Improvement of microalgae lipid productivity and quality in an ion-exchange-membrane photobioreactor using real municipal wastewater

Chang Haixing[1,2], Fu Qian[1,2*], Huang Yun[1,2], Xia Ao[1,2], Liao Qiang[1,2], Zhu Xun[1,2]

(1. *Key Laboratory of Low-grade Energy Utilization Technologies and Systems, Chongqing University, Ministry of Education, Chongqing 400030, China*; 2. *Institute of Engineering Thermophysics, Chongqing University, Chongqing 400030, China*)

Abstract: To improve the productivity and quality of microalgae-based biodiesel when using municipal wastewater (MW) as nutrients source, an ion-exchange-membrane photobioreactor (IEM-PBR) was used in this study to eliminate the negative effects of pollutants in MW on microalgae *Chlorella vulgaris* and *Scenedesmus obliquus*. In the IEM-PBR, the real MW and microalgae cultures were separated in two chambers by the ion-exchange-membranes (IEMs). Nutrients (N, P, etc.) in the MW permeated into microalgae cultures through the IEMs, while pollutants (suspended solids, competitors, etc.) in the MW could hardly permeate into microalgae cultures. As a result, the lipid productivity in the IEM-PBR was improved to 85.7 mg/(L d) for *C. vulgaris* and 111.8 mg/(L d) for *S. obliquus*, which was slightly higher than that in the traditional photobioreactor (T-PBR) with real MW after centrifugation (82.5 mg/(L d) for *C. vulgaris* and 105.8 mg/(L d) for *S. obliquus*), but much higher than that in the T-PBR with untreated MW and primary MW (with lipid productivity of 20-30 mg/(L d)). Besides, the lipid quality obtained in the IEM-PBR had higher proportion of cetane number (ca. 60%) and lower linolenic acid content (ca. 8%), which showed a superior quality in the IEM-PBR to that in the T-PBR. It demonstrated that the IEM-PBR is an effective approach to improve the productivity and quality of microalgae biodiesel.

Keywords: microalgae, photobioreactor, lipid productivity, real municipal wastewater, ion-exchange-membrane

DOI: 10.3965/j.ijabe.20171001.2706

Citation: Chang H X, Fu Q, Huang Y, Xia A, Liao Q, Zhu X. Improvement of microalgae lipid productivity and quality in an ion-exchange-membrane photobioreactor using real municipal wastewater. Int J Agric & Biol Eng, 2017; 10(1): 97–106.

1 Introduction

Microalgae are sunlight-driven cell factories that can sequestrate CO_2 and then be used to produce biodiesel through photosynthesis, which simultaneously relieves environmental and energy pressure[1]. Microalgae can be cultivated in non-agricultural land with a photosynthetic efficiency ten or more times higher than the conventional oil crops, such as sunflower, soybean and palm[2]. Therefore, biodiesel from microalgae has intrinsic advantages over the oil crops and has attracted particular interests. However, the large-scale production of microalgae-based biodiesel is not economically viable currently due to the high costs on microalgae cultivation. In particular, the vast utilization of commercial fertilizers (nitrogen, phosphorus, etc.), which constitute a big part of the cost in microalgae cultivation, severely hinders the commercialization of microalgae-based biofuels production[3]. On the contrary, the cost on microalgae cultivation can be reduced when microalgae is cultivated

Received date: 2016-09-16 **Accepted date:** 2016-12-18

Biographies: Chang Haixing, PhD candidate, research interests: wastewater treatment and biofuel production, Email: changhx@ cqu.edu.cn; **Huang Yun,** PhD, research interests: wastewater treatment and biofuel production, Email: yunhuang@cqu.edu.cn; **Xia Ao,** Professor, research interests: biofuel production, Email: aoxia@cqu.edu.cn; **Liao Qiang,** Professor, research interests: microbial energy conversion technology, Email:lqzx@ cqu.edu.cn; **Zhu Xun,** Professor, research interests: microbial energy conversion technology, Email: zhuxun@cqu.edu.cn.

***Corresponding author: Fu Qian**, Professor, research interests: microbial energy conversion technology. Mailing address: Power Engineering College, Chongqing University, Chongqing, 400044. Tel/Fax: 0086-23-65102474, Email: fuqian@cqu.edu.cn.

with wastewater containing high concentrations of nutrients. It is estimated that it can generate ~23.5 billion tons of biodiesel per year, if 70% of the discharged municipal wastewater (MW) is used as culture media for microalgae cultivation[4]. At the same time, microalgae cultivation with MW can also realize the treatment of wastewater, relieving the eutrophication of water body[5,19].

In the past decades, many researches had convinced the feasibility of microalgae and microalgae-based biodiesel production with MW[4]. Unfortunately, the biomass and lipid productivity was relatively low attributing to the high concentrations of ammonium, suspended solids, competitors and other contaminants in MW[6,7]. Jiang et al.[6] successfully cultivated the microalgae *Nannochloropsis* sp. in MW under 15% CO_2 aeration, but the maximum biomass concentration and the maximum lipid productivity were only 0.21 g/L and 9.64 mg/(L·d), respectively, which were mainly limited by the high ammonium concentration in the MW. To reduce the negative effect of high ammonium concentration in MW on microalgae growth, anammonium-tolerant microalgae strain, namely *Desmodesmus sp.* was selected, which had substantial immunity on ammonium, resulting in a maximum biomass concentration of 1.09 g/L in the simulated MW containing ca. 210 mg/L of NH_4^+-N[7,8]. However, when the selected microalgae strain *Desmodesmus* sp.was cultivated in the real MW, the maximum biomass concentration was only 0.41 g/L, which was much lower than that in the synthetic MW[7]. It was mainly attributed to the complex compositions (competitors, suspended solids, etc.) in the real MW relative to the synthetic MW. To enhance the microalgae biomass production, Ebrahimian et al.[9] mixed the primary and secondary MW to relieve the detrimental effects caused by primary MW (suspended solids, high ammonium concentration, etc.) on microalgae growth. The maximum biomass concentration of *C. vulgaris* was improved from 1.0 g/L (in primary MW) to 1.16 g/L (in mixed MW containing 25% of primary MW). The study provides us a valuable approach to cultivate microalgae with MW, but the improvement of microalgae biomass was not significant since the detrimental effects of MW were not greatly removed. Therefore, an efficient approach for microalgae cultivation with MW is necessary.

