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Microalgae membrane photobioreactor for further removal of nitrogen and phosphorus from secondary sewage effluent

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Abstract−For further removal of nitrogen and phosphorus from secondary sewage effluent, two strains of microalgae, *Chlorella* sp. *ADE4* and *Chlorella vulgaris*, were selected for cultivation in the membrane photobioreactor. The *Chlorella* sp. *ADE4*, isolated from wastewater illustrated higher removal efficiency of T-N and T-P, and faster algal growth than the *Chlorella vulgaris* in a batch experiment using treated sewage effluent. The T-N and T-P removal efficiency was 66.5% and 94.5%, respectively, within HRT of two days when the photobioreactor of *Chlorella* sp. *ADE4* was operated in continuous mode. The effluent water quality was 6.3 mg/L and 0.044 mg/L for T-N and T-P. It was estimated that the algal biomass productivity was 55 mg/L·d with T-N and T-P uptake rates of 6.25 and 0.483 mg/L·d, respectively, in the system. Operational flux below 58 LMH was found to be effective for separation of algal cell from effluent in membrane system.

Keywords: *Chlorella*, Microalgae, Membrane Photobioreactor, Nutrient Removal, Sewage Effluent

INTRODUCTION

Discharge of effluent from sewage treatment plants containing high level of nutrients such as nitrogen (N) and phosphorus (P) is one of the main causes of eutrophication. To minimize it, the nutrient concentrations in treated sewage effluent must be minimal. Recently, the effluent standard in South Korea for total phosphorus (T-P) was standardized to 0.2 mg/L and total nitrogen (T-N) to 20 mg/L. Therefore, sewage treatment plants have been required to upgrade the water quality with more efficient and economic technology. Biological nutrient removal (BNR) and chemical precipitation systems had been previously implemented in sewage treatment plants [1,2]. However, these systems have limitations in meeting the effluent standard of nitrogen and phosphorus, which is more stringent, requiring precise operation, high energy input, chemical consumption and sludge disposal. Accordingly, there was an effort to develop novel materials, such as iron-loaded zeolites which could be applied to remove ammonium and phosphate from aqueous solution with no sludge production [3].

Microalgae-based technology has gained interest as an alternative post-secondary treatment method owing to its high capacity for N and P uptake and providing benefits such as biofuel production, greenhouse gas fixation from the atmosphere and use in manufacture of cosmetic products as well [4,5]. Several types of microalgae cultivation-based wastewater treatment systems have been studied, including both open pond and closed reactor system. However, the open pond system requires large space; recent researches have mainly focused on the closed reactor system such as photobioreactor, which is effective for post treatment of wastewater along with small footprint, high algal biomass production, reduced risk of contamination and efficient control of the system operation [6].

A number of studies on microalgae cultivation have utilized synthetic media containing high concentrations of nitrogen and phosphorus, while few studies investigated the use of wastewater at low level nutrients. Secondary sewage effluent has been considered as an alternative nutrient source for microalgae cultivation because it still contains sufficient amount of nitrogen and phosphorus, which is required for further removal to prevent eutrophication in natural water bodies. However, since bacteria in the secondary sewage effluent have an adverse effect on the algal growth, the selection of bacteria-tolerant species of microalgae should be carefully considered.

Another consideration of microalgae application for nutrient removal from wastewater is the separation of algal biomass from the treated water. A considerably small size of microalgal cell results in very low settling velocity, requiring large surface area and chemicals for flocculation. Thus, existing technologies, such as sedimentation, filtration and centrifugation, are difficult to apply for microalgae harvesting in real scale. Membrane filtration has become a promising technology for microalgae harvesting due to various reasons such as low chance of chemical contamination and less biomass washout [7-13]. To date, very limited studies have been conducted to investigate the applicability of M-PBR under continuous cultivation conditions of microalgae using sewage effluent as nutrient source.

We investigated the potential of two different microalgae strains, *Chlorella vulgaris* and *Chlorella* sp. *ADE4*, for nutrient removal from wastewater provided, biomass growth under continuous culture conditions and nature of membrane filtration for biomass collection.

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MATERIALS AND METHODS

1. Culture Conditions of Microalgae

The domestic strains cultured using anaerobic digestion effluent, hereafter called *Chlorella* sp. *ADE4* and *Chlorella vulgaris*, were used for this experimental study. *Chlorella vulgaris* (*C. vulgaris* KMMC9) was purchased from Korea Marine Microalgae culture center, and *Chlorella* sp. *ADE4* was kindly provided by the Department of Microbiology, Pusan National University, South Korea [14]. Prior to the experiment, both strains were maintained in BG11 medium as previously described [15]. The medium was adjusted to a pH of 7.1 before being introduced for sterilizing in an autoclave. The microalgae samples were kept at 25±2 °C under a light intensity of 50 μmol/(m^2·s) for the entire experiment.

