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A novel algal biofilm membrane photobioreactor for attached microalgae growth and nutrients removal from secondary effluent

Feng Gao [a,b,*], Zhao-Hui Yang [b,c], Chen Li [a], Guang-Ming Zeng [b,c], Dan-Hui Ma [a], Li Zhou [a]

[a] College of Marine Science, Zhejiang Ocean University, Zhoushan 316000, China
[b] College of Environmental Science and Engineering, Hunan University, Changsha 410082, China
[c] Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, China

HIGHLIGHTS

- A novel algal biofilm membrane photobioreactor was developed to culture *C. vulgaris*.
- Biomass production of the reactor was enhanced with the addition of solid carriers.
- Most of the produced biomass was immobilized as algal biofilm in BMPBR.
- Higher biomass production in BMPBR enabled the reactor to remove more nutrients.
- Algae were completely isolated from the effluent in this attached culture system.

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ABSTRACT

In this study, a novel algal biofilm membrane photobioreactor (BMPBR) equipped with solid carriers and submerged membrane module was developed for attached growth of *Chlorella vulgaris* and secondary effluent treatment. The volumetric microalgae production achieved in BMPBR was 0.072 g L^{-1} d^{-1}, which was 1.44-fold larger than that in suspended growth membrane photobioreactor (MPBR). Furthermore, 72.4% of the total produced algal biomass was immobilized as algal biofilm in BMPBR. Advanced nutrients removal from secondary effluent was achieved both in BMPBR and MPBR, with average reduction of about 85% for PO_4^{3-}-P in the stable stage. Additionally, BMPBR showed better nitrogen removal performance than MPBR due to its higher algal biomass productivity. Moreover, with the filtration effect of the submerged membrane module in the reactor, suspended microalgae could be completely isolated from the effluent and a low average SS concentration of 0.28 mg L^{-1} was achieved in the effluent of BMPBR.

1. Introduction

In recent years, with growing concerns of global climate change and fossil fuels shortage, algal biodiesel as one of the most promising renewable biofuels have caught worldwide attention (Mata et al., 2010). Microalgae are ideal feedstock for biofuel production due to their high growth rate, high lipid content, and the possibility of year-round cultivation, then can provide more biodiesel than typical oilseed crops with using less water and land (Stephens et al., 2010). More importantly, cultivation of algal biomass can be performed on non-agricultural land, thus avoiding competition with agricultural production.

But at present, algal biofuel is not economically competitive with petrodiesel. The cultivation and harvest costs of microalgae are still too high. Using wastewater as the cultivation medium can offset the cost of fertilizers and water otherwise needed for the production of algal biomass. In addition, many of the recent studies have reported that the concentration of N and P pollutants in many kinds of wastewater can be reduced to a very low value through the assimilation of algal cells (Ruiz-Martinez et al., 2012; Singh and Thomas, 2012; Sacristán de Alva et al., 2013; Gao et al., 2014). Moreover, compared to chemical/physical nutrients removal process, microalgae assimilation can remove nutrients in a less expensive and ecological safer way (Oswald, 2003). Thereby using microalgae to simultaneously produce biofuel and treat wastewater has attracted increasing attention (Pittman et al., 2011). But before this coupled technology can be utilized, suspended algal biomass must be completely separated and harvested from the water to improve the effluent quality, and to produce

* Corresponding author at: College of Marine Science, Zhejiang Ocean University, Zhoushan 316000, China. Tel.: +86 0580 2550753; fax: +86 0580 2556515.
E-mail address: gf_hd@126.com (F. Gao).

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feedstock for biofuel production. However, it has proven to be difficult to separate and harvest the suspended microalgae in a cost-effective way (Molina Grima et al., 2003).

