



Large-scale cultivation of *Spirulina* in a floating horizontal photobioreactor without aeration or an agitation device

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Abstract

A low-cost floating photobioreactor (PBR) without the use of aeration and/or an agitation device, in which carbon was supplied in the form of bicarbonate and only wave energy was utilized for mixing, was developed in our previous study. Scaling up is a common challenge in the practical application of PBRs and has not yet been demonstrated for this new design. To fill this gap, cultivation of *Spirulina platensis* was conducted in this study. The results demonstrated that *S. platensis* had the highest productivity at 0.3 mol L⁻¹ sodium bicarbonate, but the highest carbon utilization (104 ± 2.6%) was obtained at 0.1 mol L⁻¹. Culture of *Spirulina* aerated with pure oxygen resulted in only minor inhibition of growth, indicating that its productivity will not be significantly reduced even if dissolved oxygen is accumulated to a high level due to intermittent mixing resulting from the use of wave energy. In cultivation using a floating horizontal photobioreactor at the 1.0 m² scale, the highest biomass concentration of 2.24 ± 0.05 g L⁻¹ was obtained with a culture depth of 5.0 cm and the highest biomass productivity of 18.9 g m⁻² day⁻¹ was obtained with a depth of 10.0 cm. This PBR was scaled up to 10 m² (1000 L) with few challenges; biomass concentration and productivity during ocean testing were little different than those at the 1.0 m² (100 L) scale. However, the larger PBR had an apparent carbon utilization efficiency of 45.0 ± 2.8%, significantly higher than the 39.4 ± 0.9% obtained at the 1 m² scale. These results verified the ease of scaling up floating horizontal photobioreactors and showed their great potential in commercial applications.

Keywords Bicarbonate · *Spirulina platensis* · Floating PBR · Scale up · Wave energy

Introduction

Microalgae are a promising feedstock for third-generation biofuels due to their high lipid content and high growth rate. Additionally, they have great potential in the production of biochemicals directly from photosynthesis, rather than from the indirect utilization of glucose derived from it. However, producing economically viable algal biofuel still presents a huge challenge, despite the remarkable progress that has been achieved during the past decade, including in strain screening

(Chi et al. 2014), metabolic engineering evolution (Chen and Blankenship 2011), medium optimization (Dragone et al. 2011), parameter control (Cheirsilp and Torpee 2012), biomass harvesting (Barros et al. 2015), and fuel processing (Kusdiana and Saka 2004). Technoeconomic analysis has revealed that the high cost of production includes expenditures related to photobioreactor (PBR) manufacturing, CO₂ supply, energy consumption, nutrients, and labor (Acien et al. 2012; Banerjee and Ramaswamy 2017; Hoffman et al. 2017). However, in the authors' opinion, the deeper reason for this high cost is the absence of an advanced photobioreactor that can systematically reduce it; this is the most challenging bottleneck for the algal biofuel industry.

Bioreactors are at the heart of all biochemical processes (Najafpour 2007), and innovation in their design plays a key role in the advancement of bioengineering. Historically, progress in aeration and agitation technology was able to well address problems stemming from oxygen transfer in deep culture and greatly improved the productivity of fermentors. This progress significantly accelerated the commercial application of antibiotics and boosted the development of fermentation

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engineering (Daniel et al. 1979). However, the growth of phototrophic microalgae presents challenges that are significantly different from those of heterotrophic culture. Instead of oxygen, the key limiting factors for phototrophic growth are CO₂ and light, which supply carbon and energy separately; this is different from the case of heterotrophic microbes, which use organic carbon feedstock as both an energy and carbon source. Due to its fast assimilation of organic carbon, the productivity of heterotrophic biomass can range up to 3.7 g L⁻¹ h⁻¹ (Doucha and Lívanský 2012). Due to this high efficiency, bioreactors with aeration and stirring are used in most fermentation processes, and the cost of the bioreactor usually does not account for a major portion of the total production cost of heterotrophic culture (Lee Chang et al. 2015; Lowrey et al. 2016; Smetana et al. 2017). Early researchers of microalgae cultivation adopted this design and developed various photobioreactors, such as bubble column, flat panel, and horizontal tubular PBRs. Unfortunately, these innovations have not proved to be successful in biofuel feedstock production, as the cost for these PBRs is a major portion of the total production costs for algal biomass (Acien et al. 2012; Norsker et al. 2011). It can be predicted that even if the photosynthesis efficiency is improved to 6%, which is rarely reached in practical cultivation, the PBR cost would still be a large portion of the total production cost (Acien et al. 2012). Thus, solely improving the process efficiency in the current high-cost PBRs will not provide economically viable algal biofuel production. Instead, a low-cost microalgae cultivation system has to be developed that should systematically reduce the operating costs for carbon supply and energy consumption and minimize labor use.