To effectively exploit nutrients in MW for microalgae cultivation, a novel annular ion-exchange-membrane photobioreactor (IEM-PBR) was proposed in our previous work[10]. In that work, three different characteristics of wastewater (excess nutrients, high turbidity and excess heavy metals) were simulated to evaluate the performance of the IEM-PBR. As a result, the biomass production and nutrients removal efficiencies were greatly improved in the IEM-PBR than that in the traditional PBR. However, the previous works were mainly focused on the synthetic wastewater and real wastewater was not investigated. As we know, the compositions of real MW are distinctly different from the synthetic MW. The results obtained from the synthetic MW may not be well adapted to the microalgae cultivated in real MW. In addition, only microalgae growth and nutrients removal were investigated in the previous work. The lipid productivity and quality were not discussed, which are important parameters for large-scale production of microalgae-based biodiesel in the IEM-PBR. A comprehensive understanding on the lipid productivity and quality in the IEM-PBR with real MW as nutrients source can help us to maximize the economic efficiency of microalgae-based biofuels production with wastewater.

Therefore, to explore the optimal strategy for microalgae cultivation with wastewater, real MW collected from different stages of municipal wastewater treatment plant was used as nutrients source in the IEM-PBR to produce biodiesel from microalgae *C. vulgaris* and *S. obliquus.* The microalgae lipid productivity and quality in the IEM-PBR were compared with that cultivated in the traditional PBR (i.e., microalgae cells directly cultivated in the wastewater). The IEM-PBR provided us an effective approach to improve the productivity and quality of microalgae biodiesel when cultivated with real MW.

2 Materials and methods

2.1 Microalgae strain

Microalgae strains, *Chlorella vulgaris* FACHB-31

and *Scenedesmus obliquus* FACHB-417, were purchased from the Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China. The experimental temperature was (25±0.5)℃. Continuous illumination of 90 μmol/(m^2 s) was provided to the microalgae cultures. Mixed gas containing 5% CO_2 in air was bubbled into the microalgae cultures at aeration rate of 0.3 vvm. The flow rates of CO_2 and air were controlled by mass flow meters.

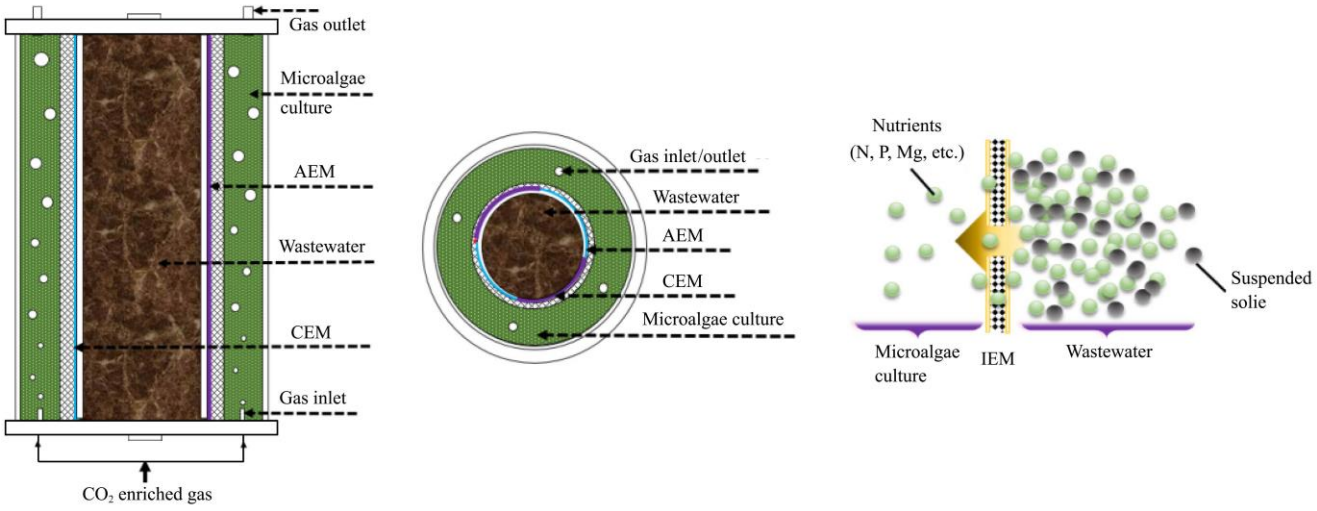
2.2 Experimental design

In the study, untreated MW, primary MW and the MW after centrifugation were used as nutrients sources for microalgae cultivation in both experimental and control groups. Untreated MW was collected from the injection point of the setting tank in Baihan wastewater treatment plant (Chongqing, China). Primary MW was collected from the injection point of the anaerobic digestion tank in the Baihan wastewater treatment plant. The MW after centrifugation obtained by centrifuging the untreated MW (Xiangyi Centrifuge Instrument Co. Ltd. GL-21M, China) at 4000 r/min for 5 min to remove the suspended solids. Key characteristics of untreated MW, primary MW and the MW after centrifugation are shown in Table 1. It can be seen that there was little difference on pH, COD, NH_4^+-N and PO_4^{3-}-P concentration between these three types of MW. However, the suspended solids concentration greatly decreased from 3200± 103 mg/L in the untreated MW to 609±45 mg/L in the primary MW and 0 in the MW after centrifugation. It resulted in the decrease of turbidity from 662.5±7.5 NTU for the untreated MW to 96.7±6.3 NTU for the primary MW and to 7.4±1.5 for the MW after centrifugation.