One recently proposed method to solve the aforementioned problem is to employ microfiltration (MF) membrane module in photobioreactors, which acted as a solid–liquid separator thereby enabled the membrane photobioreactor (MPBR) to completely isolate suspended algal cells from the effluent (Singh and Thomas, 2012; Bilad et al., 2014a). In addition, the solid retention time (SRT) and hydraulic retention time (HRT) of the reactor can be independently controlled during the culture interval (Honda et al., 2012; Bilad et al., 2014b; Gao et al., 2014), and the algal biomass concentration can be free from the growth rate of algal cells and hydraulic loading of the reactor, thus maintained at high level. Therefore a better performance of algal biomass production and nutrients removal efficiency can be achieved in MPBR (Gao et al., 2014). Despite these advantages, however, the algal cells in MPBR were grown in suspension of traditional, and the biomass concentrations achieved in recent studies were generally below 2 g L^{-1} (Honda et al., 2012; Bilad et al., 2014b; Gao et al., 2014). Therefore, to harvest the algal biomass from the culture liquor, the dilute algal culture should be further concentrated to hand over most of the water through one or more subsequent operations such as sedimentation, centrifugation and flocculation. These operations usually are time consuming and expensive, thus hindering the development and application of MPBR.

Another popular strategy is to employ attached algal culture system, in which algal cells are grown on the surface of some solid supporting materials (Johnson and Wen, 2010; Christenson and Sims, 2012; Ozkan et al., 2012; Gross et al., 2013; Zhuang et al., 2014). In these attached culture systems the algal biomass can be naturally concentrated and easily harvested. This can lead to a low cost of harvesting, and less expensive downstream dewatering process. Moreover, compared with the traditional suspension culture system, attached algal culture system usually can achieve higher algal biomass productivity (Johnson and Wen, 2010; Christenson and Sims, 2012; Zhuang et al., 2014). But, besides these advantages, the suspended algal cells remained in the water may affect the water quality of effluent, when attached microalgae culture is used for both biomass production and wastewater treatment. In the research of Zhuang et al. (2014), part of the algal cells could attach and grow on the solid carriers, and the density of suspended algal cells was similar between the reactors with and without solid carriers. So the microalgae content in the effluent should be controlled, or a subsequent operation is needed to completely isolate the suspended algal cells from the effluent, when attached microalgae culture is used for wastewater treatment. But, at present, the research on this field is scarce.

Here, a novel algal biofilm membrane photobioreactor (BMPBR) equipped with solid carriers and submerged membrane module was constructed for attached microalgae growth and nutrients removal form secondary effluent. In BMPBR membrane module acted as solid–liquid separator to completely isolate the suspended algal cells from the effluent, and the solid carriers acted as supporting material to culture attached algal biomass. Thus, this novel photobioreactor has the potential to simultaneously produce attached algal biomass and achieve high quality effluent in terms of nutrients and suspended solids.

2. Methods

2.1. Experimental-scale reactor

The experimental-scale flat-plate BMPBR of dimension 1.0 m (L) × 0.4 m (W) × 0.7 m (H) was constructed in plexiglass, as schematically shown in Fig. 1. In the experiment, the working deep of the reactor was 0.5 m. The surface to volume ratio (S/V), which is defined as a ratio of the lighted surface area to working volume of the photobioreactor, was 7 m^{-1}. The reactor body was divided into two zones. In the main zone (0.7 m × 0.4 m × 0.7 m) of the reactor, flexible fiber bundles that used as solid carriers were submerged in middle of the reactor for algal cells to attach on. At present, flexible fiber bundle as one of ideal biofilm carriers is widely used in traditional biofilm wastewater treatment process because of its large surface area, high adsorption capacity, low cost, and so on. When the attached algal biomass increased to a certain amount, the flexible fiber bundles with the rope could be pulled out from the cultivation solution. Then the attached microalgae could be easily harvested through a separator by mechanical separation, solvent extraction or some other ways.

In the outlet zone (0.3 m × 0.4 m × 0.7 m) of the reactor, a polyvinylidene fluoride (PVDF) hollow-fiber MF membrane module that used as a solid–liquid separator was submerged in the reactor, from which permeate can be continuously withdrawn from the reactor. The pore size of the membrane was 0.1 μm and the effective area of membrane surface in the module was 2.5 m^2.

At the bottom of both the main zone and outlet zone of the reactor, gas distributors were installed to provide bubbles, which provided agitation in the reactor and reduced the algal cell adsorption by the membrane. The reactor was illuminated with two LED lamps (red/blue light ratio of 4:1) placed at a distance of 5 cm from the 1.0 m (L) × 0.4 m (W) wall of the reactor. The power of each LED lamp was 9 w, and the maximum light intensity on the wall of the reactor was about 8000 lux. As a control, a MPBR without solid carriers was also constructed. Other conditions of the MPBR were the same as the BMPBR.

