Optimization of Hydraulic Retention Time and Biomass Concentration in Microalgae Biomass Production from Treated Sewage with a Membrane Photobioreactor

doi: 10.2965/jwet.15-085 Journal of Water and Environment Technology, Vol.15, No.1: 1–11, 2017

Original Article

Optimization of Hydraulic Retention Time and Biomass Concentration in Microalgae Biomass Production from Treated Sewage with a Membrane Photobioreactor

Ryo Honda[a], Yuta Teraoka[b], Mana Noguchi[a], Sen Yang[b]

[a] *Institute of Science and Engineering, Kanazawa University, Kanazawa, Japan*
[b] *Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Japan*

ABSTRACT
Treated sewage is a promising source of nitrogen and phosphorus in microalgae biomass production for carbon-neutral biofuel and chemical products. In this study, *Chlorella vulgaris* was continuously cultivated in membrane photobioreactors (MPBRs) under short hydraulic retention times (HRTs) and with different numbers of submerged membrane modules to investigate potential microalgae productivity when treated sewage was used as a nutrient source. Microalgae biomass concentrations were independent of HRT in MPBRs with one membrane module owing to microalgae biomass deposition on the membrane. Installation of an additional submerged membrane module effectively reduced deposition on the submerged membrane, resulting in increased microalgae biomass concentration and volumetric productivity. Growth kinetics suggested that HRT is the essential parameter influencing the volumetric productivity of microalgae under nutrient-limited conditions, and that optimization of the biomass concentration, which depends on the surface/volume ratio of the photobioreactor and initial light intensity, is critical to maximization of the volumetric productivity under light-limited conditions.

Keywords: membrane photobioreactor, microalgae, resource recovery from wastewater

INTRODUCTION

Microalgae is a promising carbon-neutral biomass resource to be utilized for production of biofuel and bio-based valuable products [1,2]. However, estimated costs of producing biofuel from microalgae are still 10 times higher than those of petroleum [3]. To make biofuel production from microalgae feasible, substantial reductions in the cost of cultivation, dewatering and extraction are required. During cultivation of microalgae, it is necessary to supply nitrogen, phosphorus and carbon dioxide. However, use of chemical fertilizers as nutrient sources would diminish the benefits of microalgae biomass because production of fertilizer requires fossil energy for fixation of atmospheric nitrogen. Accordingly, utilization of wastewater as a source of nitrogen and phosphorus has the potential to reduce cultivation costs and energy consumption. Secondary treatment effluent from activated sludge processes could serve as a good cultivation medium because it also contains high levels of carbonate, as well as nitrogen and phosphorus. However, nutrient contents in wastewater and treated wastewater are much lower than those in cultivation media that has typically been used in past studies of microalgae cultivation [4–8].

Application of membrane photobioreactors (MPBRs) has recently been proposed to realize highly efficient microalgae production utilizing treated wastewater as a source of nutrients [9–11]. In a simple open pond or photobioreactor (PBR) system, achievable nutrient loading is limited when medium containing low levels of nutrients (such as wastewater) is used. This is because a limited range of hydraulic retention times (HRTs) can be applied to avoid washout of microalgae. Installation of a submerged membrane filtration system in a PBR decouples HRT from solids retention time (SRT), enabling operation with a long SRT to maintain a

Corresponding author: Ryo HONDA Email: rhonda@se.kanazawa-u.ac.jp
Received: September 29, 2015, Accepted: August 19, 2016, Published online: February 10, 2017

high concentration of microalgae and short HRT to achieve high nutrient loading with low-nutrient media or wastewater. In our previous study, combination of a short HRT (24 hours) and a long SRT (18 days) generated the highest microalgae productivity in cultivation using simulated secondary effluent of wastewater treatment [10]. However, the results indicated that higher productivity could be achieved by increasing the nutrient supply since productivity was limited because of phosphorus starvation.

Installation of submerged membrane filtration in a PBR enabled substantial improvement of nutrient supply loading by operation of short HRT, which cannot be operated in a simple overflow PBR. However, the range of HRT applied in previous studies on MPBR process was 1 − 10 days [9–11]. Microalgae growth shown in those studies were still slower than their potential. Theoretically, a higher microalgae productivity than those past studies can be achieved by applying much shorter HRT and more nutrient supply loading. Meanwhile, shortening of HRT results in increase of flux and may cause severe membrane fouling. Since operation of an MPBR process with HRT shorter than 1 day has not been attempted so far, optimized operation and possible failure of the process is still unknown.

In this study, MPBRs were operated under short HRT of 24 hours and 8 hours to investigate its effects on microalgae productivity and membrane fouling. Moreover, the MPBR was operated with different numbers of submerged membrane modules to clarify the effects of microalgae biomass deposition on submerged membranes. The performance of the MPBR was then evaluated in terms of microalgae productivity. In addition, the essential operating parameters to maximize the volumetric microalgae productivity were discussed based on growth kinetics in a PBR under limited light and nutrient conditions. Removal performance of nitrogen and phosphorus in treated sewage was also evaluated as the expected secondary effect.

MATERIALS AND METHODS

Membrane photobioreactor

A flat-plate PBR made from acrylic plastic **(Fig. 1)** with dimensions of 30 cm (width) × 10 cm (length) × 50 cm (height) was filled to a depth of 13 cm with 4 L of simulated treated sewage. The surface/volume ratio (SVR), which is defined as the ratio of the lighted surface area to the effective volume, was 26 m^{-1}. A polyvinylidene difluoride hollow-fiber microfiltration membrane module (Sterapore Sade Series, Mitsubishi Rayon, Tokyo, Japan) was submerged in the reactor. The pore size of the membrane was 0.1 μm, and the membrane surface area in the module was 0.085 m^2. Two steel plates each equipped with 12 red-light-emitting diodes (LEDs) (627 nm, LXML-PD01-00040, Phillips, Amsterdam, the Netherlands) and 12 blue LEDs (455 nm, LXML-PR01-0275, Phillips, Amsterdam, the Netherlands) were placed 2.5 cm from the 30 cm wide wall of the reactor. The light intensity on the illuminated wall of each PBR was 20 W/m^2. Thickness of the acrylic plate used for the photobioreactor was 10 mm. The absorbance of the acrylic plate at 627 and 455 nm was low enough to be negligible.