2. Wastewater Characterization

Artificial sewage effluent (ASE) was prepared by adjusting the concentration of $NaNO_3$ and K_2PO_4 in the BG11 medium to provide T-N and T-P levels of 20 and 2 mg/L, respectively.

Treated sewage effluent (TSE) was collected from the Jinhae sewage treatment plant in Changwon City, South Korea. The average concentrations (n=3) of chemical oxygen demand (COD), suspended solid (SS), T-N, and T-P in the secondary effluent were 10.5, 2.3, 18.8, and 1.01 mg/L, respectively. Since the TSE is secondary effluent, T-N and T-P are mostly in the form of NO_3 and PO_4 as the chemicals used in ASE.

3. Experimental Set-up

3-1. Batch Culture

Chlorella sp. *ADE4* and *C. vulgaris* were cultivated in 10 L PBRs with a working volume of 7 L, in a double column-type reactor made of 7-mm thick transparent PVC. The inner diameters of the internal and external column were 10 and 20.6 cm, respectively, with a height of 50 cm. Two 20 W fluorescent lamps of diameter 2.5 cm were placed in the inner column of the reactor (Fig. 1) providing illumination of 50 μmol/m^2·s at a mode of light (14 h) and dark (10 h) period. The algal mixture was agitated by constant air flow rate of 4 L/min through fine bubble diffusers placed at the bottom of PBR. pH was maintained in the range of 7.5-8.5 without artificial control during the experimental period. To determine the most suitable microalgae for nutrient removal, two PBRs with two different sewage effluents (ASE and TSE) were examined for seven days. Each experiment was carried out twice at room temperature of 25±2 °C.

3-2. Continuous Culture

The most suitable sewage-cultured microalgae were used in the bioreactors running under similar operating conditions as batch operation, in a continuous mode with membrane separation. A high-density polyethylene (HDPE) hollow fiber microfiltration membrane with a pore size of 0.4 μm and a surface area of 0.04 m^2 (Econity, South Korea) was submerged in the reactor. During the first two days, microalgae suspensions were incubated in the sewage without replacement. Subsequently, the permeate was withdrawn at a constant flux of 3.75 L/m^2·h and flow rate of 2.5 mL/min to maintain a hydraulic retention time (HRT) of two days. Secondary sewage effluent without pretreatment was continuously fed into the reactor at the same flow rate. Samples were taken at fixed intervals from the feed water and effluent of M-PBR to evaluate nutrient removal. The algal suspension taken from the reactor was filtered using membrane of pore size 0.45 μm to measure the biomass concentration. The operation of the M-PBR was carried out for 18 days.

3-3. Filtration Performance

Filtration tests were set up independently with a microalgae concentration of 1 g/L. The filtration test was carried out at different constant flux variation, where the transmembrane pressure (TMP) was monitored during first 60 min. The rate of TMP increase was reported using the equation by Clech et al. Eq. (1), where TMP2