2.2. Alga strain and inoculation

Alga strain was *Chlorella vulgaris* obtained from the Culture Collection of Algae, Institute of Hydrobiology, Chinese Academy of Sciences. It was pre-cultivated in BG11 medium under stationary condition at 25 °C, continuous white fluorescent light illumination (about 12,000 lux) and shaking at 100 rpm. Then *C. vulgaris* cells in logarithmic growth phase were collected by centrifugation (8000 rpm, 15 min). The collected algal cells, after washed by 15 mg L^{-1} $NaHCO_3$ solution, were seeded to the BMPBR and MPBR to give an initial suspended microalgae concentration of 40 mg L^{-1} (dry weight).

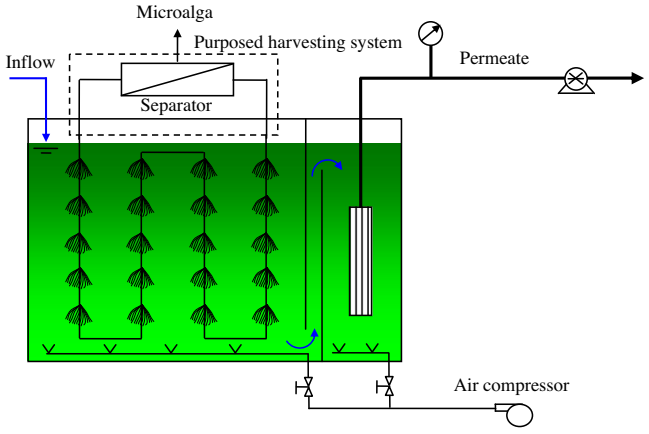

Fig. 1. Schematic diagram of the lab-scale biofilm membrane photobioreactor (BMPBR).

2.3. Reactor operation

During the experiment, simulated secondary effluent was continuously fed to BMPBR and MPBR as cultivation medium with HRT of 2 days. Meanwhile, from the membrane module submerged in the reactors, permeate was intermittently withdraw by suction pumps in a 12-min on/3-min off cycle. The simulated secondary effluent consisted of glucose, NH_4Cl, $NaNO_3$, KH_2PO_4 and mineral solution containing $FeCl_3$, $CaCl_2{\cdot}4H_2O$, $MgSO_4$, H_3BO_3, $MnCl_2{\cdot}4H_2O$, $ZnSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$, $NaMoO_4{\cdot}2H_2O$. The initial influent contained about 40.0 mg L^{-1} of chemical oxygen demand (COD), 5.0 mg L^{-1} NH_4^+-N, 15.0 mg L^{-1} total inorganic N (TIN), and 0.8 mg L^{-1} PO_4^{3-}-P. The composition of the simulated secondary effluent was designed based on three municipal wastewater treatment plants in Zhejiang Province, China. CO_2-mixed air (contained 4% of CO_2) was pumped into the rector at an aeration rate of 2.0 L min^{-1}. The pH of the culture liquor in the reactors was in the range 6.8–7.5. No temperature control was employed in the course of the experiment, resulting in culture temperatures varying in the range 25–28 °C. The experiment was repeated three times under the same conditions. At the beginning of each replicate, the reactors were rinsed thoroughly and equipped with unused flexible fiber bundles and MF membrane module.

2.4. Analyses

Suspended algal cells grown in both MPBR and BMPBR were centrifugally collected (7000*g*, 10 min, 4 °C) and freeze dried daily, then the dry biomass concentration of suspended microalgae was determined gravimetrically. At the end of the experiment, algal cells attached on the carriers in BMPBR were completely dissolved in water in an ultrasonic cleaner (500 W, 40 KHz, 30 min), and then were collected by centrifuging (7000*g*, 10 min, 4 °C). The collected algal biomass were also freeze dried and weighed, and then the attached algal biomass yield and total algal biomass yield in BMPBR would be counted and expressed as gram per unit of volume uniformly. Meanwhile, the Chlorophyll contents in suspended algal biomass and attached algal biomass were also analyzed according to Li et al. (2008).