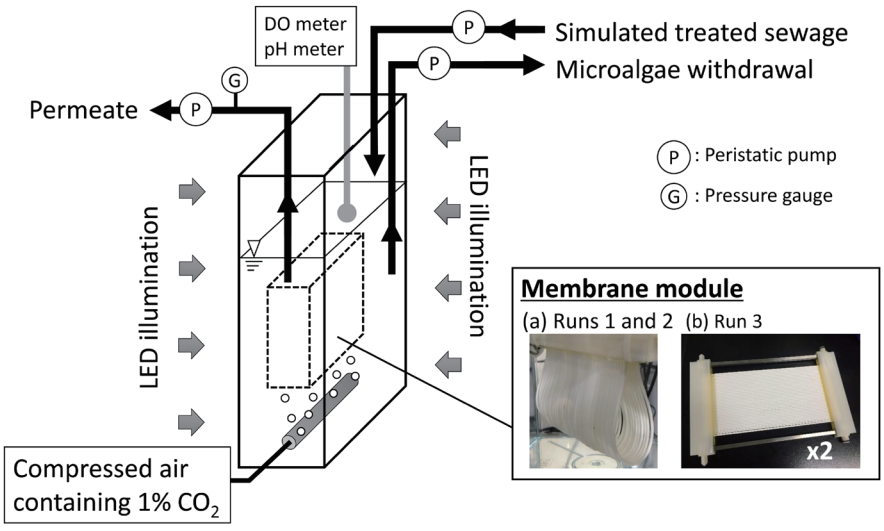

Fig. 1 Schematic diagram of the membrane photobioreactor.

Reactor operation

Simulated treated sewage was continuously supplied as a cultivation medium into an MPBR under different HRTs and numbers of membrane modules. Three runs were conducted: (i) an HRT of 24 hours with one submerged module (Run 1); (ii) an HRT of 8 hours with one submerged module (Run 2); and (iii) an HRT of 8 hours with two submerged modules (Run 3). In Runs 1 and 2, the hollow-fiber membrane module was bent to submerge in the MPBR **(Fig. 1)**. In Run 3, two modules were submerged without bending to generate more efficient cleaning by air scrubbing. The SRTs were set at 12 days in all runs, because SRT between 9 − 18 days were found to be optimum in our previous study [10]. *Chlorella vulgaris* NIES-2170 was used as the inoculant. Simulated treated sewage contained 10 mg of peptone, 1.5 mg of beef extract, 210 g of $NaHCO_3$, 57.3 mg of NH_4Cl, 1.51 mg of $NaH_2PO_4{\cdot}2H_2O$, 70 mg of $CaCl_2$ and 40 mg of $MgSO_4{\cdot}7H_2O$ per liter, and micronutrients including 0.1 µg of vitamin B12, 0.1 µg of biotin, 10 µg of thiamine HCl, 3 mg of Na_2EDTA, 0.59 mg of $FeCl_3{\cdot}6H_2O$, 0.11 mg of $MnCl_2{\cdot}4H_2O$, 0.03 mg of $ZnSO_4{\cdot}7H_2O$, 0.01 mg of $CoCl_2{\cdot}6H_2O$ and 7.5 µg of $Na_2MoO_4{\cdot}2H_2O$ per liter. The composition of the simulated treated sewage was designed based on the water quality of secondary treatment effluent of a sewage treatment plant in Kanazawa, Japan. The simulated treated sewage contained 5.0 mgC/L total organic carbon (TOC), 30 mgC/L inorganic carbon (IC), 15 mgN/L total nitrogen (TN) as ammonium, and 0.3 mgP/L total phosphorous as phosphate. The MPBR was purged with compressed air mixed with CO_2 to 1% of the partial pressure, which simulated off-gas from an aeration tank at a sewage treatment plant [10]. The flow rate of the purge gas was set at 200 mL/min. Membrane permeate was intermittently withdrawn by suction pumps in a 5 min on/ 1 min off cycle. Membrane modules were physically cleaned occasionally when transmembrane pressure (TMP) increased by +20 kPa from that of virgin membrane. Physical cleaning was performed by backwashing on days 43 and 67 in Run 1, and on days 4, 17, 43 and 67 in Run 2, while it was accomplished by offline flushing on day 52 in Runs 1 and 2. Backwashing was conducted by pumping the permeate back to the module at the same flux as withdrawal of permeate for 30 min in Runs 1 and 2. During offline flushing, biomass removed from the membrane was returned to the MPBR. In Run 3, the membrane was cleaned by backwashing on days 15, 34, 45, 52 and 58 by pumping the permeate back to the module at the same flux as withdrawal of permeate for 60 min. The temperature was maintained at 24 ± 4°C in Runs 1 and 2, and 19 ± 3°C in Run 3.

Sample analysis

Biomass concentration was analyzed as suspended solids (SS). Chlorophyll *a* (chl. *a*) and SS concentrations were analyzed according to Eaton *et al*. [12]. Dissolved inorganic nitrogen of ammonium (NH_4-N), nitrite (NO_2-N) and nitrate (NO_3-N) and phosphate-phosphorus (PO_4-P) concentrations were analyzed by ion chromatography (LC-10AD, Shimadzu, Kyoto, Japan).

RESULTS AND DISCUSSION

Effects of HRT

In runs in which one membrane module was submerged, both nitrogen and phosphorus supplies were sufficient under the shorter HRT of 8 hours, while phosphate became deficient under the longer HRT of 24 hours (**Table 1**; **Figs. 2 and 3**). However, microalgae biomass concentration was nearly comparable, regardless of HRT conditions **(Fig. 4)**. More growth of microalgae was implied under shorter HRT because a higher consumption of inorganic carbon was observed (**Table 1**). However, a portion of the microalgae was retained as cake on the membrane. More biomass was likely to be present as cake deposited on the membrane under the shorter HRT since the flux was larger. In runs with one membrane module, microalgae were retained inside the loop structure formed by hollow-fiber membrane strings **(Fig. 1)**. This also probably caused low concentrations of microalgae in the bulk solution. Because the major part of microalgae biomass to be harvested would be in the bulk solution in a practical process, control of biomass deposition on membrane is also suggested to be significant. After day 52, when the microalgae retained in the module were detached by offline flushing, biomass concentration increased, reaching a maximum of 780–870 mg/L. These results suggest that not only nutrient supply, but also control of microalgae deposition on the membrane affect biomass productivity.