A microalgae cultivation system known as the Bicarbonate-based Integrated Carbon Capture and Algae Production System (BICCAPS) was developed in our previous study (Chi et al. 2011, 2013), in which carbon was supplied in a bicarbonate form. No aeration or agitation device was necessary for this system, as the high concentration of bicarbonate provided sufficient carbon, and the mixing process was able to be driven with natural forces such as water power (Zhu et al. 2017) or wave energy (Zhu et al. 2018). Simple horizontal PBRs that float in the ocean were developed using inflatable membranes and were named the Bicarbonate-based Integrated Carbon Capture and Algae Production System on Ocean (BICCAPSO), which significantly reduced the cost of PBR manufacturing and installation (Norsker et al. 2011). The use of this system also systematically reduced the operating costs, as the temperature is controlled by sunlight heating and surrounding water cooling, dissolved oxygen is controlled via wave energy mixing, the pH is controlled by the buffering effects of bicarbonate/carbonate, and no interval feeding is required. Because of these advantages, no on-duty personnel are required during the entire algal cultivation process, except during inoculation and harvesting, just as in agricultural production.

Despite these advances, the use of floating horizontal photobioreactors for BICCAPSO faces similar challenges in the scaling-up process as do traditional photobioreactors, which significantly limits their application in commercial-scale production. For example, the largest vertical flat panel PBR has a volume of no more than 100 L (Quinn et al. 2012), and its scaling up is limited by the small gas exchange area produced by the aerating equipment for each PBR (Garcia-Gonzalez et al. 2005). The largest working volume for a horizontal tubular PBR was reported to be 25 m³ (Olaizola 2000), and its scaling up was limited by high levels of accumulated dissolved oxygen (Licheng et al. 2013). Regarding horizontal photobioreactors, an offshore photobioreactor was able to be scaled-up to 83.6 m², with a working volume of 2 m³. However, cell growth in this so-called “photobioreactor” was mainly dependent on organic carbon, as serious biofouling blocked most of the sunlight radiation (Novoveska et al. 2016). Although a recent study of a floating horizontal photobioreactor predicted its flow field at a scale of 200 m² via modeling (Pirasaci et al. 2017), the actual photobioreactor was only 1 m² in volume (Dogaris et al. 2015). It is notable that its mixing process was driven by a paddle wheel on an open pond, rather than by wave energy (Pirasaci et al. 2017).

Spirulina is a filamentous cyanobacterium with a high content of protein and vitamins that is commercially produced as feed or for food supplementation (Lu et al. 2011). Currently, it is mainly cultivated in open pond systems, which have the drawbacks of low biomass density and low productivity. This may be improved by cultivation using a floating horizontal PBR. Thus, *Spirulina* was selected for cultivation with BICCAPSO to evaluate the potential for commercial application of this system (Soni et al. 2017). Its growth under different concentrations of bicarbonate was investigated, and the optimal concentration of bicarbonate was determined. Since intermittent mixing can be a problem during cultivation driven by wave energy, leading to high levels of accumulated dissolved oxygen, this algae was cultivated in the presence of pure oxygen bubbling; bubbling of air was used as a control to test the inhibitory effects of oxygen. Subsequently, the effect of the culture depth on biomass productivity was evaluated in the ocean at a scale of 1 m². Finally, this system was tested at the scale of 10 m² to prove the feasibility of the scaling up of floating horizontal PBRs and to evaluate their performance on a larger scale.

Materials and methods

Microalgae strains and culture medium

The cyanobacterium strain *Spirulina platensis* FACHB-314 was purchased from the Institute of Hydrobiology, Chinese

Academy of Sciences (Wuhan, China). It was cultivated in culture medium containing 25.2 g L⁻¹ NaHCO₃, 2.5 g L⁻¹ NaNO₃, 1.0 g L⁻¹ NaCl, 0.04 g L⁻¹ CaCl₂·2H₂O, 1.0 g L⁻¹ K₂SO₄, 0.5 g L⁻¹ K₂HPO₄, 0.2 g L⁻¹ MgSO₄·7H₂O, 0.01 g L⁻¹ FeSO₄·7H₂O, and 1 mL L⁻¹ A5 trace elemental solution (Deamici et al. 2017).

Effect of bicarbonate concentration on algal growth

To test the effect of bicarbonate concentration on the growth of *S. platensis*, batch and semi-continuous cultures were grown with 0.1, 0.3, 0.5, and 0.7 mol L⁻¹ NaHCO₃. The cultures were continuously illuminated at a light intensity of 141.5 μmol m⁻² s⁻¹. Experiments were carried out in 1000 mL Erlenmeyer flasks with 400 mL of medium at 140 rpm, and the temperature was maintained at 25 ± 1 °C. The biomass productivity (P_{Biomass}) and specific growth rate (μ) were calculated as:

$$P_{\text{Biomass}} (\text{g L}^{-1} \text{d}^{-1}) = \frac{DCW_2 - DCW_1}{t_2 - t_1} \quad (1)$$

$$\mu (\text{d}^{-1}) = \frac{\ln(DCW_2/DCW_1)}{t_2 - t_1} \quad (2)$$

where P_{Biomass} is the biomass productivity (mg L⁻¹ d⁻¹), μ is the specific growth rate (d⁻¹), and DCW_2 and DCW_1 are the dry cell weight (DCW) at times t_2 and t_1 , respectively.

For the measurement of DCW, triplicate samples of 40 mL were first acidulated with hydrochloric acid prior to centrifugation, then washed twice and re-suspended in ammonium bicarbonate. Then, the algae pellets were dried overnight at 105 °C until they reached a stable weight. DCW was calculated by subtracting the empty dish weight from the total weight of the dish and dried biomass.