In practical application, complete harvest of attached algal cells from carriers is not necessary. The algal cells still adhering to surface of the solid carriers can be used as "seed" for the regrowth of algal biofilm. Therefore some low-consumption methods such as mechanical separation or water extraction can be used to harvest most of the attached algal cells from the solid carriers.

Water samples were taken daily from the inflow and outflow of the reactors to evaluate the concentration of NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^{3-}-P and suspended solids (SS) according to Chinses standard analytical methods for the examination of water.

3. Results and discussion

3.1. Microalgae growth

Fig. 2 presents the suspended algal biomass inside the reactors as a function of culture time during the whole operation period. MF membrane module submerged in the photobioreactor can effectively prevent the algal cells from wash out, and then enable the reactor to operate with large supply and high microalgae productivity (Honda et al., 2012; Gao et al., 2014). In this study, the volumetric microalgae productivity of MPBR in the whole culture interval was 49.89 mg L^{-1} d^{-1} (calculated from the data in Fig. 2), which is slightly higher than our previous report when a column MPBR and real treated sewage were used to culture the same algal strain (Gao et al., 2014).

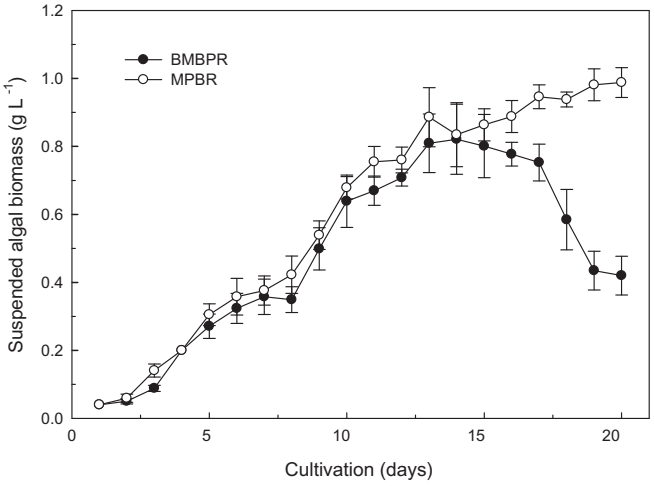

Fig. 2. The suspended algal biomass as a function of culture time in the reactors of biofilm membrane photobioreactor (BMPBR) and membrane photobioreactor (MPBR). The data are mean ± standard deviation of three independent experiments.

Compared with MPBR, the difference of BMPBR proposed in this study is the addition of flexible fiber bundles that used as solid carriers. As shown in Fig. 2, the suspended algal biomass in BMPBR was similar to that in MPBR during the early 14 days operation, which meant the penetration of light was not dramatically interfered by the flexible fiber bundles fixed in the middle of the reactor, and then the growth of suspended microalgae in the reactor was not significantly influenced. Thus the microalgae attached on the solid carriers could be considered as an increment on the basis of suspended algal biomass.

At day 15, suspended algal biomass in BMPBR reached the maximum value. After that, the algal concentration declined (Fig. 2), and the color of the microalgae culture liquor also could be observed changing from dark green to green gradually. The decline of the suspended algal biomass in BMPBR may be caused by two characteristics of the culture system. First, with the cultivation going on, suspended algal cells in the culture medium more and more quickly transferred to the surface of the solid carriers. Some studies have reported that the growth rate of microalgae on the supporting materials with attached microalgae colonies were faster than that on the fresh supporting materials (Johnson and Wen, 2010; Christenson and Sims, 2012; Gross et al., 2013). Secondary, as shown in Fig. 2, the growth of suspended microalgae in MPBR slowed down in high concentration of suspended algal biomass (from day 13). Similar phenomenon might also happen in BMPBR because of their similar structure. Further research is needed to confirm this hypothesis.