In an MPBR process for microalgae cultivation, air supply necessary for membrane scouring is much smaller than in a typical MBR process for wastewater treatment, in which air is supplied mainly for the purpose of membrane scouring. This is probably because SS concentration is much lower in microalgae cultivation process than in an MBR. Severe increase of TMP was not observed for about 40 days when it was operated under 24 hours of HRT as observed in the previous study where the MPBR was operated under 24 hours of HRT [10]. However, more frequent cleaning became necessary when HRT was shorter. Air supply for membrane scouring also should be optimized dependent on HRT.

Table 1 Microalgae productivity and nutrient removal under different hydraulic retention times (HRTs) and with varying numbers of submerged membrane modules.

(a) Microalgae productivity

		Suspended solids [mg/L]		Chlorophyll a		Volumetric biomass productivity [g/(m^3·d)]	
HRT [hrs]	No. of modules	Average*	Max	Concentration [mg/L]	Content [mg/g-SS]	Average*	Max
24	1	314 (214)	786	1.74	4.53	26.2	65.5
8	1	368 (256)	872	1.44	3.60	30.7	72.7
8	2	468 (199)	856	9.01	18.0	39.0	71.3

(b) Consumption of nutrients and inorganic carbon

		Inorganic nitrogen				PO_4-P				Inorganic carbon			
HRT [hrs]	No. of modules	Permeate [mgN/L]	Loading [mgN/(L·d)]	Consumption rate [mgN/(L·d)]	% consumed	Permeate [mgP/L]	Loading [mgP/(L·d)]	Consumption rate [mgP/(L·d)]	% consumed	Permeate [mgC/L]	Loading [mgC/(L·d)]	Consumption rate [mgC/(L·d)]	% consumed
24	1	5.6 (3.2)	15.0	10.5 (3.1)	70.0	0.0082 (0.019)	0.30	0.29 (0.02)	96.7	7.2 (3.3)	30.0	23.3 (3.7)	77.7
8	1	6.1 (3.3)	45.0	30.3 (11.5)	67.5	0.066 (0.034)	0.90	0.74 (0.12)	82.5	4.0 (2.5)	90.0	78.9 (7.8)	87.7
8	2	5.1 (3.1)	45.0	29.6 (9.3)	65.9	0.012 (0.028)	0.90	0.87 (0.076)	96.8	7.7 (12.1)	90.0	69.4 (34.7)	77.1

*Average was calculated from the data after day 12 until the end of operation to exclude start-up period. The values in parentheses indicate the standard deviation.

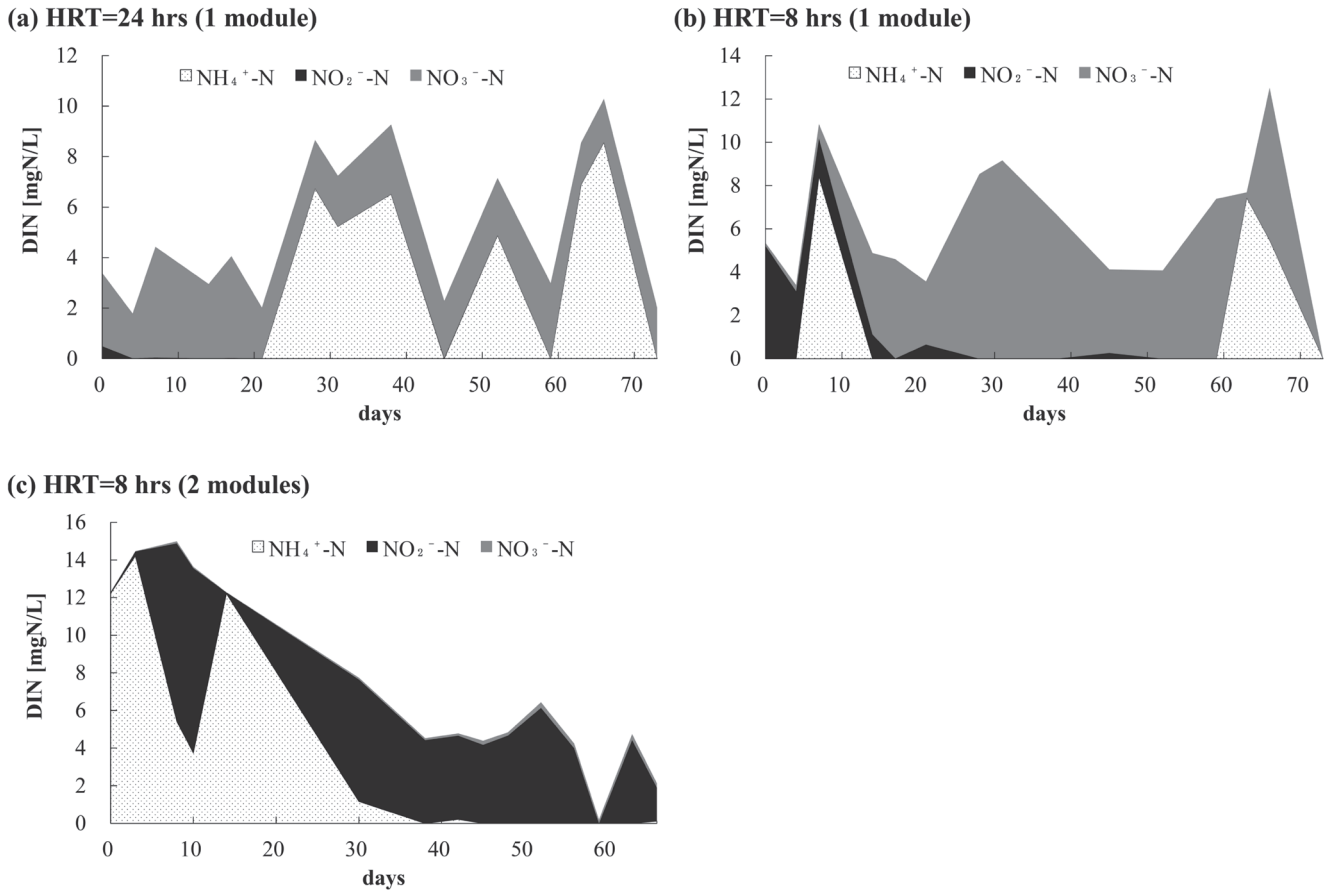

Fig. 2 Variations in dissolved inorganic nitrogen (DIN) concentrations under different hydraulic retention times and numbers of submerged membrane modules.