Calculation of apparent carbon utilization efficiency (%)

The apparent carbon utilization efficiency was calculated using Eq. (3)

$$\text{Efficiency}(\%) = \frac{\Delta DCW \times C_C}{\Delta Tic} \times \% \quad (3)$$

where ΔTic is the change in the total inorganic carbon (Tic) during the cultivation process and ΔDCW represents the change in biomass concentration. C_C represents the carbon content in the dry cell weight of the algal biomass, for which a value of 50% was used in this study (Sánchez Mirón et al. 2003).

Since the alkalinity of the culture media does not change with either consumption or replenishment of CO₂ (Wolf-

Gladrow et al. 2007), Tic was calculated at the measured pH according to Eq. (4) (Da et al. 2015):

$$Tic = \frac{Alc}{\alpha_1 + 2\alpha_2} \quad (4)$$

where Alc is the alkalinity of the medium and α_1 and α_2 are the ionization fractions of HCO₃⁻¹ and CO₃²⁻, respectively, which are obtained as a function of the pH and equilibrium constants k_1 and k_2 , as shown in Eq. (5) and Eq. (6):

$$\alpha_1 = \frac{1}{\left(1 + \frac{H^+}{k_1} + \frac{k_2}{H^+}\right)} \quad (5)$$

$$\alpha_2 = \frac{1}{\left(1 + \frac{H^+}{k_2} + \frac{(H^+)^2}{k_1 k_2}\right)} \quad (6)$$

where the equilibrium constants k_1 and k_2 were theoretically calculated based on the salinity and temperature according to Millero et al. (2006).

In addition, the concentration of dissolved CO₂ (CO_{2D}) can also be calculated from the measured pH value and Tic, as shown in Eq. (7).

$$CO_{2D} = Tic \times \frac{1}{\left(1 + \frac{k_1}{H^+} + \frac{k_1 k_2}{(H^+)^2}\right)} \quad (7)$$

In this study, pH* is defined as the pH at which the concentration of dissolved CO₂ is equal to CO₂^{*}, which represents the saturated concentration in the culture medium. This equation was solved using Matlab 14.0.

CO₂^{*} was calculated according to Eq. (8):

$$CO_2^* = H_{CO_2} P_{CO_2} \quad (8)$$

where P_{CO_2} is the partial pressure of saturated CO₂ and H_{CO_2} represents Henry's constant for CO₂.

Effect of oxygen concentration on algal growth

To test the effect of oxygen concentration on algal growth, *S. platensis* was cultured in a bubble column reactor with a culture volume of 500 mL, in which the reactor had a diameter of 5 cm and a total volume of 600 mL. One reactor was bubbled with 100% oxygen, and the other was bubbled with air filtered through 5 M NaOH to remove CO₂. The gas was supplied to the reactor through a sparger at the bottom with a flow rate of 100 mL min⁻¹. The room temperature was maintained at 25 ±

1 °C using an air conditioner. The cultures were illuminated at a light intensity of $188.7 \mu\text{mol m}^{-2} \text{s}^{-1}$.

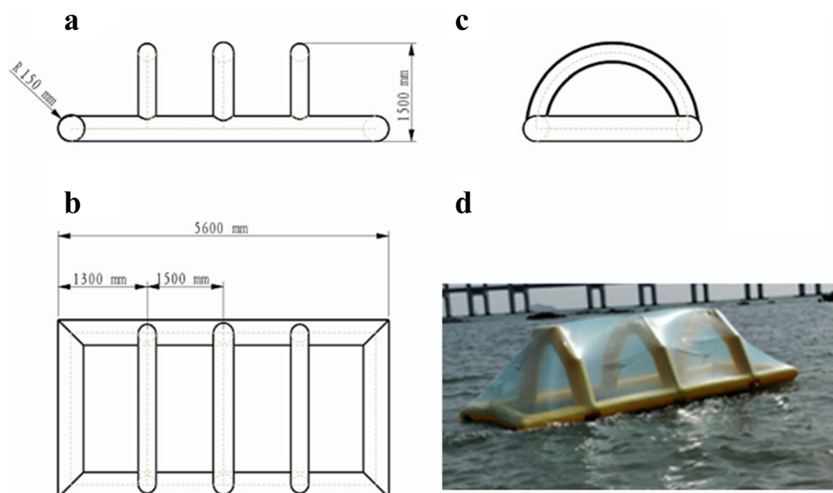
Inflatable PBRs for BICCAPSO

In this study, polyvinyl chloride (PVC) membrane was used to make an inflatable PBR as described in our previous study (Zhu et al. 2018). Briefly, the frame and arch structure were constructed using an inflatable tube and covered and sealed with anti-fogging PVC, with the sampling and gas exchange ports left uncovered and unsealed. To anchor the PBR in the ocean, beackets were attached to the inflated tube. Air was blown into the inflatable tube through a valve to maintain a firm structure. Two PBRs with surface areas of 1.0 m^2 ($1.0 \text{ m} \times 1.0 \text{ m}$) and 10.0 m^2 ($5.0 \text{ m} \times 2.0 \text{ m}$) were made using this design. The 10.0 m^2 PBR had three arched structures (Fig. 1), while the 1.0 m^2 PBR had only one, and the height of the arch structure(s) for each was 1.5 and 0.5 m, respectively. Experiments with these PBRs were conducted in an off-shore test field at the Lingshui Bay in Dalian ($38^\circ 87' \text{N}$, $121^\circ 55' \text{E}$), China.