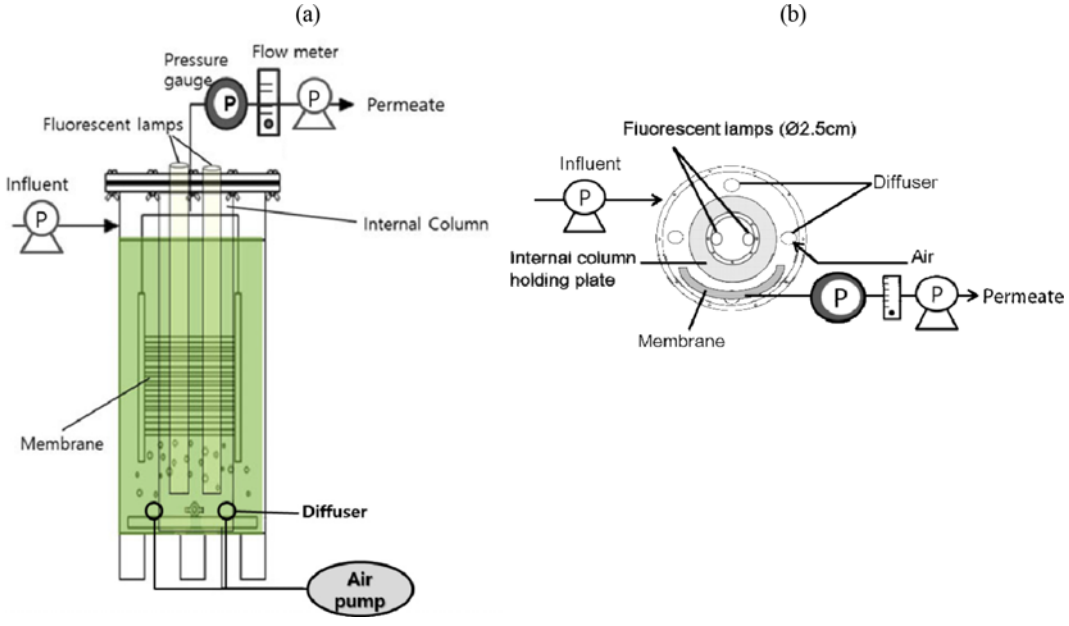

Fig. 1. Schematic of set-up of the membrane photo bioreactor (a) side view (b) top view of the reactor.

and TMP1 are defined as the TMP at the end and the initial filtration tests at times t2 and t1, respectively [16].

$$\frac{dP}{dt} = \frac{(TMP2 - TMP1)}{t2 - t1} \quad (1)$$

4. Microalgae Growth Characteristics

Growth characteristic of the microalgae was measured as biomass in dry weight, which was determined by gravimetric filtration of the cultured microalgae through a glass microfiber filter (GF/C, Whatman®) and drying at 105 °C for 24 h.

The specific growth rate at a given time interval ($\mu\Delta t$, d^{-1}) was expressed as Eq. (2).

$$\mu\Delta t(d^{-1}) = \frac{\ln X2 - \ln X1}{t2 - t1} \quad (2)$$

where, X1 and X2 represent dry weight at time t1 and t2, respectively.

The cultured microalgae were centrifuged at 12,000 g for 15 min. The supernatant was filtered through membrane of pore size 0.22 μm. The filtrate was collected as soluble EPS in terms of carbohydrate and protein concentrations. Carbohydrate concentration in the extracted EPS was measured using a phenol-sulfuric method with glucose as the standard [17]. Sample absorbance was measured at 490 nm. Meanwhile, protein concentration was measured by the Bradford method [18], using bovine serum albumin (BSA) (Biorad, USA) as the standard. The color developed at the absorbance of 595 nm. All measurements were done in duplicate and data reported in this study represents the mean value of the duplicates.

RESULTS AND DISCUSSION

1. Algal Growth and Nutrient Removal under Batch Conditions

Growth of the two microalgal strains was monitored for seven days. Biomass dry weights were measured for microalgal growth as shown in Fig. 2.

Chlorella sp. *ADE4* and *C. vulgaris* illustrated higher growth rates in ASE (0.23 d^{-1} and 0.17 d^{-1}, respectively) than in TSE (0.16 d^{-1} and 0.11 d^{-1}, respectively). This is due to presence of indigenous bacteria and protozoa in the treated sewage effluent (TSE), and the differences in the chemical composition between artificial and real sewage effluent interfere with the microalgal growth [19]. Wastewater-isolated *Chlorella* sp. *ADE4* showed a faster growth rate than commonly used microalgae *C. vulgaris* in both artificial and real sewage effluent, suggesting that the newly isolated *Chlorella* sp. *ADE4* was more adaptable and viable than *C. vulgaris* in the low-nutrient conditions of the treated sewage effluent.

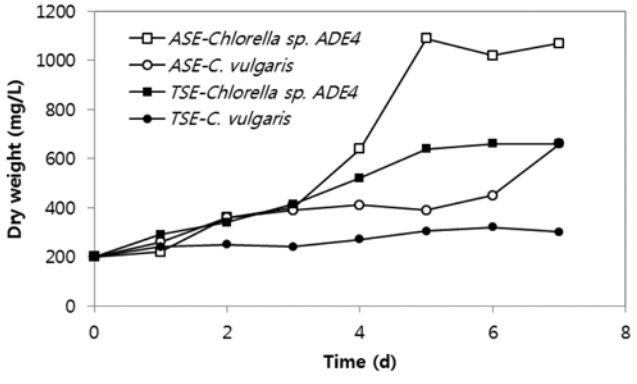

Fig. 2. Growth curves of *Chlorella* sp. *ADE4* and *C. vulgaris* in the batch culture (ASE, artificial sewage effluent; TSE, treated sewage effluent).