Table 1 Characteristics of the untreated MW, primary MW and the MW after centrifugation (mean ± SD)

Parameter	Untreated MW	Primary MW	MW after centrifugation
pH	6.8±0.4	6.7 ±0.3	7.0 ±0.2
COD/mg Ł$^{-1}$	310 ±11	270 ±22	260 ±18
-N/mg Ł$^{-1}$	40.5 ±1.1	40.3 ±0.8	39.7 ±1.2
-P/mg Ł$^{-1}$	9.3 ±0.6	9.2 ±0.3	9.2 ±0.4
Suspended solids /mg Ł$^{-1}$	3200 ±103	609 ±45	0
Turbidity /NTU	662.5±7.5	96.7 ±6.3	7.4 ±1.5

For the experimental groups, microalgae was cultivated in the IEM-PBR, which is shown in Figure 1[10]. Anion exchange membrane (AEM, AMFOR INC, USA) and cation exchange membrane (CEM, AMFOR INC, USA) were adhered on the wall of the inner cylinder. Microalgae inoculated in deionized waster and cultivated in the microalgae cultivating chamber, i.e., the space between the inner cylinder and the outer cylinder. The wastewater stream was injected in the wastewater chamber, i.e., the space inside the inner cylinder. Nutrients (NH_4^+, PO_4^{3-}, etc.) contained in the MW continuously permeated from the MW to microalgae culture attributing to the permselectivity of the IEMs, while the suspended solids and competitors in the MW could hardly permeate into the microalgae culture. In this way, the detrimental effects of contaminants in MW on microalgae growth could be effectively eliminated in the IEM-PBR.



a. Front view of the IEM-PBR b. Top view of the IEM-PBR c. Working principle of the IEMs

Figure 1 Front view (a) and top view (b) of the ion-exchange-membrane photobioreactor, and the working principle of the ion-exchange-membranes (c)[10]

For the control groups (i.e., microalgae directly cultivated in the MW), the experiments were conducted in the traditional PBR (T-PBR). The configuration of the T-PBR was same as the IEM-PBR, while microalgae were directly inoculated in the MW and cultivated in the cultivating chamber. No MW or culture medium was injected in the wastewater chamber of the T-PBR, so that no nutrients exchange occurred between the wastewater chamber and the microalgae cultivating chamber for the control groups.

2.3 Analysis

2.3.1 Determination of biomass concentration

Biomass concentration (g/L) of microalgae was determined using gravimetric method. Microalgae biomass in 10 mL broth was harvested by centrifugation at 8000 r/min for 8 min and washed twice with deionized water. Then the biomass was dried at 85 ℃ in an oven till constant weight.

2.3.2 Determination of wastewater compositions and light attenuation

Nutrients concentration (mg/L) in wastewater and culture medium was detected with ion chromatograph (ICS-5000, ThermoFisher, USA). Cation analytical column (4×250 mm, CS12A, ThermoFisher, USA) and cation self-regenerating suppressor (4 mm, CSRS 300, ThermoFisher, USA) were used to detect cation (ammonium, etc.) concentration. Anion analytical column (4×250 mm, AS11-HC, ThermoFisher, USA) and anion self-regenerating suppressor (4 mm, ASRS 300, ThermoFisher, USA) were used to detect anions (phosphate, etc.) concentration.

COD concentration (mg/L) in culture medium and MW was detected with a COD detector (5B-3C, Lianhua Tech. Co., Ltd) after filtration of microalgae cells and suspended solids with a filter.

Turbidity (NTU) of the MW and culture medium was detected with scatter light turbidimeter (WGZ-B, Shanghai Xinrui Instrument & Meters Co., Ltd). Standard formazin solution (100 NTU) and deionized water (0) were used to calibrate the instrument.

Suspended solids concentration (mg/L) was detected using gravimetric method. Briefly, suspended solids in 100 mL MW was collected by centrifuging and dried at 85°C in an oven till constant weight. To ensure the accuracy, each result was the mean value of five replications.

Light attenuation curve was determined by measuring the local light intensity (μmol/(m^2 s)) in microalgae suspension with a waterproof probe of irradiatometer (T-10WsA, Konica Minolta Inc, Japan). The starting point was chosen as the front point of the PBRs facing the incident light.

2.3.3 Determination of microalgae biomass compositions

Total lipids were gravimetrically quantified using the method proposed by Bligh and Dyer[11] with some modifications. Briefly, 100 mg of dry algae powder was mixed with 7.6 mL of chloroform/methanol/water (1:2:0.8, v/v/v), ultrasonicated for 30 min at 100 W with ultrasonic cell crusher (JY92-2D, Xinzhi Instrument Ltd., NingBo, China), and then vibrated for 1 h at ambient temperature of 35 ℃ in water bath. Then the mixer was centrifuged at 6500 r/min for 5 min to collect the supernatant and the residual biomass was extracted twice more. Chloroform (6 mL) and water (6 mL) were added to the combined supernatant to ensure a final volume ratio of 1:1:0.9 (chloroform/methanol/water). The chloroform phase was carefully transferred to a new tube and evaporated over 24 h in a drying oven at 60 ℃. Finally, total lipids were gravimetrically measured. The lipid composition was analyzed as fatty acid methyl esters (FAMEs) by acidic transesterification of the lipids extracted previously[12]. The FAMEs were dissolved in hexane and nonadecanoic acid (C19:0) was added as an internal standard. After then, the mixture was incubated at 85 ℃ for 2.5 h and 1 μL of the mixture after incubation was injected into gas chromatograph (GC-2010, SHIMADZU, Japan) for determination of individual FAMEs.