Nitrogen and phosphorus consumption rates were 2.0 − 2.5 times higher when the HRT was shorter (**Table 1**), which was similar to the results reported by Honda *et al*. [10]. These findings demonstrated that nutrient consumption rates could be enhanced by reducing the HRT, while ratios of consumed nutrients were larger when the HRT was longer. Therefore, higher nutrient removal ratios were expected as the secondary effect when the process is operated under a longer HRT. The removal ratio achieved here was comparable to those reported in previous studies [13,14]. Under the longer HRT of 24 hours, over 95% of the phosphorus was removed. Removal of dissolved nitrogen was 45 − 71%. The ratios of consumed nitrogen to phosphorus were 41.7 and 48.0 under HRTs of 24 hours and 8 hours, respectively. Phosphorus became the primary limiting factor under longer HRT in this study, because the N/P ratio of the influent was 56.7, which simulated secondary treatment effluent of a wastewater treatment plant in Kanazawa City. However, nitrogen could be the limiting nutrient if the treated sewage with a lower N/P ratio was used as the influent.

These results suggested that MPBR process would be also effective for biodiesel production from microalgae by applying a sequential operation of short and long HRTs. Although the focus of this study is to increase nutrient loading to maximize productivity of microalgae biomass, operation under nutrient-limited condition is also necessary for biodiesel production by microalgae to let microalgae accumulate carbohydrates in their cells. By employing an MPBR, nutrient-sufficient condition and nutrient-limited condition can be controlled only by changing HRT. Therefore, it would be possible to carry out the production of microalgae cells as well as the accumulation of carbohydrates in a single reactor.

Effects of addition of membrane modules

In the run with two membrane modules, hollow-fiber strings were set up straight to enable more efficient cleaning by air scrubbing **(Fig. 1)**. The biomass concentration in the bulk solution became larger when two membrane modules

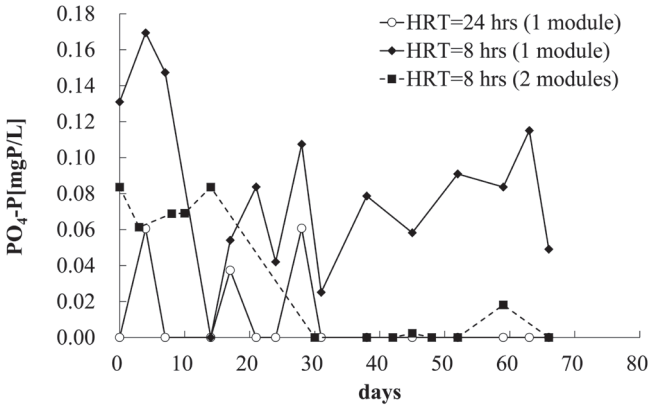

Fig. 3 Variations in phosphate-phosphorus (PO_4-P) concentrations under different hydraulic retention times and numbers of submerged membrane modules.

were installed in the PBR **(Fig. 2**; **Table 1**). This is probably because less microalgae biomass was deposited on the membrane owing to lower flux and more efficient membrane cleaning by air scrubbing in the PBR with two modules. A higher chl. *a* concentration also indicated that more microalgae existed in the bulk solution and grew photosynthetically in the PBR with two modules **(Fig. 3)**. A higher microalgae concentration in the bulk solution is preferable for higher microalgae productivity because microalgae in the bulk solution are not only expected to grow better than those in the cake deposits owing to higher light availability, but are also easily harvested. These results show that reduction of microalgae deposition on the membrane is important to increase microalgae biomass concentration in bulk solution, and hence the volumetric productivity. Setup of the membrane module would be important in reducing stagnant dark spaces, where microalgae become less competitive with other chemoautotrophic bacteria such as nitrifying bacteria. Increasing the membrane modules, which results in increased surface area, is also effective because it can reduce cake deposition by lowering flux through the membrane.

Nitrification was observed in all runs, suggesting presence of nitrifying bacteria in the PBR **(Fig. 2)**. However, trends of the nitrification varied among the runs. In runs operated with one module, nitrate was the dominant inorganic nitrogen species when SS was low, while ammonium became dominant after SS increased to exceed 400 mg/L. Meanwhile, partial nitrification to nitrite became dominant almost throughout the operation period in Run 3, which was operated under 8 hours of HRT with two modules. There was no oxygen depletion since dissolved oxygen was kept at 6 − 8 mg/L almost throughout the operation in all runs. Pollice *et al*. (2002) reported that partial nitrification to nitrite occurs in shorter SRT of 10 days even when oxygen is not limited, because nitrite-oxidizing bacteria prefer longer SRT [15]. In this study, complete nitrification to nitrate occurred in runs with one module probably because nitrite-oxidizing bacteria could be retained with longer SRT in the dead space inside the loop structure of membrane strings. However, after the membrane setup was improved, only partial nitrification to nitrite occurred because there was no dead space inside the module where nitrite-oxidizing bacteria were retained.