Table 1. Mean TN and TP removal efficiencies using *Chlorella* sp. *ADE4* and *C. vulgaris* in artificial sewage effluent (ASE) and treated sewage effluent (TSE) in batch conditions

Microalgae strain	Culture	TN removal (%)			TP removal (%)		
		1 d	2 d	7 d	1 d	2 d	7 d
Chlorella ADE4	ASE	0	60.8	95.4	89.1	89.6	97.5
	TSE	39.4	62.3	67.5	65.4	100	100
Chlorella vulgaris	ASE	0	4.8	93.3	19.2	23.4	96.5
	TSE	10.6	61.7	63.6	46.9	59.1	78.5

N and P removal efficiencies are shown in Table 1. The real sewage effluent sample used in this experiment had average T-N and T-P concentrations of 18.8 and 1.01 mg/L, respectively. The highest N and P uptake was observed for *Chlorella* sp. *ADE4* when treated with TSE, where 62.3% of T-N and 100% of T-P were removed after two days. The T-N removal in this study was less efficient compared to that in the study conducted by Cho et al. using *Chlorella* sp. *ADE-5* for high concentration nutrient uptake, which reported more than 85% of T-N removal within 100 h of operation [14].

The treated sewage effluent in this study contained a low concentration of T-P and the culture could completely remove T-P within 2 d resulting in depletion of the P source. Depletion of the P source is associated with N assimilation observed from rapid reduction of the T-N concentration in wastewater in the first two days, which ceased thereafter. In a previous study, N and P were suggested to be biochemically dependent nutrients [20]. Davies and Sleep showed that *Skeletonema costatum* was significantly simulated when both NO_3^- and PO_4^{3-} were added, but was repressed when only NO_3^- was added [21]; this could explain why the N concentration was still high after P exhaustion, once the P became limiting.

2. Algal Growth and Nutrient Removal under Continuous Conditions

Chlorella sp. *ADE4* was more adaptable and more effective in removing nutrients than *C. vulgaris* in batch conditions. These results suggest that *Chlorella* sp. *ADE4* might be a better choice for further testing in continuous conditions. Under batch conditions, *Chlorella* sp. *ADE4* appeared to grow slowly owing to P limitation. Growth of *Chlorella* sp. *ADE4* was monitored in continuous mode when fresh sewage effluent was fed continuously at the same flow rate as treated water withdrawal from the system (Fig. 3).

Chlorella sp. *ADE4* adapted well to low-nutrient concentrations in secondary sewage effluent under continuous mode at HRT of 2 d showing the algal growth rate of 0.153 d^{-1} and average biomass productivity of 55 mg/L·d. The biomass productivity was higher compared with other studies operated at an HRT of 1 d; Honda et

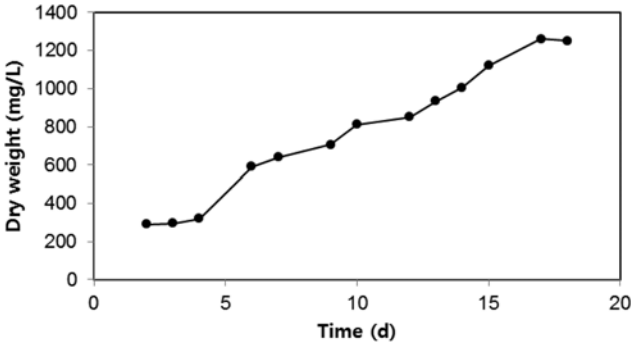

Fig. 3. Dry weight of *Chlorella* sp. *ADE4* in continuous mode using real wastewater.

al. reported biomass productivity of 48 mg/L·d in a submerged membrane PBR using ASE [22], and Xu et al. reported production of 32.5 mg/L·d using an algae-based membrane bioreactor (A-MBR) [13].

The variation of T-N and T-P concentrations in influent and effluent is illustrated in Fig. 4. The influent T-N concentration slightly fluctuated in the range of 17.3-20.6 mg/L (average 18.8 mg/L) during operation periods of the system (Fig. 4(a)) and the effluent concentration was maintained stably between 5.5 and 7.3 mg/L (average of 6.3 mg/L). Consequently 66.5% of T-N removal was achieved from the treated sewage effluent. While the average T-P removal was 94.5% producing an average effluent T-P concentration of 0.044 mg/L as shown in Fig. 4(b). However, since the influent T-P concentration was relatively low at average 1.01 mg/L (0.75-1.5 mg/L), there was severe P-limitation in the PBR, causing limited N removal and less biomass productivity. Adding P source may alleviate the P scarcity, allowing much higher N removal and biomass productivity.