Effects of culture depth and scaling up

Culture depths of 5.0, 7.5, and 10 cm were investigated using small PBRs at a 1 m^2 scale, with culture volumes of 50, 75, and 100 L, respectively. Based on the results, a culture depth of 10.0 cm was selected for testing with the 10 m^2 PBR (1000 L), with a 1 m^2 PBR (100 L) used as the control. These outdoor cultures were carried out from September 2017 to October 2017. Every three days, 120 mL of culture broth was sampled to obtain three repeated measurements of biomass concentration. Light intensity in the outdoor cultures was measured with a data logging light meter (Zhituo Instruments, Hanzhou, China). Temperature was recorded with a digital thermometer probe (Sinomeasure, Hanzhou, China).

Fig. 1 Diagram of the inflatable membrane floating PBR at the 10 m^2 scale. **a** Side view; **b** top view; **c** front view; **d** overview



Results

Effect of bicarbonate concentration on algal growth

Biomass growth in batch cultures with different concentrations of bicarbonate is shown in Fig. 2a. The highest biomass concentrations of 1.21 ± 0.03 , 1.55 ± 0.03 , 1.38 ± 0.05 , and $0.97 \pm 0.08 \text{ g L}^{-1}$ were achieved in cultures with bicarbonate concentrations of 0.1, 0.3, 0.5, and 0.7 mol L^{-1} , respectively. The corresponding average volumetric productivity values during the first 5 days were 0.23 ± 0.004 , 0.27 ± 0.01 , 0.23 ± 0.01 , and $0.16 \pm 0.01 \text{ g L}^{-1} \text{ d}^{-1}$, respectively. The specific growth rates were 1.39 ± 0.02 , 1.76 ± 0.01 , 1.28 ± 0.04 , and $1.53 \pm 0.23 \text{ d}^{-1}$, respectively (Table 1). This indicates that *S. platensis* can survive in a bicarbonate concentration of up to 0.7 mol L^{-1} , although it showed optimal growth at 0.3 mol L^{-1} .

Since pH plays a significant role in algal growth, the pH change was also measured during the entire culturing process. As shown in Fig. 2b, the highest pH value of 12.3 was measured in the culture with 0.1 mol L^{-1} bicarbonate, and a sharp increase in pH was observed from day 4 to day 5. Due to the high pH of this culture, the biomass concentration decreased after day 5, and similar results were observed in our other studies. For the other three groups, the pH was never greater than 10.5, indicating that higher concentrations of bicarbonate/carbonate had better pH buffering effects.

Apparent carbon utilization efficiency in cultures with different bicarbonate concentrations

The apparent carbon utilization efficiency showed great variation between cultures with differing concentrations of bicarbonate. As shown in Fig. 3, the apparent carbon utilization efficiency decreased with increased bicarbonate concentration, and overall apparent carbon utilization efficiencies of

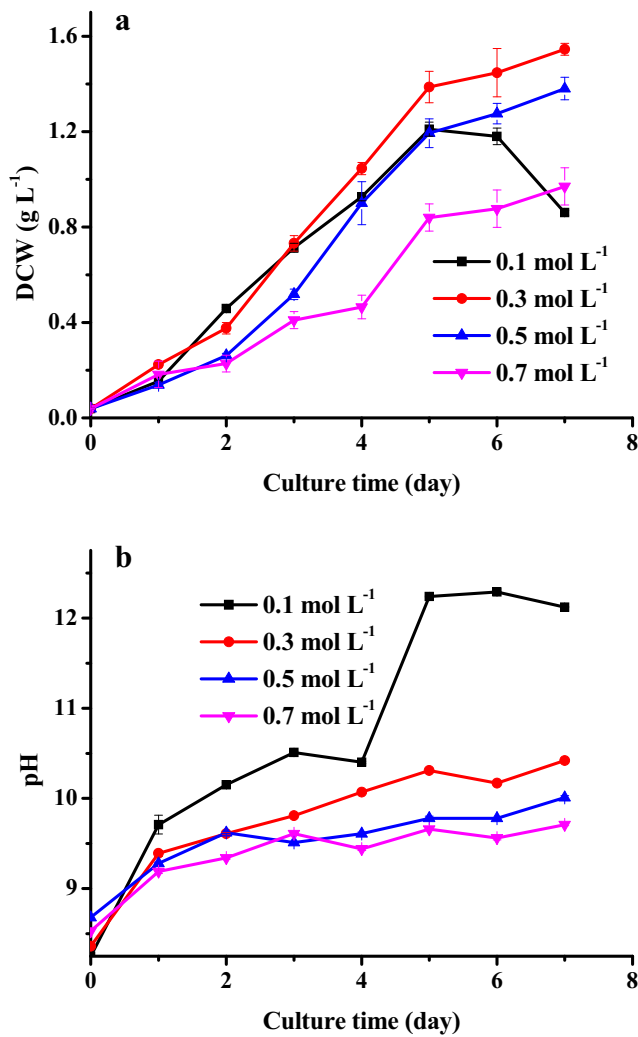


Fig. 2 Growth of *S. platensis* in different concentrations of NaHCO₃ in batch culture. **a** Biomass concentration; **b** pH. Values are the mean ± SD of three independent measurements