Phosphate became limited when two modules were submerged **(Fig. 3)**, which likely occurred because more microalgae grew in the illuminated bulk solution and consumed more phosphate than in the PBR with one module. The phosphorus consumption rate by *C. vulgaris* was 0.3 − 0.9 mgP/(L·d) in this study (**Table 1**). However, *C. vulgaris* has a higher potential growth rate because it was comparable or lower than other studies using cultivation media with a high nutrient concentration. Therefore, it is suggested that a higher microalgae biomass productivity can be achieved by increasing phosphorus loading, i.e. by shortening HRT. Consequently, when treated sewage is utilized for microalgae production, it was suggested that an HRT shorter than 8 hours was required to supply phosphate at levels sufficient to achieve potential microalgae productivity. In runs operated with an HRT of 8 hours and one module, TMP did not recover well when backwashing alone was conducted, even though it was performed more frequently than in the run with the 24-hour HRT (**Fig. S1**). In the run with two modules, only backwashing was needed to recover the TMP for 60 days of operation. This is probably because deposition of microalgae biomass was reduced by improvement of membrane setup. Under one-module setup, microalgae biomass was deposited in the loop structure of membrane strings, and air scouring could not reach in the loop. Under two-module setup, since the membrane strings were set straight, biomass deposition was reduced and air scouring was also improved.

Optimum microalgae concentration in a membrane photobioreactor

The maximum microalgae biomass concentration achieved in this study was 800 − 900 mg/L. In the majority of previous studies of cultivation of *C. vulgaris*, the maximum biomass concentration ranged from 800 to 1,200 mg/L [10,11,16–21], although some studies reported much higher concentrations of 1,500 to 12,000 mg/L [22–25]. However, a range of 800 to 1,200 mg/L can be considered a practically achievable

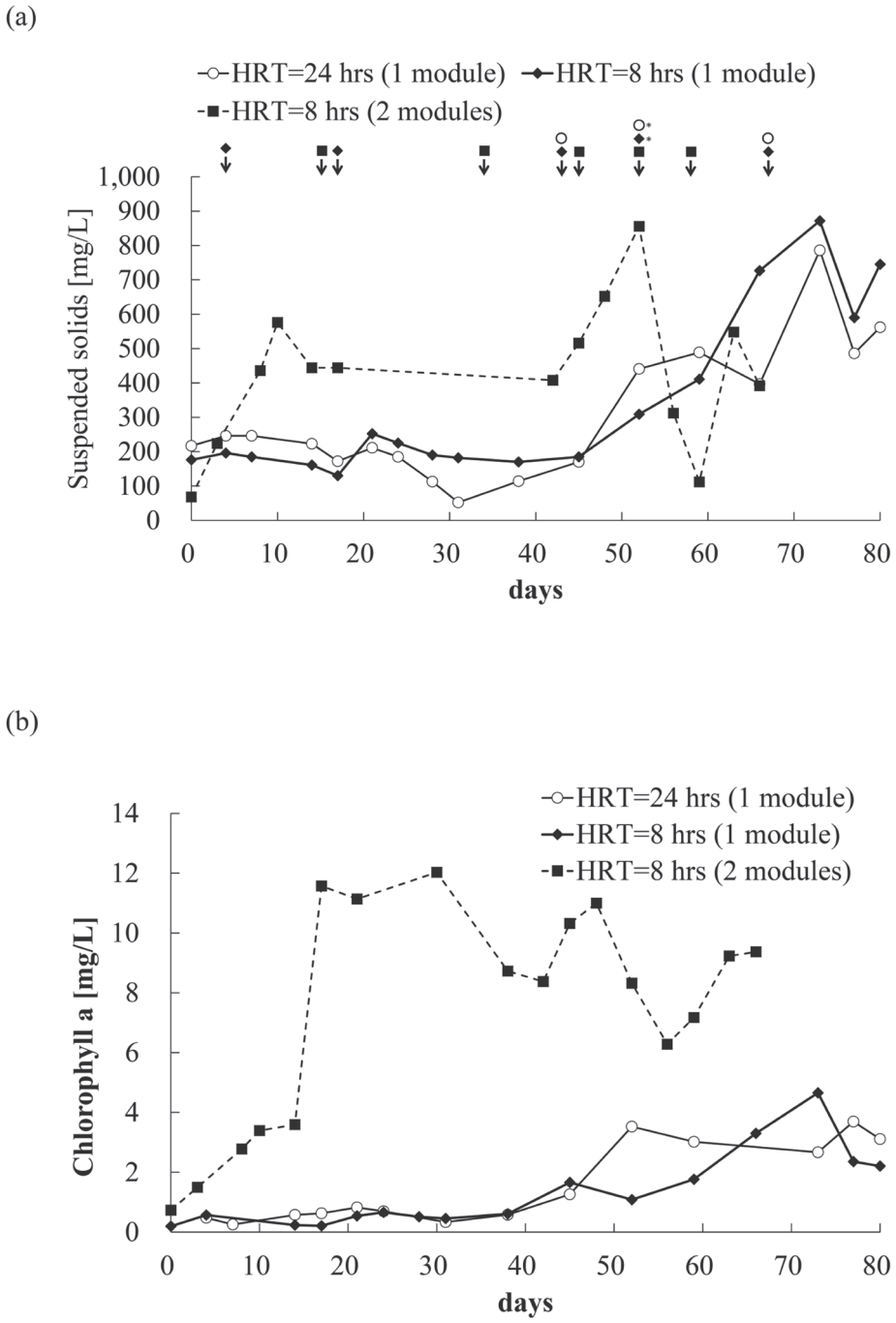

Fig. 4 Variations in (a) biomass concentration and (b) chlorophyll *a* concentration under different hydraulic retention times and numbers of submerged membrane modules. The arrows stand for timing of membrane cleaning; symbols with asterisks are for offline flushing and symbols without asterisks are for backwashing.

concentration of microalgae in a PBR if we exclude some of the previous cases in which an extremely high biomass concentration was achieved. A possible reason for the limited concentration of microalgae is constraints of light penetration. Since light intensity decreases exponentially in a PBR, the fraction in which there is sufficient light for microalgal growth is limited to a few centimeters from the light source. However, increasing the light intensity does not simply increase the growth area because high intensity light is known to cause photo-inhibition [26]. Therefore, a better

way to improve lighting efficiency is to design a PBR with a high surface/volume ratio (SVR) and/or control biomass concentration.

