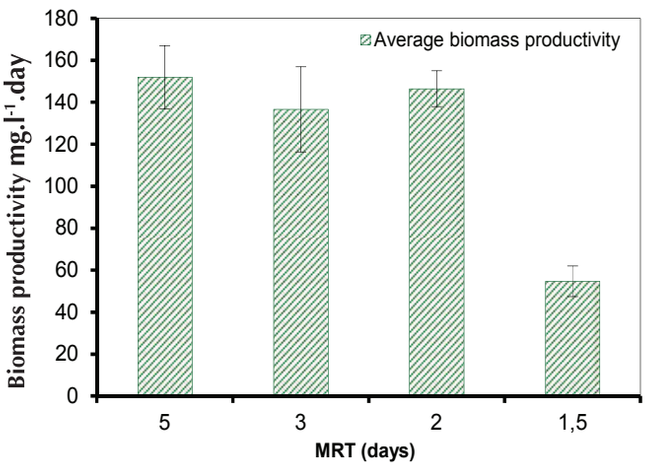


Fig. 3. Biomass productivity of *Chlorella vulgaris* at different MRTs.

At MRT of 3 days, average biomass concentration and biomass productivity reached 410 mg/l and 136.67±20.34 mg.l^{-1}.day, respectively. The system was stable after several days and operated for 50 days at 3-day MRT.

At MRT of 2 days, microalgae biomass concentration achieved a steady state quickly for several days. During 15 days of operation, average biomass concentration and biomass productivity were 292.86 mg/l and 146.43±8.52 mg.l^{-1}.day, respectively.

When MRT was controlled at MRT of 1.5 days, the biomass concentration began to decrease significantly from 310 mg/l (day 194) to 80 mg/l (day 203); it then became steady at this value. At this stage, average biomass concentration and biomass productivity achieved 82 mg/l and 54.67±7.30 mg.l^{-1}.day, respectively.

The biomass growth in MPBR was measured as MLSS. This value included living, dead algae, protozoa and bacteria. However, based on cell counts and microscopic observation, living algae was observed to be dominant in the biomass mixture during the cultivation period, which ranged from 0.3×10^6 to 28.5×10^6 cells/ml (Fig. 2). Flocs formation of microalgae occurred in MPBR at the beginning of the stationary phase; therefore, the counting number of algae was hardly estimated because flocs formation was occurred in the reactor. The appearance of flocs in MPBR could be due to the competition of bacteria and their extracellular polymeric substance [14] and the intracellular substances was released by dead algae [8]. Bacteria growth could not cause a 'shut down' of the photobioreactor and the microalgae dominant, although bacteria, protozoa, and flocs formation occurred in the MPBR at almost MRTs. Moreover, the influence of bacteria was effectively prevented by withdrawal of biomass and a microfiltration membrane module in the photobioreactor.

The longer MRT corresponded with high biomass concentration (Table 1), which may lead to the rapid removal of nitrogen [15, 16]. However, the high concentration indicates low nutrient loading rates or low F/M ratios. In this study, these ratios were 0.13, 0.22, 0.3, and 1.21 for nitrogen and 0.01, 0.01, 0.02, and 0.04 for phosphorus corresponding with MRT of 5, 3, 2, and 1.5 days, respectively. Therefore, at MRT of 5 days, MPBR performed the optimum biomass productivity; the productivity at 2 days was then 136.67±20.34 mg.l^{-1}.day. Relative to MRT of 2 days, the lower biomass productivity was achieved at MRT of 3 days due to lower F/M ratio. In contrast to MRT of 3 days, the lowest microalgae productivity occurred at 1.5 days because of the overly high F/M ratios. In addition, light may limit the microalgal growth due to self-shading at high biomass concentration; therefore, dark respiration of algae occurs in MPBR [17]. This was not proved in this study.

Based on the observed results, it is clear that the MRT as short as 1.5 days could cause the biomass productivity to decrease significantly due to low algal biomass concentration retained in the reactor. MRT of lower than 2 days strongly affects the biomass concentration and biomass productivity of the MPBR. In addition, the suitable MRTs for MPBR in this study ranged between 2 and 5 days. The average biomass productivicty ranged between 146.43±8.52 and 151.93±15.05 mg.l^{-1}.day for MRT of 2 to 5 days (Table 1).

Table 1. Comparison of performance of MPBRs.