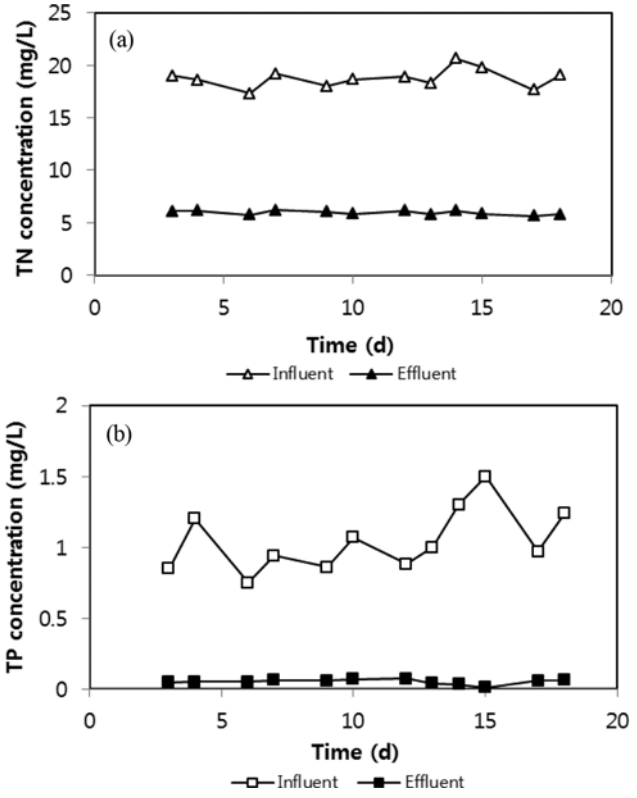

Fig. 4. Removal of TN (a) and removal of TP (b) in continuous mode.

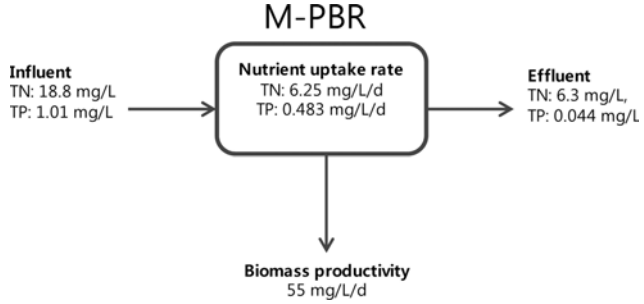

Fig. 5. Flow diagram of nutrient mass balance in a membrane photobioreactor (M-PBR) system.

The nutrient mass balance in the M-PBR system is summarized in Fig. 5. *Chlorella* sp. *ADE4* utilized N and P from sewage effluent and produced biomass at a rate of 55 mg/L·d. From a nutrient balance aspect, T-N and T-P uptake rates were 6.25 and 0.483 mg/L·d, respectively, which are similar to those of the other studies. Although the types of nitrogen and microalgae species are different, Park et al. reported 5.20-6.46 mg/L·d removal of NH_4-N, dependent on the seed concentration of *Scenedesmus* sp. in an anaerobic digestion effluent obtained from a pig farm [23]. Kapdan and Aslan found the saturation value constant of the Stover-Kincannon model for NH_4-N removal by *C. vulgaris* to be 10.3 mg/L/d [24]. A relationship between biomass production and nutrient demand in this study was assumed to be in such a manner that to generate 1 g dry weight of microalgae, 113 mg of T-N and 8.782 mg of T-P are needed. Cho et al. reported that 1 g of *Chlorella* sp. corresponded to 78.9 mg of N in BG11 medium and 133.1 mg of N in sewage effluent mixture from anaerobic digestion and sludge concentrate tanks [14].