104 ± 2.6, 43.9 ± 2.1, 31.6 ± 1.3, and 28 ± 1.4% were achieved in cultures with bicarbonate concentrations of 0.1, 0.3, 0.5, and 0.7 mol L⁻¹, respectively. To the authors’ knowledge, this was the first demonstration of a carbon utilization efficiency greater than 100%, with the previously reported highest efficiencies being 91.40% in bicarbonate cultivation (Kim et al. 2017) and 85.6% in 15% CO₂ (Cheng et al. 2013). This is probably due to the transfer of CO₂ from air into the

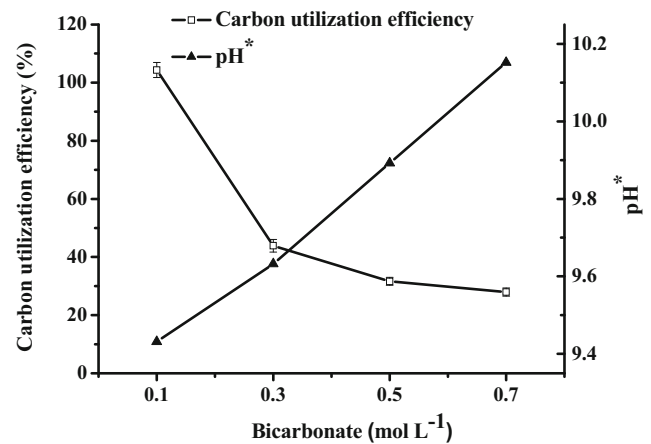


Fig. 3 Effect of bicarbonate concentration on apparent carbon utilization efficiency and pH*. Values are the mean ± SD of three independent measurements

culture medium when the pH of the medium is higher than pH*. To prove this, the pH* values for culture medium with different concentrations of sodium bicarbonate were calculated and found to be 9.43, 9.63, 9.89, and 10.15 for 0.1, 0.3, 0.5, and 0.7 mol L⁻¹, respectively. The pH of the culture with 0.1 mol L⁻¹ bicarbonate exceeded pH* after the 1st day. Therefore, CO₂ should have been transferred into the medium after this time point, leading to an increase in Tic. This should explain why its apparent carbon utilization efficiency exceeds 100% (Fig. 3). Compared with the 0.1 mol L⁻¹ culture, the other three cultures had a low utilization efficiency. This was because the Tic decreased due to CO₂ being lost to air when the culture pH was less than pH*, and this loss increased over the period of time that the culture pH less was than pH* as well as with the bicarbonate concentration.

Effect of oxygen concentration on algal growth

Due to the intermittent nature of wave energy, a high level of dissolved oxygen may be accumulated in a floating photobioreactor, which can decrease biomass productivity and even lead to the collapse of the culture. Thus, the effect of a high level of oxygen on *S. platensis* growth was tested prior to cultivation in the ocean. As shown in Fig. 4a, there was no difference in the biomass concentration of cultures bubbled with pure O₂ or air during the first four days of

Table 1 Summary of *S. platensis* growth with different concentrations of bicarbonate in batch culture (5 days)

| Bicarbonate (mol L ⁻¹) | Average productivity (g L ⁻¹ d ⁻¹) | Maximum productivity (g L ⁻¹ d ⁻¹) | μ _{max} (d ⁻¹) |
|------------------------------------|---|---|-------------------------------------|
| 0.1 | 0.23 ± 0.00 | 0.31 ± 0.00 | 1.39 ± 0.02 |
| 0.3 | 0.27 ± 0.01 | 0.36 ± 0.01 | 1.76 ± 0.01 |
| 0.5 | 0.23 ± 0.01 | 0.38 ± 0.06 | 1.28 ± 0.04 |
| 0.7 | 0.16 ± 0.01 | 0.38 ± 0.01 | 1.53 ± 0.23 |

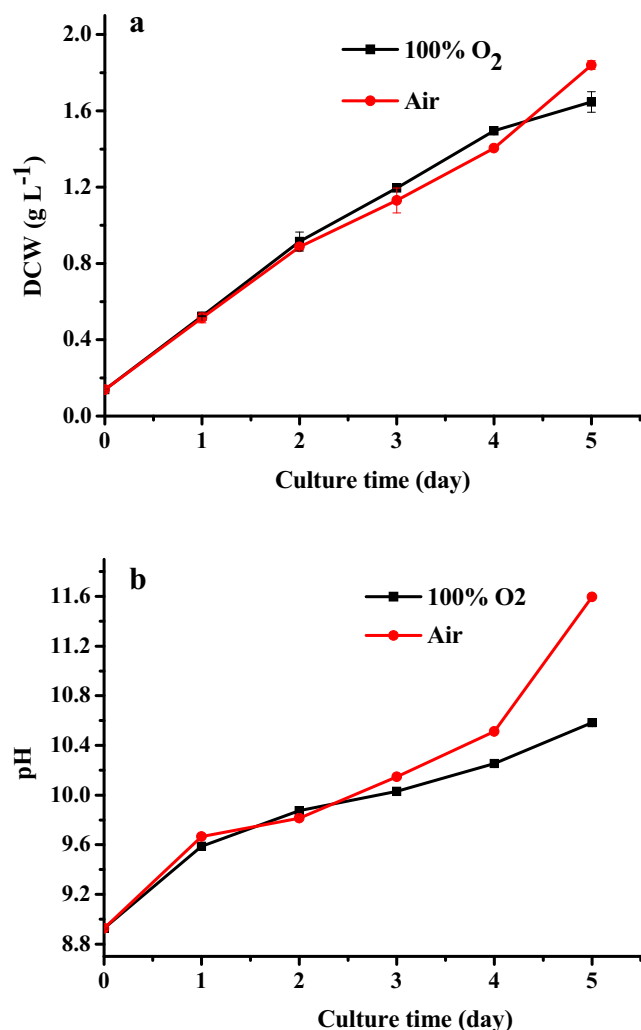


Fig. 4 Effect of pure oxygen on *S. platensis*. **a** Biomass concentration; **b** pH. Values are the mean \pm SD of three independent measurements