References	MPBR	Influent concentrations		Nutrients loading		Growth of microalgae	
	SVR (m^{-1})	*TN (mg N/l)*	*TP (mg P/l)*	*TN (mgN.l^{-1}.day)*	*TP (mgP.l^{-1}.day)*	*MLSS (mg/l)*	*Microalgae productivity (mg.l^{-1}.day)*
5-day MRT (this study)	39.2	200.1	10.2	86.30	5.13	759	151.93±15.05
3-day MRT (this study)		184.0	9.4	92.01	4.70	410	136.67±20.34
2-day MRT (this study)		176.5	12.5	88.28	6.29	292	146.43±8.52
1.5-day MRT (this study)		198.8	9.3	99.44	4.66	82	54.67±7.30
Marbelia, et al. (2014) [11]	20.0	7.4	1.6	3.74	0.84	590	27.00
Gao, et al. (2014) [3]	32.3	19.1	1.24	8.39	0.56	-	39.93
Gao, et al. (2016) [13]	57.5	13.3	0.72	6.66	0.36	1724	50.72
Gao, et al. (2016) [18]	56.2	6.8	0.42	6.81	0.42	1100	42.60

Remarks: SVR = surface volume ratio; TN = total nitrogen; TP = total phosphorus; MLSS = mixed liquor suspended solids.

Because of the high nutrient media in this study, which were 10- to 28-fold and 6- to 24-fold higher than these wastewaters respectively, the microalgae productivity in this study was higher than in previous studies [3, 11, 13, 18]. Relative to other studies, the nutrient loading in this study was higher. This

proved that the 1:30-diluted human urine provided sufficient nutrients for microalgae production, while Jaatinen, et al. (2016) reported that the 1:25-diluted urine was the optimal medium for *Chlorella vulgaris* cultivation [8]. The submerged membrane demonstrated the effectiveness in preventing wash-out of biomass and improvement of nutrient loading. The highest biomass concentration of 759 mg/l at MRT of 5 days was achieved.

In this study, the MPBR exposed an illumination area of 0.32 m^2 and yielded the surface to volume (S/V) ratio of m^2/m^3, which was lower than the optimum S/V ratios of 80-100 m^2/m^3 in PBR [11]. However, the reactor's biomass and biomass productivity were respectively 759 mg/l and 151.93±15.05 $mg.l^{-1}$.day. This value was higher than that yielded by other MPBRs [3, 11]. Therefore, the performance of MPBR could be minimised by effective mixing of air bubbles. Moreover, the S/V ratio was smaller than the ratio in previous studies by Gao, et al. [13, 18]; nevertheless, the higher production was achieved in this study due to the lower biomass concentration (Table 1). The high concentration of algae could cause the respiration in the dark [17] and the smaller production in these studies.

The N/P ratio of diluted human urine in this study was 20:1, which was higher than the ratio of microalgal biomass ($CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$) [5] and Redfield ratio (16:1) [18]; therefore, P was the limiting factor for microalgal growth. In addition, the N/P ratio of 15:1 was regarded as the optimum ratio for microalgal growth with maximum biomass concentration of 3568 mg/l [19]. Additionally, other types of wastewater containing the lower N/P ratio can be mixed with human urine for microalgal cultivation. For example, the shrimp farming wastewater containing TN and TP was 159 and 19.6 kg/ha.crop (the N/P ratio was 8:1), which is one of the potential sources for eutrophication in the Mekong Delta [20].

Conclusions

This study illustrates the potential of applying human urine for biomass production. Urine can be an ideal nutrient to cultivate microalgal biomass. The average biomass productivity was as high as 146.43 to 151.93 $mg.l^{-1}$.day at the operated MRT of 2 to 5 days. The MRT shorter than 1.5 day caused a significant reduction of biomass productivity.

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The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] T. Karak, P. Bhattacharyya (2011), "Human urine as a source of alternative natural fertilizer in agriculture: A flight of fancy or an achievable reality", *Resour. Conserv. Recycl.*, **55**, pp.400-408.

[2] K. Tuantet, H. Temmink, G. Zeeman, M. Janssen, R.H. Wijffels, C.J.N. Buisman (2014), "Nutrient removal and microalgal biomass production on urine in a short light-path photobioreactor", *Water Res.*, **55**, pp.162-174.

[3] F. Gao, Z.H. Yang, C. Li, Y. Wang, Y. Jie Jin, W. Hong, Deng Y. Bing (2014), "Concentrated microalgae cultivation in treated sewage by membrane photobioreactor operated in batch flow mode", *Bioresour. Technol.*, **167**, pp.441-446.