The optimum microalgae concentration to maximize the volumetric productivity can be predicted from the HRT, SRT, and SVR. The volumetric microalgae productivity is defined as the concentration of harvested microalgae (x) divided by the SRT (θ_s). In a chemostat, this is equal to the apparent growth of microalgae in a PBR under continuous cultivation (Eq. (1)),

$$\frac{X}{\theta_S} = \mu' X = (\mu - b) X \tag{1}$$

where μ' is the apparent specific growth rate [1/d], μ is the true specific growth rate [1/d], and b is the decay rate [1/d]. In a chemostat, the amount of nutrients consumed by growth is equal to the nutrient supply load, which is derived from the nutrient concentration in influent (s_0) [g/L] and HRT (θ_H) [d] (Eq. (2)),

$$\frac{S_0 - S}{\theta_H} = \frac{\mu X}{Y} \tag{2}$$

where S is the nutrient concentration in the PBR [g/L] and Y is the growth yield of microalgae to the limiting nutrient [g/g]. When a nutrient is the limiting factor of growth, i.e., $S = 0$, microalgae concentration and volumetric productivity (X / θ_s) in the PBR are predicted by Eqs. (3) and (4), respectively:

$$X = \frac{S_0 Y}{1 + b\theta_S} \cdot \frac{\theta_S}{\theta_H} \approx S_0 Y \cdot \frac{\theta_S}{\theta_H} \qquad (b \approx 0) \tag{3}$$

$$\frac{X}{\theta_S} = \frac{S_0 Y}{1 + b\theta_S} \cdot \frac{1}{\theta_H} \approx S_0 Y \cdot \frac{1}{\theta_H} \qquad (b \approx 0) \tag{4}$$

When the decay rate (b) is negligible relative to the true specific growth rate (μ), microalgae concentration depends on SRT and HRT, while volumetric microalgae productivity is independent of SRT, but dependent solely on HRT.

When there are sufficient nutrients and light is the limiting factor of growth, the true specific growth rate of microalgae limited by light intensity is given by the Monod model as follows:

$$\mu = \mu_m \cdot \frac{I - I_{\min}}{K_I + (I - I_{\min})} \tag{5}$$

$$I = I_0 \cdot 10^{-Al} \tag{6}$$

where μ_m is the maximum specific growth rate [1/d], I is the light intensity at a depth of l from the illuminated surface [W/m^2], $I_{\min}$ is the minimum light intensity required for growth [W/m^2], K_I is the half-saturation coefficient of the light intensity [W/m^2], I_0 is the light intensity at the lighted surface [W/m^2], and A is the optical density of microalgae culture [-]. The specific growth rate (μ) is not uniform in the PBR, but depends on the depth from the illuminated surface (l) [m] since light intensity decreases as a result of absorbance by microalgae cells. When we assume a simple case of a flat-plate PBR illuminated from one side, the light distribution inside the reactor is expected to have the following results: (i) the entire PBR is illuminated with greater light intensity than the minimum required for their growth ($I > I_{\min}$), or (ii) a dark fraction in which the light intensity is lower than the minimum level required for growth is present ($I \leq I_{\min}$) **(Fig. 5)**. In the former light-sufficient case (i), the thickness of the PBR (L) is shorter than the critical depth (L_0) [m], where light intensity reaches the minimum light intensity for their growth ($I_{L_0} = I_{\min}$). In this case, the average true specific growth rate in the PBR ($\overline{\mu_S}$) is derived as follows:

$$\begin{aligned}\overline{\mu_S} &= \mu_m \cdot \frac{1}{L}\int_0^L \frac{I - I_{\min}}{K_I + (I - I_{\min})} dl \\ &= \frac{\mu_m}{AL \cdot \ln 10}\left[I_m \left(\frac{1}{I_L} - \frac{1}{I_0} \right) \right. \\ &\quad \left. + K_I \left\{ \frac{\ln(K_I + I_L - I_{\min})}{I_L} - \frac{\ln(K_I + I_0 - I_{\min})}{I_0} \right\} \right] \\ &\qquad (L < L_0)\end{aligned} \tag{7}$$

where I_L is the light intensity at depth L from the illuminated side of the PBR, i.e., the light intensity at the farthest fraction from the light sources. In the latter light-deficient case (ii), when the thickness of the PBR (L) is longer than the critical depth (L_0), the average of the true specific growth rate in the PBR ($\overline{\mu_d}$) is derived by:

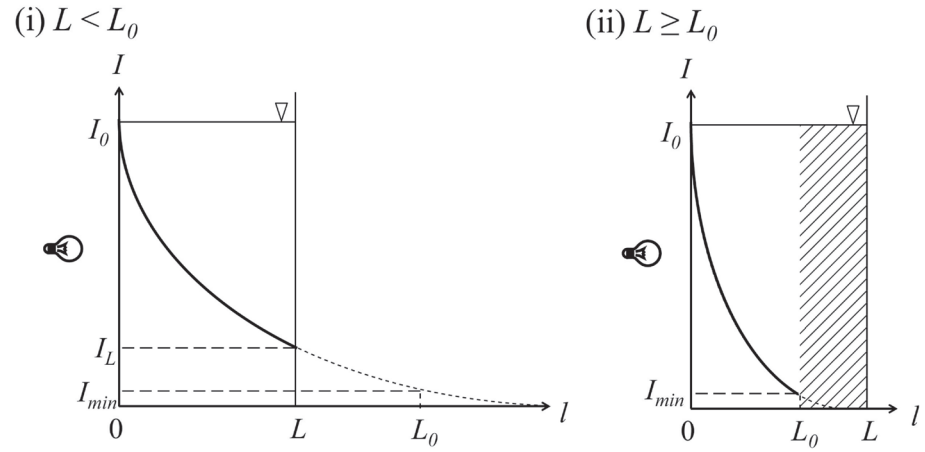

Fig. 5 Expected profile of light intensity in a rectangular photobioreactor illuminated by one side.

$$\overline{\mu_d} = \mu_m \cdot \frac{1}{L}\int_0^{L_0} \frac{I - I_{\min}}{K_I + (I - I_{\min})} dl = \frac{\mu_m}{AL \cdot \ln 10}\left[\frac{I_0 - I_{\min}}{I_0} + K_I\left\{\frac{\ln K_I}{I_{\min}} - \frac{\ln(K_I + I_0 - I_{\min})}{I_0}\right\}\right] \quad (L \geq L_0) \tag{8}$$