3.2. Algal biomass production and chlorophyll content

At the end of the cultivation, attached algal cells were completely dissolved in water by an ultrasonic cleaner. The color of this microalgae suspension was dark green. Microscopic observations showed that *C. vulgaris* was the only observed alga species in this microalgae suspension. Attached and suspended microalgae in the reactors was harvested to analysis its production and chlorophyll content (mg g^{-1}, dry biomass). As shown in Table 1, the total biomass yield and total biomass productivity in BMPBR were obviously higher than that in MPBR. The total amount of algal biomass productivity in BMPBR was 0.072 g L^{-1} d^{-1}, which was 1.44-fold larger than that in MPBR. This means that the volumetric microalgae production of the reactor was enhanced by about 44% with the addition of the solid carriers. Moreover, it can be calculated from

Table 1
Production and chlorophyll content of algal biomass harvested from BMPBR and MPBR.[a]

	BMPBR	MPBR
Algal biomass yield		
Suspended microalgae (g L^{-1})	0.380 ± 0.060	0.948 ± 0.044
Attached microalgae (g L^{-1})[b]	0.994 ± 0.142	/
Total microalgae (g L^{-1})	1.373 ± 0.198	0.948 ± 0.044
Algal biomass productivity[c]		
Suspended microalgae (g L^{-1} d^{-1})	0.020 ± 0.003	0.050 ± 0.002
Attached microalgae (g L^{-1} d^{-1})	0.052 ± 0.007	/
Total microalgae (g L^{-1} d^{-1})	0.072 ± 0.010	0.050 ± 0.002
Chlorophyll content		
Suspended microalgae (mg g^{-1}, dry biomass)	24.5 ± 2.1	23.6 ± 2.0
Attached microalgae (mg g^{-1}, dry biomass)	28.6 ± 2.9	/

[a] The data are expressed as mean ± SD of three independent experiments.
[b] The attached microalgae yield is calculated by dividing the total attached algal biomass by the effective volume of the reactor.
[c] Algal biomass productivity is calculated by dividing the algal biomass yield by the culture time.

Table 2
Nutrients removal performance and effluent SS concentration of BMPBR and MPBR after stabilization.

	BMPBR	MPBR
NH_4^+-N		
Membrane permeate (mg L^{-1})	0.20 ± 0.08	0.24 ± 0.09
Reduction (%)	96.0 ± 1.6	95.2 ± 1.8
Removal rate (mg L^{-1} d^{-1})	2.40 ± 0.04	2.38 ± 0.05
TIN		
Membrane permeate (mg L^{-1})	2.62 ± 0.63	5.28 ± 0.95
Reduction (%)	82.5 ± 4.0	64.9 ± 6.2
Removal rate (mg L^{-1} d^{-1})	6.19 ± 0.30	4.87 ± 0.47
PO_4^{3-}-P		
Membrane permeate (mg L^{-1})	0.11 ± 0.03	0.12 ± 0.02
Reduction (%)	85.9 ± 2.3	85.2 ± 3.1
Removal rate (mg L^{-1} d^{-1})	0.35 ± 0.02	0.34 ± 0.01
SS		
Membrane permeate (mg L^{-1})	0.28 ± 0.15	0.31 ± 0.15

Note: the data are mean ± standard deviation.
TIN (total inorganic N) = NH_4^+-N + NO_3^--N + NO_2^--N. Permeate concentration of NO_2^--N was below 0.05 mg L^{-1} during the culture interval.
Removal rate = $\frac{(C_{inf}-C_{eff})\times Q}{V}$ where C_{inf} and C_{eff} were the concentration (mg L^{-1}) of NH_4^+-N (or TIN, PO_4^{3-}-P) in the influent and effluent, respectively. Q was the flow rate (100 L d^{-1}). V was the working volume (200 L) of the reactor.

the data in Table 1 that the attached microalgae yield accounted for 72.4% of the total microalgae yield in BMPBR. So it can be concluded that most of the increased algal cells were immobilized as algal biofilm in the reactor. This is advantageous for the harvest of microalgae. Therefore, compared with MPBR, higher microalgae production and concentrated algal biomass was simultaneously achieved in BMPBR by using flexible fiber bundles as biological carriers.

Regarding chlorophyll content, as Table 1 presents, the chlorophyll content of the attached algal cells was higher than that of the suspended algal cells both in MPBR and BMPBR. This result is comparable with past studies about embedded algae system (Robinson et al., 1985; Bailliez et al., 1986; Pane et al., 1998; Lau et al., 1998), they reported that immobilization of algal cells in embedding medium such as calcium alginate and carrageenan gives rise to higher chlorophyll content relative to suspended cultures. The higher chlorophyll content in immobilized algal cells is usually regarded as compensating for the self-shading or gel-screening effect (Lau et al., 1998; Ruiz-Marin et al., 2010). And the retention effect of chlorophyll by the embedding medium may also be the reason for the relatively higher chlorophyll content in immobilized algal cells. Some studies have reported that high levels of chlorophyll can be retained in alginate-beads even during long dark storage of algal cells, while a fast decrease occurs in free controls (Tamponnet et al., 1985; Gaudin et al., 2006). The present study shows that attached culture of microalgae also gives rise to higher chlorophyll content, relative to suspended culture. Similar influence of immobilization on chlorophyll content presented above may also happen in attached culture system.