3 Results and discussion

3.1 Microalgae growth in IEM-PBR and T-PBR

The turbidity of MW had significant influence on light attenuation in the cultures, and the variations of light penetration in deionized MW, untreated MW, primary MW and the MW after centrifugation are shown in Figure 2. It can be observed that the light intensity at distance

of 1 cm (i.e. the middle point of the culture) from light incident surface in the untreated MW and the primary MW had dropped to almost 0 attributing to the light shading effect caused by suspended solids. After removal of the suspended solids in the MW by centrifugation, the light penetration in the MW after centrifugation was greatly improved, with light intensity of 41.4 μmol/(m^2 s) at the distance of 2 cm (i.e. the innermost point of culture) from the light incident surface. However, the process of centrifugation consumed a large quantity of energy, reducing the economy efficiency of microalgae cultivation with MW. In contrast, the energy cost on suspended solids removal by centrifuging can be avoided by using the IEM-PBR. In the IEM-PBR, the suspended solids in MW were totally isolated in the wastewater chamber and only the nutrients (N, P, etc.) could permeate into microalgae cultivating chamber through IEMs. As seen in Figure 2, the light intensity in microalgae culture medium of the IEM-PBR (i.e., the deionized water) at distance of 2 cm from the light incident surface was 60.1 μmol/(m^2 s), which was higher than that in the MW after centrifugation. It demonstrated that the light shading effect caused by suspended solids can be substantial eliminated in the IEM-PBR.

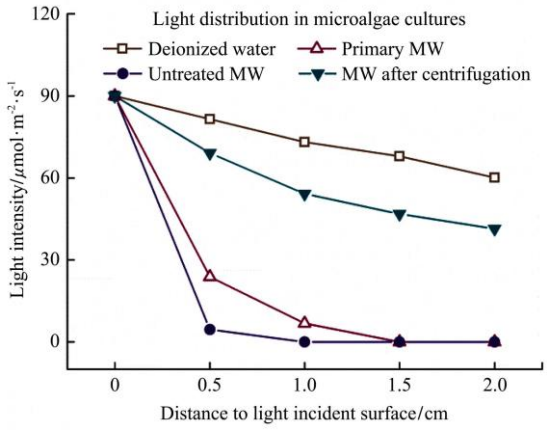
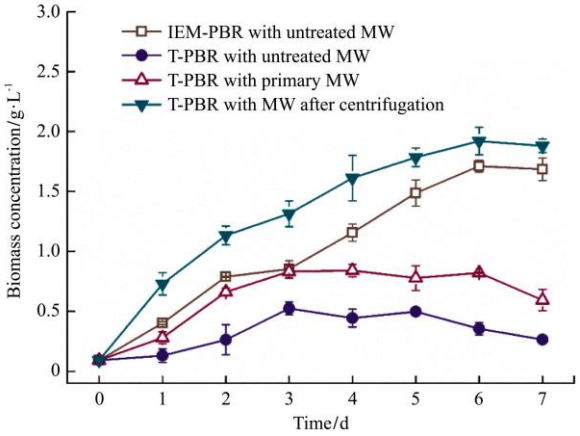
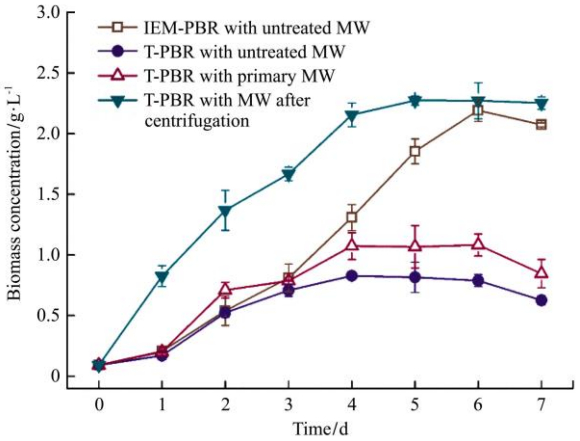


Figure 2 Variation of light penetration in deionized water, untreated MW, primary MW and the MW after centrifugation

Light intensity is the driving force for microalgae growth and metabolism. Insufficient light can severely limit the growth of microalgae[1]. Variations of microalgae *C. vulgaris* and *S. obliquus* biomass concentration in the IEM-PBR and the T-PBR are shown in Figure 3. Compared with the cases when cultivated in the T-PBR with untreated MW and primary MW, the maximum biomass concentration were greatly improved to 1.71 g/L for *C. vulgaris* and to 2.19 g/L for *S. obliquus* when cultivated in the IEM-PBR with untreated MW, which were similar with the values when cultivated in the T-PBR with MW after centrifugation (with the maximum biomass concentration of 1.92 g/L for *C. vulgaris* and to 2.27 g/L for *S. obliquus* (Figure 3)).



a. *Chlorella vulgaris*



b. *Scenedesmusobliquus*

Figure 3 Variations of microalgae *Chlorella vulgaris* (a) and *Scenedesmus obliquus* (b) biomass concentration in the IEM-PBR with untreated MW and in the T-PBR with untreated MW, primary MW and the MW after centrifugation

When microalgae were cultivated in the T-PBR with untreated MW and primary MW, most of incident light was shaded by the suspended solids (Figure 2). The microalgae biomass increased rapidly at initial four days attributing to the existence of COD. The maximum biomass concentrations of 0.52 g/L for *C. vulgaris* and 0.83 g/L for *S. obliquus* were obtained when cultivated with untreated MW, and 0.84 g/L for *C. vulgaris* and 1.07 g/L for *S. obliquus* were obtained when cultivated with primary MW (Figure 3). After the initial four days,