cultivation. However, a slightly lower biomass concentration of $1.65 \pm 0.05 \text{ g L}^{-1}$ was obtained in the culture bubbled with O₂ on the 5th day, compared with the value of $1.84 \pm 0.02 \text{ g L}^{-1}$ in the culture bubbled with air. These results demonstrated that high dissolved oxygen does affect the growth of *S. platensis*, but its inhibition is limited and does not lead to cell death. This indicates that high oxygen levels due to intermittent mixing caused by the use of wave energy would not lead to serious inhibition of growth of *S. platensis*. The change in pH in these two cultures was also determined. As shown in Fig. 4b, the final pH values were 10.58 and 11.59 in the cultures bubbled with pure oxygen and air, respectively. A higher pH correlated with a higher biomass concentration, which is consistent with the results shown in Fig. 4a.

Effect of culture depth on cultivation on the ocean

Cultivation of *S. platensis* on the ocean was conducted during this study, and the results demonstrated that the biomass

production of *S. platensis* was significantly affected by the culture depth. As shown in Fig. 5a, the highest biomass concentrations of 2.24 ± 0.05 , 1.74 ± 0.02 , and $1.41 \pm 0.06 \text{ g L}^{-1}$ were achieved in the PBRs with culture depths of 5.0, 7.5, and 10.0 cm, respectively. In contrast, the average areal productivity increased with the increase in culture depth, as the average

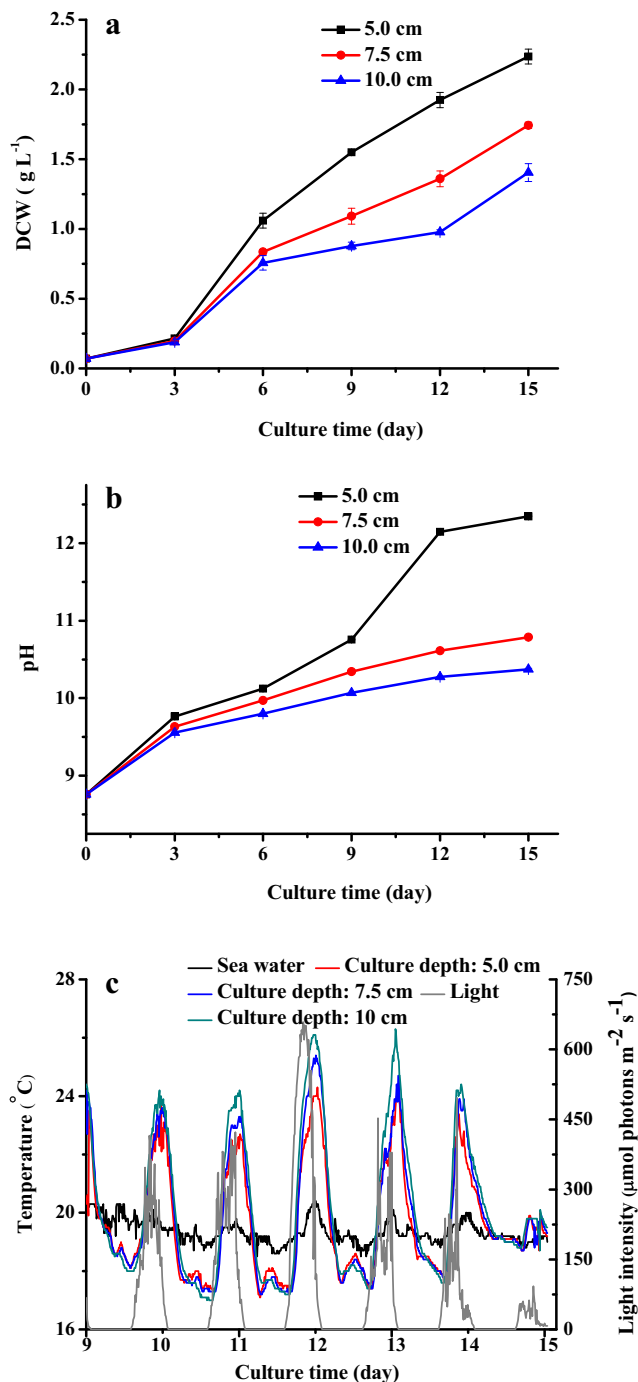


Fig. 5 Growth of *S. platensis* in a horizontal floating PBR at the 1.0 m^2 scale with different culture depths. **a** Biomass concentration; **b** pH; **c** culture temperature in the PBR. Values are the mean \pm SD of three independent measurements

areal productivities at culture depths of 5.0, 7.5, and 10.0 cm were 7.22, 8.36, and 8.89 $\text{g m}^{-2} \text{d}^{-1}$, respectively; the corresponding highest daily productivity values were 14.1, 15.6, and 18.9 $\text{g m}^{-2} \text{d}^{-1}$, respectively. The pH change in these three cultures was also determined. As shown in Fig. 5b, the final pH values were 12.4, 10.8, and 10.4 for the cultures with depths of 5.0, 7.5, and 10 cm, respectively. The corresponding average apparent carbon utilization efficiency values were 83.3 ± 2.0 , 66.1 ± 0.9 , and $55.0 \pm 2.0\%$ in cultures with depths of 5.0, 7.5, and 10 cm, respectively.