3.3. Nutrients removal and effluent SS concentration of the reactors

Analysis of water samples taken daily from the membrane permeate shows that nutrients removal efficiency of the reactors initially improved in the first five days and then stabilized in the rest of the cultivation time. The nutrients removal performance of BMPBR and MPBR after stabilization was presented in Table 2. Advanced nutrients removal from secondary effluent could be achieved by microalgae cultivation both in BMPBR and MPBR after stabilization (Table 2). In particular, the average effluent PO_4^{3-}-P concentrations of the reactors were below 0.12 mg L^{-1} after stabilization, with the corresponding reduction of about 85%. As the culture pH in this study was in the range 6.8–7.5, chemical precipitation could be considered as insignificant for the P removal. And then it could be deduced that phosphorus was eliminated mainly by the assimilation of algal cells. The mass N:P ratio in the influent was 18.8 on average, which was obviously higher than the calculated mass N:P ratio (5.0) of the algal cells according to the approximate microalgal molecular formula $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti, 2007). Therefore, phosphorus could be considered as the limited nutrients for the growth of the algal cells in this study, and then was reduced to a lower level of concentration. However, the effluent PO_4^{3-}-P concentration of the reactors was not reduced to an undetectable level. This may be resulted from some other limitation such as light intensity.

In addition as Table 2 shows, compared to MPBR, BMPBR showed better N removal performance with a lower effluent concentration and a higher reduction and removal rate of NH_4^+-N and TIN (NH_4^+-N + NO_3^--N + NO_2^--N). Usually, N in the wastewater can be eliminated mainly through volatilization and biological process. Based on the culture pH of 6.8–7.5 during the experiment, it can be calculated that the ratio of free ammonia to total ammonia in the wastewater was below 2.2% by the expression of Anthonisen et al. (1976). Therefore, it can be deduced that levels of volatilization were very low in the reactors, and then the N elimination in the culture interval was due mainly to biomass growth. Meanwhile, the ratios of eliminated N to increased algal biomass also could illustrate the main way of N removal in the reactors, which were 8.58% in BMPBR and 9.82% in MPBR, respectively, and were both close to the theoretical N content (6.59%) according to the approximate microalgal molecular formula $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti, 2007).

The average N removal rate of BMPBR in the stable operation interval was 6.19 mg L^{-1} d^{-1}, which was 1.27-fold larger than that of MPBR. As the algal biomass productivity in BMPBR was 1.44-fold larger than that in MPBR (calculated from the data in Table 1), it can be deduced that the better performance of N removal in BMPBR was mainly due to its higher algal biomass productivity. Therefore, it can be concluded that using solid carriers in photobioreactor is conducive not only to the production and harvest of microalgae, but also to the nutrients removal from the wastewater.

SS concentration in the effluent of the reactors was also investigated during the culture interval. As shown in Table 1, complete isolation of suspended algal cells from the effluent could be achieved by the filtration of the membrane module both in BMPBR and MPBR. The effluent SS concentration of the reactors was below

0.5 mg L^{-1}. Thereby, membrane module submerged in the reactor not only prevented the washing out of suspended algal cells, but also improved the effluent quality in terms of SS.

4. Conclusions

A novel algal biofilm membrane photobioreactor was developed in this work for attached algal biomass production and nutrients removal from secondary effluent. When compared to suspended growth membrane photobioreactor, the BMPBR achieved higher volumetric microalgae production and nutrients removal rate due to the developed algal biofilm on the surface of solid carriers submerged in the reactor. Moreover, compared with traditional attached algal culture system, BMPBR has the advantage of completely isolation of the algal cells from the effluent. Thus attached algal biomass production and high quality effluent in terms of nutrients and SS were simultaneously achieved in BMPBR.

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