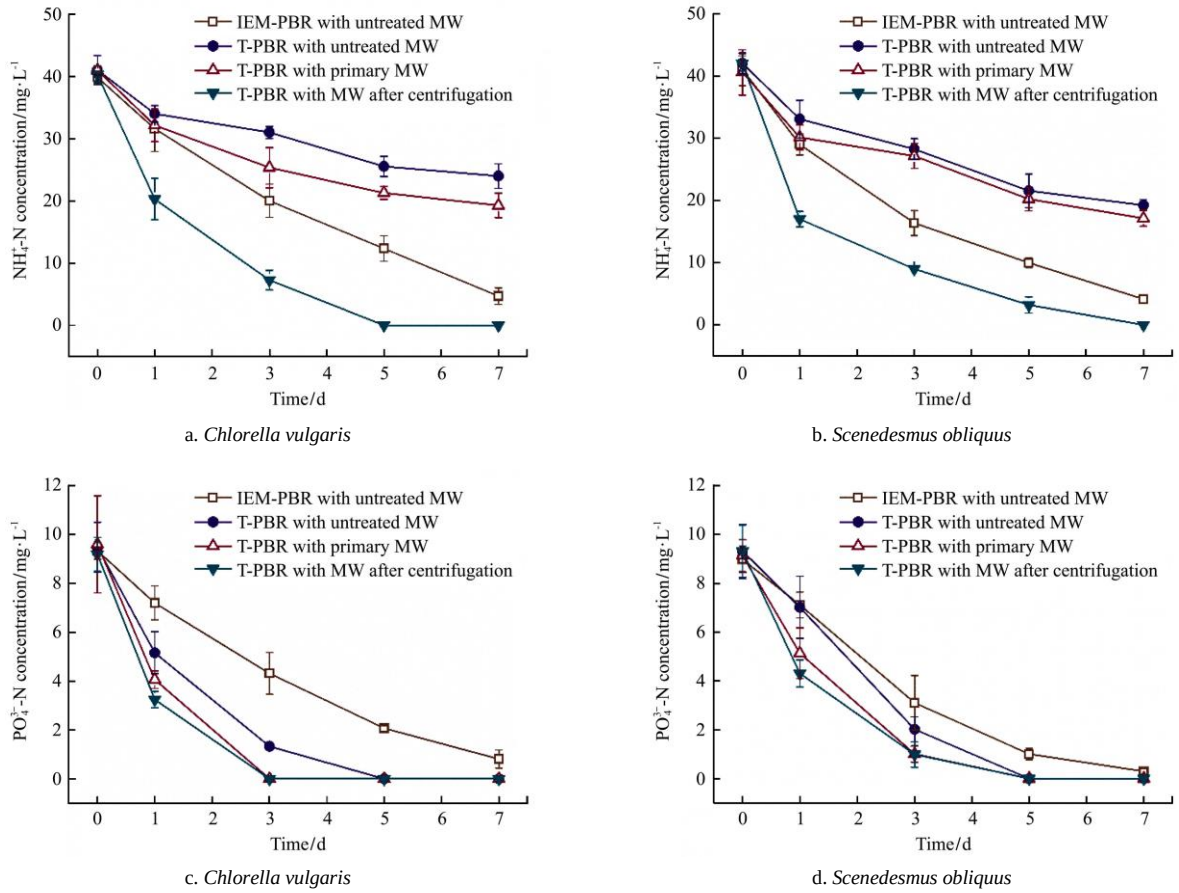
the COD in the MW was used up (which was detected as zero) and the light could hardly penetrate into microalgae culture (Figure 2). The lack of energy in the T-PBR with untreated MW and with primary MW led to little increase of microalgae biomass after the fourth day. In contrast, when microalgae were cultivated in the IEM-PBR, much more light could penetrate into microalgae culture for microalgae growth relative to the T-PBRs with untreated MW and primary MW. In addition, nutrients (N, P, etc.) continuously permeated from wastewater chamber to microalgae cultivating chamber. As a result, the maximum biomass concentration in the IEM-PBR increased to 1.71 g/L for *C. vulgaris* and to 2.07 g/L for *S. obliquus*. However, the biomass concentration obtained in the IEM-PBR was lower than that in the T-PBR with MW after centrifugation, especially for the initial five days. The possible reason was that the microalgae cultivated in the T-PBR with MW after centrifugation grew under mixotrophic mode, while the microalgae cultivated in the IEM-PBR could only grew under photoautotrophic mode since COD could hardly permeate through the IEM. The diversity energy source for microalgae in the T-PBR with MW after centrifugation contributed to higher growth rates over that in the IEM-PBR. As seen in Figure 2, there was 41.4 μmol/(m^2 s) of light at distance of 2 cm from light incident surface in the T-PBR with MW after centrifugation, which was similar with that in the deionized water (i.e., the microalgae culture medium in the IEM-PBR). Besides, when microalgae were directly cultivated in the MW after centrifugation in the T-PBR, the existence of organic matters (Table 1) in the T-PBR provided organic carbon for microalgae. However, the microalgae cultivated in the IEM-PBR could only grow in photoautotrophic mode because the microalgae and the MW were separated in two chambers (i.e., the microalgae was in the microalgae cultivating chamber and the MW was in the wastewater chamber of the IEM-PBR). The organic matters in the MW of the IEM-PBR could hardly permeate into the culture medium attributing to the permselectivity of the IEMs. As a result, the maximum biomass concentration in the T-PBR with MW after centrifugation was higher than that in the IEM-PBR, with values of 1.92 g/L for *C. vulgaris* and 2.27 g/L for *S. obliquus*. However, the improvements were not significant (11.6% for *C. vulgaris* and 8.7% for *S. obliquus*), which were far not enough to compensate the energy cost on MW centrifugation.

3.2 Nutrients removal in the IEM-PBR and the T-PBR

The variations of NH_4^+-N and PO_4^{3-}-P concentration in the T-PBR and the IEM-PBR are shown in Figure 4. The removal efficiencies of NH_4^+-N in the IEM-PBR (85.7% for *C. vulgaris* and 89.9% for *S. obliquus*) were much higher than that in the untreated MW (41.5% for *C. vulgaris* and 54.3% for *S. obliquus*) and the primary MW (52.9% for *C. vulgaris* and 57.8% for *S. obliquus*), but slightly lower than that in the T-PBR with MW after centrifugation (with NH_4^+-N removal efficiency of 100%) (Figures 4a and 4b). Compared with the removal efficiency of NH_4^+-N, there was little difference on P removal efficiencies between the T-PBR and the IEM-PBR (Figures 4c and 4d), with P removal efficiencies of ca. 100% for all cases.