When the optical density is proportional to the biomass concentration (X) [g/L], it is described as $X = cA$, where c is the proportional coefficient [g/L]. As shown in Eq. (1), the apparent specific growth equals the reciprocal of the SRT. Therefore, the microalgae concentration and the volumetric productivity are predicted by Eqs. (9) and (10), respectively:

$$X = \mu_m \frac{c\lambda}{L} \cdot \frac{\theta_S}{1 + b\theta_S} \approx \mu_m \frac{c\lambda}{L} \cdot \theta_S \qquad (b \approx 0) \tag{9}$$

$$\frac{X}{\theta_S} = \mu_m \frac{c\lambda}{L} \cdot \frac{1}{1 + b\theta_S} \approx \mu_m \frac{c\lambda}{L} \qquad (b \approx 0) \tag{10}$$

where

$$\lambda = \frac{1}{\ln 10}\left[\frac{I_0 - I_{\min}}{I_0} + K_I\left\{\frac{\ln K_I}{I_{\min}} - \frac{\ln(K_I + I_0 - I_{\min})}{I_0}\right\}\right].$$

The above equations hold true in PBRs of any shape when the thickness of the PBR (L) is represented by the reciprocal of the SVR (R_{sv}) [m^{-1}]. Consequently, the microalgae concentration under light-limited condition is proportional to the SRT and SVR, while the volumetric productivity is solely dependent on the SVR, as described by Eqs. (11) and 12, respectively:

$$X = \mu_m c\lambda \cdot \frac{\theta_S}{1 + b\theta_S} \cdot R_{SV} \approx \mu_m c\lambda \cdot \theta_S \cdot R_{SV} \qquad (b \approx 0) \tag{11}$$

$$\frac{X}{\theta_S} = \mu_m c\lambda \cdot \frac{1}{1 + b\theta_S} \cdot R_{SV} \approx \mu_m c\lambda \cdot R_{SV} \qquad (b \approx 0) \tag{12}$$

where R_{sv} is the surface/volume ratio of the PBR (SVR). When the decay rate (b) is not negligible, the maximum volumetric productivity is observed at $L = L_0$ (**Fig. S2**), where the illuminated light energy is fully utilized. At this point, the light intensity at the darkest fraction of the PBR equals the minimum light intensity for microalgae growth. Therefore, the optimum biomass concentration (X_e) and SVR have the following relationship:

$$X_e = c \cdot \log_{10}(I_0 / I_{\min}) \cdot R_{SV}. \tag{13}$$

Since practically achievable SVR is limited in a scaled-up PBR, the biomass concentration would be the critical operating parameter for maximization of the volumetric productivity when sufficient nutrients are supplied and light is the primary limiting factor.

Practically, the biomass concentration is controlled by the SRT. In a simple PBR without membrane separation, it is not

always possible to control the biomass concentration at optimum levels because the SRT is constrained by the HRT. An MPBR has a good advantage because the SRT can be controlled independently from the HRT to achieve the optimum biomass concentration. Combination of a pre-cultivation PBR with an MPBR also has potential to maintain the optimum biomass concentration [27]. In the two-stage cultivation process, *C. vulgaris* was cultivated to approximately 800 mg/L in the first PBR (without a submerged membrane) for pre-cultivation, then sent to the second PBR equipped with a submerged membrane for further concentrated cultivation to approximately 2,000 mg/L. Interestingly, during this process, recirculation of retentate from the second-stage MPBR to the first pre-cultivation PBR enables control of the biomass concentration in the pre-cultivation PBR at optimum levels to achieve the highest microalgae productivity.

Consequently, the following conditions were required to optimize microalgae biomass productivity from treated sewage: (i) a short HRT to supply sufficient nitrogen, phosphorus and dissolved carbonate; and (ii) control of the SRT or circulation of membrane retentate to achieve the optimum biomass concentration where the light intensity reaches the minimum light intensity for microalgae growth at the darkest fraction of the PBR.

CONCLUSIONS

Optimum conditions of a MPBR process were investigated to maximize microalgae biomass production by utilizing nitrogen and phosphorus in treated sewage. With one submerged membrane module, biomass concentrations were almost comparable, regardless of HRT, because of microalgae biomass deposition on the membrane. Installation of an additional submerged membrane module effectively increased microalgae biomass concentration and volumetric productivity. Under nutrient-limited conditions, a short HRT is essential for the determination of the volumetric productivity of microalgae. Under light-limited conditions, optimization of the biomass concentration, which depends on SVR and initial light intensity, is essential to maximization of the volumetric productivity. Setup of the membrane modules for low flux and efficient air scrubbing is also important in the control of biomass concentration. Meanwhile, higher removal of nutrients is expected as the secondary effect when the process is operated under a longer HRT.

ACKNOWLEDGMENTS

This study was supported by JSPS Grant-in-Aid for Young Scientists B (Grant No. 24760428), the Steel Foundation for Environmental Protection Technology, the Center of Innovation Program by the Japan Science and Technology Agency (JST), Ams Corporation, and the Kurita Water and Environment Foundation.

SUPPLEMENTARY MATERIALS

Supplementary figures for this article can be accessed at the journal website (https://www.jstage.jst.go.jp/article/jwet/15/1/15_15-085/_article).

REFERENCES

[1] Lam MK, Lee KT: Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnol. Adv.*, **30**(3), 673–690, 2012. [Medline] [CrossRef]

[2] Pires JC, Alvim-Ferraz MC, Martins FG, Simões M: Carbon dioxide capture from flue gases using microalgae: Engineering aspects and biorefinery concept. *Renew. Sustain. Energy Rev.*, **16**(5), 3043–3053, 2012. [CrossRef]

[3] Chisti Y: Constraints to commercialization of algal fuels. *J. Biotechnol.*, **167**(3), 201–214, 2013. [Medline] [CrossRef]

[4] Acién Fernández FG, Fernández Sevilla JM, Sánchez Pérez JA, Molina Grima E, Chisti Y: Airlift-driven external-loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance. *Chem. Eng. Sci.*, **56**(8), 2721–2732, 2001. [CrossRef]