[4] Y. Luo, P. Le-Clech, R.K. Henderson (2016), "Simultaneous microalgae cultivation and wastewater treatment in submerged membrane photobioreactors: a review", *Algal Res.*, **24**, pp.425-437.

[5] Y. Chisti (2007), "Biodiesel from microalgae", *Biotechnol. Adv.*, **25**, pp.294-306.

[6] A.M. Kunjapur, A.R.B. Eldridge (2010), "Photobioreactor design for commercial biofuel production from microalgae", *Ind. Eng. Chem. Res.*, **49(8)**, pp.3516-3526.

[7] M.R. Bilad, V. Discart, D. Vandamme, I. Foubert, K. Muylaert, I.F.J. Vankelecom (2014), "Coupled cultivation and pre-harvesting of microalgae in a membrane photobioreactor (MPBR)", *Bioresour. Technol.*, **155**, pp.410-417.

[8] S. Jaatinen, A.-M. Lakaniemi and J. Rintala (2016), "Use of diluted urine for cultivation of Chlorella vulgaris", *Environ. Technol.,* **37(9)**, pp.1159-1170.

[9] S. Zhang, C.Y. Lim, C.-L. Chen, H. Liu, and J.-Y. Wang (2014), "Urban nutrient recovery from fresh human urine through cultivation of Chlorella sorokiniana", *J. Environ. Manag.,* **145**, pp.129-136.

[10] C. Safi, B. Zebib, O. Merah, P.-Y. Pontalier, and C. Vaca-Garcia (2014), "Morphology, composition, production, processing and applications of Chlorella vulgaris: a review", *Renew. Sust. Energ. Rev.*, **35**, pp.265-278.

[11] L. Marbelia, M.R. Bilad, I. Passaris, V. Discart, D. Vandamme, A. Beuckels, K. Muylaert, I.F.J. Vankelecom (2014), "Membrane photobioreactors for integrated microalgae cultivation and nutrient remediation of membrane bioreactors effluent", *Bioresour. Technol.,* **163**, pp.228-235.

[12] American Public Health Association, American Water Works Association, Water Environment Federation (1992), "Standard methods for the examination of water and wastewater", *American Public Health Association*, Washington.

[13] F. Gao, C. Li, Z.H. Yang, G.M. Zeng, J. Mu, M. Liu, W. Cui (2016), "Removal of nutrients, organic matter, and metal from domestic secondary effluent through microalgae cultivation in a membrane photobioreactor", *J. Chem. Technol. Biotechnol.*, **91**, pp.2713-2719.

[14] J. Lee, D-H. Cho, R. Ramanan, B-H. Kim, H-M. Oh, H-S. Kim (2013), "Microalgae-associated bacteria play a key role in the flocculation of Chlorella vulgaris", *Bioresour. Technol.,* **131**, pp.195-201.

[15] J. Ruiz, Z. Arbib, P.D. Álvarez-Díaz, C. Garrido-Pérez, J. Barragán, J.A. Perales (2014), "Influence of light presence and biomass concentration on nutrient kinetic removal from urban wastewater by scenedesmus obliquus", *J. Biotechnol.,* **178**, pp.32-37.

[16] A.M. Åkerström, L.M. Mortensen, B. Rusten, H.R. Gislerød (2014), "Biomass production and nutrient removal by chlorella sp. as affected by sludge liquor concentration", *J. Environ. Manag.*, **144**, pp.118-124.

[17] H. Choi (2015), "Intensified production of microalgae and removal of nutrient using a microalgae membrane bioreactor (mmbr)", *Appl. Biochem. Biotechnol.*, **175**, pp.2195-2205.

[18] F. Gao, C. Li, Z.H. Yang, G.M. Zeng, L.J. Feng, J. Liu, J. Zhi, M. Liu, H. Cai (2016), "Continuous microalgae cultivation in aquaculture wastewater by a membrane photobioreactor for biomass production and nutrients removal", *Ecol. Eng.*, **92**, pp.55-61.

[19] H-N-P. Vo, X-T. Bui, T-T. Nguyen, D. Duc Nguyen, T-S. Dao, N-D-T. Cao, T-K-Q. Vo (2018), "Effects of nutrient ratios and carbon dioxide bio-sequestration on biomass growth of Chlorella sp. in bubble column photobioreactor", *J. Environ. Manage.*, **219**, pp.1-8.

[20] P. Thi Anh, C. Kroeze, S.R. Bush, A.P.J. Mol (2010), "Water pollution by intensive brackish shrimp farming in south-east Vietnam: Causes and options for control", *Agric. Water Manag.*, **97**, pp.872-882.