For the microalgae cultivated in the T-PBR with untreated MW and primary MW, the relatively low removal efficiency of NH_4^+-N (around 50%) was mainly because of the poor microalgae growth (Figure 3), which reduced the requirements of N for synthesis of intracellular nitrogenous macromolecules[13]. When microalgae were cultivated in the IEM-PBR, the microalgae biomass concentration was greatly improved (Figure 3) since the light shading effect caused by suspended solids was totally eliminated, enhancing the assimilation of N from the culture medium. As a result, the NH_4^+-N removal efficiency was improved to about 90%. However, the removal efficiency of NH_4^+-N in the IEM-PBR (ca. 90%) was slightly lower than that in the in the T-PBR with MW after centrifugation (100%). It was because that the NH_4^+-N concentration difference between microalgae culture medium and the MW was the driving force of NH_4^+-N permeation from the MW (contained in the wastewater chamber) to microalgae culture medium (contained in the microalgae cultivating chamber) in the IEM-PBR. The decrease of NH_4^+-N concentration in wastewater along with time resulted in

the decrease of driving force for NH_4^+-N permeation, which resulted in the decrease of NH_4^+-N permeation rate from the MW to microalgae culture medium. In contrast, when microalgae was cultivated in the T-PBR with MW after centrifugation, the NH_4^+-N could be totally removed since the microalgae was directly inoculated in the MW after centrifugation. However, though the removal efficiency of NH_4^+-N in the IEM-PBR was lower than 100%, subsequent processing of the residual NH_4^+-N in the MW of the IEM-PBR was not necessary since the residual amount was low (with 4.70 mg NH_4^+-N/L for *C. vulgaris* and 4.13 mg NH_4^+-N/L for *S. obliquus*), which could meet the discharge standard for NH_4^+-N (with the discharge standard of 5 mg NH_4^+-N/L)[14].



a. *Chlorella vulgaris* b. *Scenedesmus obliquus*

c. *Chlorella vulgaris* d. *Scenedesmus obliquus*

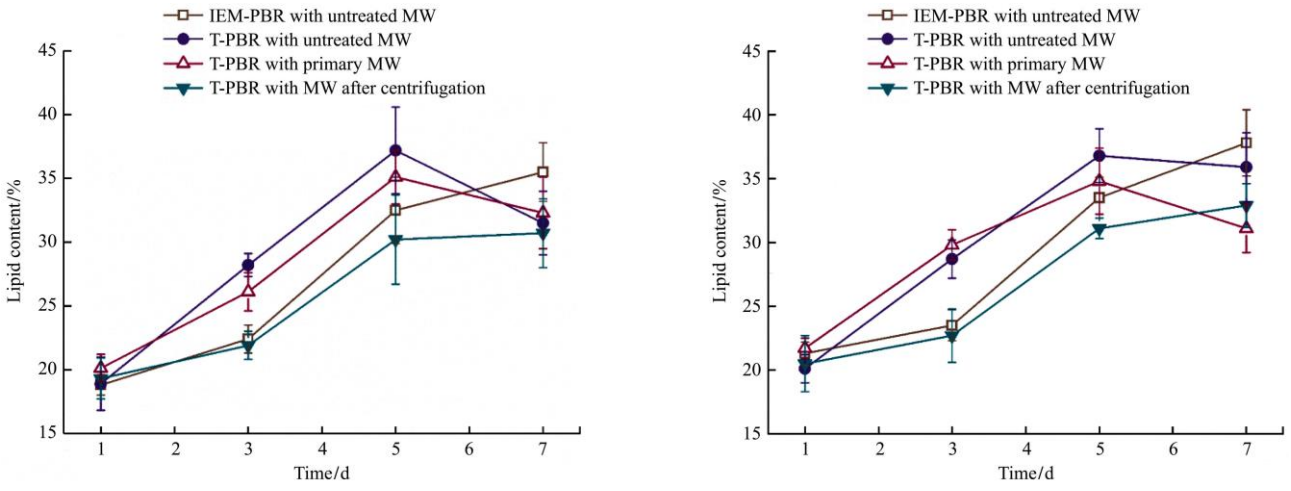
Figure 4 Variations of NH_4^+-N concentration (a and b) and PO_4^{3-}-P concentration (c and d) in the IEM-PBR with untreated MW and in the T-PBR with untreated MW, primary MW and the MW after centrifugation

3.3 Lipid productivity and quality in the IEM-PBR and the T-PBR

Variations of lipid content in microalgae biomass obtained in the IEM-PBR and the T-PBR are shown in Figure 5. In general, the lipid content of microalgae cultivated in the IEM-PBR and the T-PBR with MW after centrifugation monotonously increased with time, while the lipid content of microalgae cultivated in the T-PBR with untreated MW and the primary MW first increased and then decreased with time (Figure 5). Compared to the microalgae cultivated in the T-PBR, the microalgae cultivated in the IEM-PBR had the highest lipid productivities of 85.7 mg/(L d) for *C. vulgaris* and 111.8 mg/(L d) for *S. obliquus*, which were significantly higher than that in the T-PBR with untreated MW and primary MW (Table 2).