[5] Chini Zittelli G, Rodolfi L, Biondi N, Tredici MR: Productivity and photosynthetic efficiency of outdoor cultures of *Tetraselmis suecica* in annular columns. *Aquaculture*, **261**(3), 932–943, 2006. [CrossRef]

[6] Meiser A, Schmid-Staiger U, Trösch W: Optimization of eicosapentaenoic acid production by *Phaeodactylum tricornutum* in the flat panel airlift (FPA) reactor. *J. Appl. Phycol.*, **16**(3), 215–225, 2004. [CrossRef]

[7] Nedbal L, Trtílek M, Cervený J, Komárek O, Pakrasi HB: A photobioreactor system for precision cultivation of photoautotrophic microorganisms and for high-content analysis of suspension dynamics. *Biotechnol. Bioeng.*, **100**(5), 902–910, 2008. [Medline] [CrossRef]

[8] Richmond A, Cheng-Wu Z: Optimization of a flat plate glass reactor for mass production of *Nannochloropsis* sp. outdoors. *J. Biotechnol.*, **85**(3), 259–269, 2001. [Medline] [CrossRef]

[9] Bilad MR, Arafat HA, Vankelecom IF: Membrane technology in microalgae cultivation and harvesting: a review. *Biotechnol. Adv.*, **32**(7), 1283–1300, 2014. [Medline] [CrossRef]

[10] Honda R, Boonnorat J, Chiemchaisri C, Chiemchaisri W, Yamamoto K: Carbon dioxide capture and nutrients removal utilizing treated sewage by concentrated microalgae cultivation in a membrane photobioreactor. *Bioresour. Technol.*, **125**, 59–64, 2012. [Medline] [CrossRef]

[11] Marbelia L, Bilad MR, Passaris I, Discart V, Vandamme D, Beuckels A, Muylaert K, Vankelecom IF: Membrane photobioreactors for integrated microalgae cultivation and nutrient remediation of membrane bioreactors effluent. *Bioresour. Technol.*, **163**, 228–235, 2014. [Medline] [CrossRef]

[12] Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MA: Standard methods for the examination of water and wastewater. 21st ed. American Public Health Association, Washington DC, USA, 2005.

[13] Zhou W, Li Y, Min M, Hu B, Zhang H, Ma X, Li L, Cheng Y, Chen P, Ruan R: Growing wastewater-born microalga *Auxenochlorella protothecoides* UMN280 on concentrated municipal wastewater for simultaneous nutrient removal and energy feedstock production. *Appl. Energy*, **98**, 433–440, 2012. [CrossRef]

[14] Cho S, Luong TT, Lee D, Oh YK, Lee T: Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production. *Bioresour. Technol.*, **102**(18), 8639–8645, 2011. [Medline] [CrossRef]

[15] Pollice A, Tandoi V, Lestingi C: Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate. *Water Res.*, **36**(10), 2541–2546, 2002. [Medline] [CrossRef]

[16] Concas A, Lutzu GA, Pisu M, Cao G: Experimental analysis and novel modeling of semi-batch photobioreactors operated with *Chlorella vulgaris* and fed with 100% (v/v) CO_2. *Chem. Eng. J.*, **213**, 203–213, 2012. [CrossRef]

[17] Discart V, Bilad MR, Marbelia L, Vankelecom IF: Impact of changes in broth composition on *Chlorella vulgaris* cultivation in a membrane photobioreactor (MPBR) with permeate recycle. *Bioresour. Technol.*, **152**, 321–328, 2014. [Medline] [CrossRef]

[18] Fan LH, Zhang YT, Zhang L, Chen HL: Evaluation of a membrane-sparged helical tubular photobioreactor for carbon dioxide biofixation by *Chlorella vulgaris*. *J. Membr. Sci.*, **325**(1), 336–345, 2008. [CrossRef]

[19] de Morais MG, Costa JA: Carbon dioxide fixation by *Chlorella kessleri, C. vulgaris, Scenedesmus obliquus* and *Spirulina* sp. cultivated in flasks and vertical tubular photobioreactors. *Biotechnol. Lett.*, **29**(9), 1349–1352, 2007. [Medline] [CrossRef]

[20] Přibyl P, Cepák V, Zachleder V: Production of lipids in 10 strains of *Chlorella* and *Parachlorella*, and enhanced lipid productivity in *Chlorella vulgaris*. *Appl. Microbiol. Biotechnol.*, **94**(2), 549–561, 2012. [Medline] [CrossRef]

[21] Widjaja A, Chien CC, Ju YH: Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. *J. Taiwan Inst. Chem. Eng.*, **40**(1), 13–20, 2009. [CrossRef]

[22] Brányiková I, Maršálková B, Doucha J, Brányik T, Bišová K, Zachleder V, Vítová M: Microalgae—novel highly efficient starch producers. *Biotechnol. Bioeng.*, **108**(4), 766–776, 2011. [Medline] [CrossRef]

[23] Feng Y, Li C, Zhang D: Lipid production of *Chlorella vulgaris* cultured in artificial wastewater medium. *Bioresour. Technol.*, **102**(1), 101–105, 2011. [Medline] [CrossRef]

[24] Fu W, Gudmundsson O, Feist AM, Herjolfsson G, Brynjolfsson S, Palsson BØ: Maximizing biomass productivity and cell density of *Chlorella vulgaris* by using light-emitting diode-based photobioreactor. *J. Biotechnol.*, **161**(3), 242–249, 2012. [Medline] [CrossRef]

[25] Keffer JE, Kleinheinz GT: Use of *Chlorella vulgaris* for CO_2 mitigation in a photobioreactor. *J. Ind. Microbiol. Biotechnol.*, **29**(5), 275–280, 2002. [Medline] [CrossRef]

[26] Carvalho AP, Silva SO, Baptista JM, Malcata FX: Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. *Appl. Microbiol. Biotechnol.*, **89**(5), 1275–1288, 2011. [Medline] [CrossRef]

[27] Bilad MR, Discart V, Vandamme D, Foubert I, Muylaert K, Vankelecom IF: Coupled cultivation and pre-harvesting of microalgae in a membrane photobioreactor (MPBR). *Bioresour. Technol.*, **155**, 410–417, 2014. [Medline] [CrossRef]