The temperatures in these three cultures were also recorded. As shown in Fig. 5c, the temperature in the PBR varied from 20 to 24.4 °C at the 5.0 cm culture depth, from 19.9 to 25.4 °C at the 7.5 cm culture depth, and from 20.1 to 26.3 °C at the 10.0 cm culture depth. Correspondingly, the highest temperatures of the surrounding water varied only from 19.2 to 20.4 °C. The maximum temperature differences between the PBR and the surrounding water were 5.1, 5.3, and 6.7 °C for culture depths of 5.0, 7.5, and 10.0 cm, respectively. This indicated that the BICCAPSO PBR has the advantage of heat preservation. The maximum temperature difference between the PBR and the surrounding water was 5.8 °C on a sunny day, while it was only 1 °C on a cloudy day (Fig. 5c). During the night, however, the culture temperatures were usually lower than the water temperature, indicating the presence of heat transfer from the surrounding water into the PBR, which would maintain the temperature of the PBR higher than that of the air during cold weather.

Scaling up to 10.0 m²

To test the feasibility of scaling up, a culture was grown in a 10 m² PBR, and its performance was compared to that of a 1.0 m² PBR. As shown in Fig. 6a, there was no significant difference between the final biomass concentrations obtained using these two PBRs, with the highest biomass concentration of the 1.0 m² PBR being $1.02 \pm 0.02 \text{ g L}^{-1}$ and that of the 10 m² PBR being $1.04 \pm 0.06 \text{ g L}^{-1}$. Based on these results, the average biomass productivity during the 15-day cultivation was calculated as $6.62 \text{ g m}^{-2} \text{ day}^{-1}$ in the 1.0 m² PBR and $6.63 \text{ g m}^{-2} \text{ day}^{-1}$ in the 10 m² PBR. Moreover, the maximum areal productivity was slightly higher in the 10 m² PBR ($11.4 \text{ g m}^{-2} \text{ d}^{-1}$) than in the 1.0 m² PBR ($9.69 \text{ g m}^{-2} \text{ d}^{-1}$). These results indicated that the scaling up of the floating horizontal PBR was successful.

The pH change in these two cultures was also determined. As shown in Fig. 6b, the pH in the 1.0 m² PBR increased from 8.71 to 10.1; however, slightly lower values were found in the larger 10.0 m² PBR, where the pH increased from 8.71 to 9.82. The corresponding average apparent carbon utilization efficiency values were calculated as 39.4 ± 0.9 and $45.0 \pm 2.8\%$, respectively.

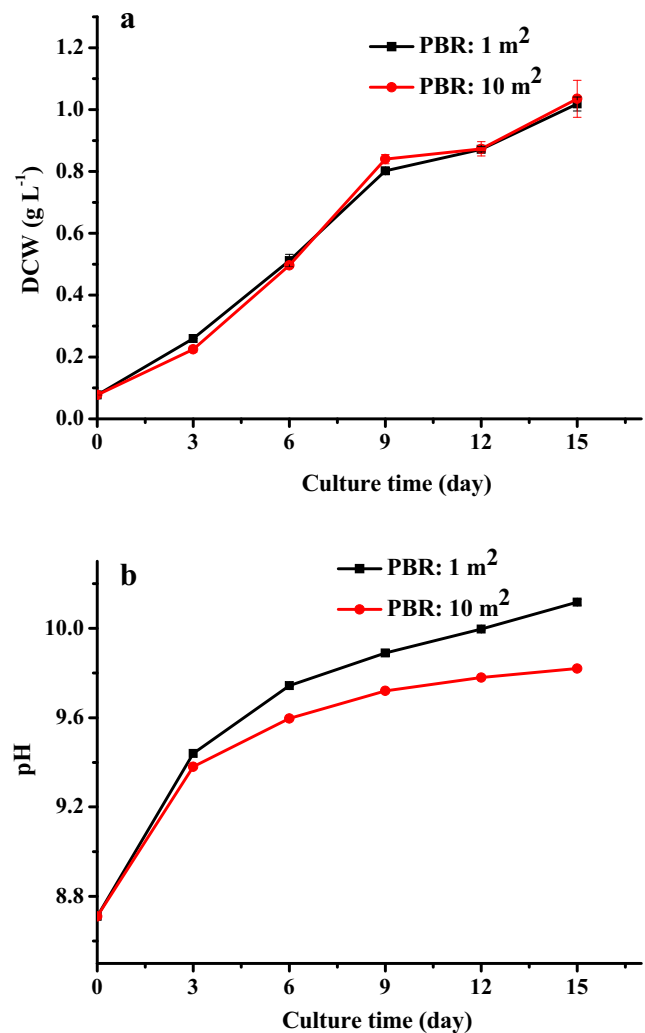


Fig. 6 Cultivation of *S. platensis* in a horizontal floating PBR at the scale of 10.0 m². **a** Biomass concentration; **b** pH. Values are the mean \pm SD of three independent measurements