3. Membrane Filtration for Microalgae Harvesting

In determining appropriate operating conditions for the microalgae harvesting using membrane filtration, flux is one of the key parameters. Higher fluxes typically give higher fouling rates, and selecting a filtration flux to allow sustained filtration is challenging. One of the most common ways to define the operational flux

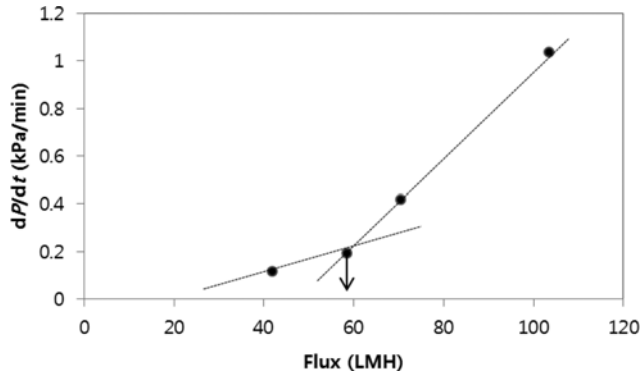

Fig. 6. Relationship between the rate of transmembrane pressure increase and flux at a microalgal concentration of 1 g/L.

Table 2. Comparison of flux and transmembrane pressure in membrane filtration of microalgae

Reference	Algae concentration	Flux [LMH]	TMP (kPa)	Membrane
Bilad et al. [8]	0.41 g/L *Chlorella vulgaris*	50	20	MF (PVDF) 0.36 μm
Chiou et al. [26]	2×10^6 cells/mL *Chlorella vulgaris*	70	172	UF (cellulose) 10 kDa
This study	1 g/L *Chlorella* sp. *ADE4*	58.5	30	MF (HDPE) 0.4 μm

is by increasing the flux for a fixed condition, where the appropriate flux will not increase TMP over a time period known as the critical flux [25]. The relationship between the rate of TMP increase and flux is shown in Fig. 6. In most cases, an increase in flux leads to an increased TMP. However, a clear difference can be observed in the fouling rate, in terms of the dP/dt obtained during the fixed permeate flux test. The rate of TMP increase for flux at 42, 58.5, 70.5, and 103.5 LMH was recorded as 0.12, 0.19, 0.41, and 1.03, respectively. The rate of TMP increase can be defined as the fouling rate, and the results suggest slight increase in fouling rate at flux values below 58.5 LMH. This flux value is the critical flux which indicates significant TMP increase or severe fouling above. Consequently, the flux below 58.5 LMH could be recommended for sustainable operation of the microalgae separation by MF. The flux and TMP results from this study were compared with those from previous studies in Table 2. Although, the flux and TMP are comparable to other studies, a flux value of 58.5 LHM seems to be quite high for filtration of treated wastewater containing 1 g/L in extended period of operation. Membrane flux is the most important factor of system operation for fouling control to be considered in membrane filtration. Therefore, the applicable flux should be much lower than the value of 58.5 LMH suggested in this study for stable operation of the separation system in M-PBR.

In the current study, carbohydrate and protein of soluble EPS after operating M-PBR for 5 d were 42.5 mg/L and 14.4 mg/L, respectively, comparable to those found in *Chlorella* sp. cultivated in primary effluent (carbohydrate of soluble EPS: 33 mg/L and protein of soluble EPS: 13 mg/L) [27]. The potential fouling factors are complicated, such as the nature of NOM, solution characteristics, membrane properties, and system operation conditions etc., and the combined effects of the factors result in more severe fouling problems [28].

CONCLUSION

The study shows that nutrient in secondary sewage effluent could be removed by microalgae effectively. *Chlorella* sp. *ADE4* was found to be a suitable strain of microalgae, showing a higher growth rate and rapid N and P uptake compared to *C. vulgaris*. T-N and T-P removal efficiencies of 66.5% and 94.5%, respectively, were achieved in 2 d when cultivated in sewage effluent, owing to the tolerance for low P concentrations of the *Chlorella* sp. *ADE4*. In sewage effluent, levels of 18.8 mg/L of T-N and 1.01 mg/L of T-P could be treated to value lower than 6.3 and 0.044 mg/L, respectively. If discharged into the receiving waterways, this is likely to reduce eutrophication. Biomass productivity of 55 mg/L·d can be expected from secondary sewage effluent in M-PBR, which was effective for facilitating biomass separation. Permeate flux below 58.5 LMH was recommended for sustaining operations in MF membrane system.

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NOMENCLATURE

ASE : artificial sewage effluent
BG11 : blue green medium
COD : chemical oxygen demand
LMH : $L/m^2 \cdot h$ (Liters per Meter sq. Hour)
M-PBR : membrane photo bioreactors.
PBR : photo bioreactors
PVC : polyvinyl chloride
TMP : transmembrane pressure
T-N : total nitrogen
T-P : total phosphorus
X : initial/final dry weight/cell number
t : time

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