When cultivated in the T-PBR with untreated MW and primary MW, the lipid content of microalgae first increased to the peak values (ca. 35%) and then decreased with time after the fifth day (Figure 5). In the initial five days, the lipid content of microalgae increased with time because that the poor cultivation condition (i.e., insufficient light and excess contaminants in the T-PBR) enhanced the accumulation of intracellular reactive oxygen species. It triggered the degradation of intracellular macromolecules and enhanced the synthesis

of lipid, leading to the increase of lipid content in the cells[15,16]. After the fifth day, the available intracellular macromolecules were completely decomposed and the lipid, which existed as energy-storage substance in the cells, was utilized by cells to supply energy for microalgae survival[16]. Such a biochemical response of microalgae led to the decrease of lipid content in the cells. As a result, the poor lipid productivities were obtained as 11.7 mg/(L d) for *C. vulgaris* and 32.3 mg/(L d) for *S. obliquus* when cultivated in the T-PBR with untreated MW, 27.2 mg/(L d) for *C. vulgaris* and 37.8 mg/(L d) for *S. obliquus* when cultivated in the T-PBR with primary MW (Table 2). When cultivated in the IEM-PBR and in the T-PBR with MW after centrifugation, the lipid content of microalgae biomass monotonously increased with time. It was worth mentioning that the increase rate of lipid content was small at initial three days but much larger at the latter days. It was probably because that the contaminants (suspended solids, competitors, etc.) in the microalgae culture of the IEM-PBR and in the T-PBR with MW after centrifugation were mostly removed. The growth environment, especially the light condition, was much better than that in the T-PBR with untreated MW and primary MW in the initial three days. Such a condition contributed more on microalgae growth than lipid synthesis, which led to a relatively small increase of lipid content, with the increase of lipid content from ca. 19% to 22% (Figure 5). After the third day, the nutrients (N, P, etc.) in microalgae cultures gradually became insufficient to microalgae, which constructed nutrients-starvation conditions for microalgae. The nutrients-starving condition activated the diacylglycerol acyl transferase and increased the intracellular content of fatty acid acyl-CoA, which could enhance the conversion of acid acyl-CoA to triglyceride hydrolysis, resulting in a significantly increase of lipid content in microalgae cells after the third day (Figure 5)[16]. As a result, the lipid productivities in the IEM-PBR were improved to 85.7 mg/(L d) for *C. vulgaris* and 111.8 mg/(L d) for *S. obliquus*, which were slightly higher than that in the T-PBR with MW after centrifugation (with values of 82.5 mg/(L d) for *C. vulgaris* and 105.8 mg/(L d) for *S. obliquus*) (Table 2).



a. *Chlorella vulgaris* b. *Scenedesmus obliquus*

Figure 5 Variations of lipid content in microalgae biomass (a) *Chlorella vulgaris*, (b) *Scenedesmus obliquus*

Table 2 Comparisons of the maximum biomass concentration, lipid content and lipid productivity between the IEM-PBR with untreated MW and the T-PBR with untreated MW, primary MW and the MW after centrifugation

	Chlorella vulgaris			*Scenedesmusobliquus*		
	Maximum biomass concentration/g Ł$^{-1}$	Lipid content /%	Lipid productivity /mg (L d)$^{-1}$	Maximum biomass concentration/g Ł$^{-1}$	Lipid content /%	Lipid productivity /mg (L d)$^{-1}$
IEM-PBR with untreated MW	1.69	35.5	85.7	2.07	37.8	111.8
T-PBR with untreated MW	0.26	31.5	11.7	0.63	35.9	32.3
T-PBR with primary MW	0.59	32.3	27.2	0.85	31.1	37.8
T-PBR with MW after centrifugation	1.88	30.7	82.5	2.25	32.9	105.8

Besides the lipid productivities, the FAMEs composition was analyzed since it ultimately affected the biodiesel quality of microalgae. The FAMEs composition is shown in Table 3. Overall, the proportions of total saturated, total mono-unsaturated and total poly-unsaturated fatty acid in the lipid were similar between the IEM-PBR and the T-PBR, with around 33% for each section. However, the quality of the lipid in the IEM-PBR was much better than that in the T-PBR. Cetane number is the most important property of the biodiesel, which is widely used as diesel-fuel quality parameter related to the combustion quality and ignition delay time. The biodiesel having a high cetane number usually has a good ignition property[17], and the cetane number should be at a minimum of 51 according to European standard EN 14214. The cetane number in the IEM-PBR and the T-PBR could all meet the standard, whereas cetane number in the IEM-PBR (60.05% for *C. vulgaris* and 58.77% for *S. obliquus*) were much higher than that in the T-PBR with MW after centrifugation (52.31% for *C. vulgaris* and 51.06% for *S. obliquus*) (Table 3). It demonstrated that the biodiesel obtained in the IEM-PBR had better ignition and combustion properties than that in the T-PBR. In addition, the linolenic acid (C18:3) content should be lower than 12% since it can cause the degradation of the lipid and then reduce the biodiesel quality[18]. It can be seen from Table 3 that the linolenic acid content of lipid obtained from the IEM-PBR could well meet the standard, with 7.35% for *C. vulgaris* and 8.84% for *S. obliquus*. However, the linolenic content in the lipid obtained from the T-PBR was higher than the standard (12%), with 15.62% for *C. vulgaris* and 13.91% for *S. obliquus*. Therefore, it demonstrated that the biodiesel obtained in the IEM-PBR was superior to that in the T-PBR.