Discussion

The annual average biomass productivity of *Spirulina* in massive cultivation on an open pond is usually in the range of 5.29 to 9.09 $\text{g m}^{-2} \text{d}^{-1}$ (Lu et al. 2011; Olguin et al. 1997). Compared to this, the 1 m² PBR used in this study had an average productivity of 7.22 to 8.89 $\text{g m}^{-2} \text{d}^{-1}$. This experiment was conducted from September 23rd to October 8th, 2017, when daylight hours are fewer than the night hours. Sunlight plays the most significant role in outdoor cultivation, and a longer irradiation time usually results in higher productivity. Thus, an annual average biomass productivity higher than that achieved on an open pond would be obtained using BICCAPSO. Additionally, batch culture was used in this study, but semi-continuous culture is usually utilized in commercial production to avoid a long lag-phase. If this mode of cultivation is adopted, a much higher average biomass productivity would be obtained using BICCAPSO, as

18.9 g m⁻² day⁻¹ was the highest daily biomass productivity achieved during this study. Thus, BICCAPSO holds promise to significantly improve the biomass productivity of *Spirulina*, which has the largest annual production volume among all microalgae species.

S. platensis was reported to be severely inhibited by a high level of dissolved oxygen (Vonshak et al. 1996), but only limited inhibition was observed in this study when cultures were bubbled with pure oxygen (Fig. 4). This indicates that different strains of *Spirulina* have varying capabilities to tolerate high dissolved oxygen levels. Although the level of dissolved oxygen in the floating PBR on the ocean was not measured due to technical difficulties, this study proved the feasibility of culturing *S. platensis* using floating PBRs driven by wave energy, even in the presence of intermittent mixing, under which dissolved oxygen may accumulate to a high level (Dogaris et al. 2015). Thus, intermittent mixing will not affect the application of BICCAPSO as long as the proper production strain is selected.

Due to its simple structure, the BICCAPSO PBR experienced few challenges in the scaling-up process and was successfully scaled up to 10 m² in this study. Compared with the 1 m² PBR, the 10 m² PBR has greater length and width (5 m × 2 m) and has three arch structures that are higher (1.5 m). It is predicted that an even larger PBR can be easily developed based on this design. However, for further scaling up, the optimal length-to-width ratio should be investigated, as the motion of a floating PBR in the presence of natural waves is significantly affected by its aspect ratio (Mani 1991). The height of the arch structure presents another challenge for the scaling-up process. Theoretically, the height of the arch structures should be increased as the PBR width increases; however, a PBR with higher arches would experience greater drag forces caused by wind. This may increase the mooring force, which may be harmful to the PBR. This should also be investigated in depth at a larger scale. In addition, a larger PBR may demonstrate poor mixing, as observed in this study, but this may be addressed by constructing an inner baffle (Kim et al. 2016). With these efforts, it is probable that an even larger PBR with better performance can be developed in the future.

Cultivation in the 10 m² PBR not only resulted in similar biomass production to that of the smaller scale PBR but also resulted in a higher apparent carbon utilization efficiency (Fig. 5a). This is probably because the smaller PBR experienced more intensive mixing than the larger one, resulting in a higher value of k_{LaCO_2} (mass transfer coefficient of CO₂) and more CO₂ escaping from the medium into the air (Gao et al. 2015). A culture with a shallower depth would have a greater k_{LaCO_2} value, and this could explain why it had a lower carbon utilization rate. However, when the pH of the medium is higher than pH*, a greater k_{La} value would improve the carbon utilization rate, as CO₂ would be transferred from the air into the

medium. Thus, it is important to achieve a balance between high pH, which can have a detrimental effect on cell growth and the carbon utilization rate (Chi et al. 2014).

The current production cost for *Spirulina* is approximately \$3 per kilogram (Lu et al. 2011); further research on BICCAPSO could lead to a reduction of this cost for a number of reasons. First, BICCAPSO has a higher productivity than the traditional open pond system and uses free wave energy for mixing, as indicated in this study. Second, carbonate produced from bicarbonate consumption can be used to capture CO₂ for recycling (Chi et al. 2011); thus, there would be no net consumption of bicarbonate, as it only functions as the carrier of CO₂, which would reduce the cost of the carbon supply. Third, CO₂ in flue gas could be used, and this would further reduce the carbon cost. In addition, seawater can provide free nutrients and water for microalgae cultures, and utilization of ocean space has a lower cost than that of land. All these efforts would further reduce the production cost and improve the economic viability of algal biofuel. Certainly, oleaginous algae strains need to be cultivated to produce biofuel. Fortunately, great progress has been made in the cultivation of oleaginous microalgae strains with bicarbonate, including *Scenedesmus* sp. (Panacha et al. 2015), *Neochloris oleoabundans* (Santos et al. 2012; Santos et al. 2013), and *Chlorella variabilis* (Patidar et al. 2016). All these strains exhibited excellent growth and lipid productivity and show promise for use in BICCAPSO for biofuel production (Chen et al. 2016).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical statement This article does not contain any studies with human or animal subjects.

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