Table 3 FAMEs compositions (w/w total fatty acid) of *Chlorella vulgaris* and *Scenedesmus obliquus* when cultivated in the IEM-PBR with untreated MW and in the T-PBR with untreated MW, primary MW and MW after centrifugation

FAME compositions	FAME content/%			
	Chlorella vulgaris		*Scenedesmus obliquus*	
	IEM-PBR with untreated MW	T-PBR with MW after centrifugation	IEM-PBR with untreated MW	T-PBR with MW after centrifugation
C16:0	32.13	29.34	35.20	28.66
C16:1	13.34	6.85	6.28	11.32
C16:2	11.42	8.77	13.48	5.67
C16:3	3.61	7.35	3.81	5.41
C18:0	2.82	3.63	2.95	3.65
C18:1	23.35	21.19	24.27	19.74
C18:2	5.98	7.25	5.17	11.64
C18:3	7.35	15.62	8.84	13.91
Cetane number	60.05	52.31	58.77	51.06
Total saturated	34.95	32.97	38.15	32.31
Total mono-unsaturated	36.69	28.04	30.55	31.06
Total poly-unsaturated	28.36	38.99	31.30	36.63

4 Conclusions

The IEM-PBR was adopted in this study to improve productivity and quality of biodiesel from microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* when using real MW as nutrients source. Compared with the lipid productivity of ca. 30 mg/(L·d) in the T-PBR with untreated MW and primary MW, the lipid productivity in the IEM-PBR was significantly improved to 85.7 mg/(L·d) for *C. vulgaris* and 111.8 mg/(L·d) for *S. obliquus*, which was slightly higher than that in the T-PBR with MW after centrifugation. Besides, the cetane number of the lipid in the IEM-PBR (60%) was much higher than that in the T-PBR (52%), and the linolenic acid content of lipid in the IEM-PBR (8%) was lower than that in the T-PBR (15%). It demonstrated that the lipid quality obtained in the IEM-PBR was better than the lipid obtained in the T-PBR with MW after centrifugation. In addition, the energy cost on MW centrifugation was avoided in the IEM-PBR. Therefore, the IEM-PBR provided us an

effective approach to improve the productivity and quality of microalgae biodiesel when cultivated with real wastewater.

Acknowledgment

The authors are grateful for the financial support provided by the State Key Program of National Natural Science of China (No. 51136007); the International Cooperation and Exchange of the National Natural Science Foundation of China (No. 51561145013); the National Science Foundation for Young Scientists of China (No. 51606020); the National Key Research and Development Program-China (2016YFB0601002); the Postdoctoral Scientific Research Project of Chongqing, China (Xm2015070); and the National Natural Science Funds for Young Scholar (51506017).

[References]

[1] Chang H X, Huang Y, Fu Q, Liao Q, Zhu X. Kinetic characteristics and modeling of microalgae *Chlorella vulgaris* growth and CO_2 biofixation considering the coupled effects of light intensity and dissolved inorganic carbon. Bioresoutrce Technology, 2016; 206: 231–238.

[2] Chisti Y. Biodiesel from microalgae. Biotechnology Advances, 2007; 25(3): 294–306.

[3] Sialve B, Bernet N, Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnology Advances, 2009; 27(4): 409–416.

[4] Abinandan S, Shanthakumar S. Challenges and opportunities in application of microalgae (*Chlorophyta*) for wastewater treatment: A review. Renewable Sustainable Energy Reviews, 2015; 52: 123–132.

[5] Pancha I, Chokshi K, Maurya R, Trivedi K, Patidar S K, Ghosh A, et al. Salinity induced oxidative stress enhanced biofuel production potential of microalgae *Scenedesmus* sp. CCNM 1077. Bioresource Technology, 2015; 189: 341–348.

[6] Jiang L, Luo S, Fan X, Yang Z, Guo R. Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO_2. Applied Energy, 2011; 88: 3336–3341.

[7] Ji F, Liu Y, Hao R, Li G, Zhou Y, Dong R. Biomass production and nutrients removal by a new microalgae strain *Desmodesmus* sp. in anaerobic digestion wastewater. Bioresource Technology, 2014; 161: 200–207.

[8] Sriram S, Seenivasan R. Biophotonic perception on *Desmodesmus sp.* VIT growth, lipid and carbohydrate content. Bioresource Technology, 2015; 198: 626–633.

[9] Ebrahimian A, Kariminia HR, Vosoughi M. Lipid production in mixotrophic cultivation of *Chlorella vulgaris* in a mixture of primary and secondary municipal wastewater. Renewable Energy, 2014; 71: 502–508.

[10] Chang H X, Fu Q, Huang Y, Xia A, Liao Q, Zhu X, et al. An annular photobioreactor with ion-exchange-membrane for non-touch microalgae cultivation with wastewater. Bioresource Technology, 2016; 219: 668–676.

[11] Bligh E G, Dyer W J. A rapid method of total lipid extraction and purification. Canadian Journal Biochemistry Physiology, 1959; 37: 911–917.

[12] Miao X, Wu Q. Biodiesel production from heterotrophic microalgae oil. Bioresource Technology, 2006; 97(6): 841–846.

[13] Beuckels A, Smolders E, Muylaert K. Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. Water Research, 2015; 77(15): 98–106.

[14] Mohan D, Sarswat A, Ok Y S, Pittman C U J. Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent—A critical review. Bioresource Technology, 2014; 160: 191–202.

[15] Pancha I, Chokshi K, George B, Ghosh T, Paliwal C, Maurya R, et al. Nitrogen stress triggered biochemical and morphological changes in the microalgae *Scenedesmus sp*. CCNM 1077. Bioresource Technology, 2014; 156: 146–154.

[16] Takagi M, Watanabe K, Yamaberi K, Yoshida T. Limited feeding of potassium nitrate for intercellular lipid and triglyceride accumulation of *Nannochloris* sp. UTEX LB1999. Applied Microbiology Biotechnology, 2000; 54: 112–117.

[17] Meher L, VidyaSagar D, Naik S. Technical aspects of biodiesel production by transesterification-a review. Renewable Sustainable Energy Review, 2006; 10: 248–268.

[18] Singh B, Guldhe A, Rawat I, Bux F. Towards a sustainable approach for development of biodiesel from plant and microalgae. Renewable Sustainable Energy Review, 2014; 29: 216–245.

[19] Zheng Y P, Huang Y, Liao Q, Zhu X, Fu Q, Xia A. Effect of wettability on the growth of *Scenedesmus obliquus* biofilm attached on glass surface coated with polytetrafluoroethylene emulsion. Int. J. Hydrogen Energ., 2016; 41(46): 21728–21735.