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Plastic bag as horizontal photobioreactor on rocking platform driven by water power for culture of alkalihalophilic cyanobacterium

He Zhu, Chenba Zhu, Longyan Cheng and Zhanyou Chi*

Abstract

Background: Mixing in traditional algae culture system consumes intensive electricity. This should be replaced by nature force to reduce energy cost and, more importantly, to realize positive energy balance of algal biofuel production. This study aims to develop a horizontal photobioreactor, in which mixing can be provided with rocking movement driven by nature force.

Results: Simple boxes were used as small-scale horizontal photobioreactors on a rocking platform for culture of alkalihalophilic *Eubalthece* sp. ZM001. There was no CO₂ gas bubbling since 1.0 M NaHCO₃ supplied sufficient inorganic carbon in it. Effect of culture depth, rocking cycle, and light intensity to algal biomass production, pH change, and DO accumulation were investigated in this system. Biomass concentration of 2.73 g/L was achieved in culture with 2.5 cm depth, and maximum productivity of 17.06 g/m²/day was obtained in culture with 10 cm depth. $k_1 a$ in PBR with different culture depths and rocking cycles was measured, and it was from 0.57 to 33.49 h⁻¹, showing great variation. To test this system at large scale, a plastic bag with a surface area of 1 m² was placed on a rocking platform driven by water power, and it resulted in a biomass concentration of 1.88 g/L.

Conclusion: These results proved feasibility of a novel photobioreactor system driven by nature force, as well as low cost of manufacturing, and easy scaling-up.

Keywords: Microalgae, Bicarbonate, Mixing, Photobioreactor, Nature force

Background

Microalgae are promising for biofuel production to replace petroleum, but limited by high production cost. Photobioreactor (PBR) is the key component of algae culture system, and it is crucial for cost-effective algae culture process development. Traditional PBRs such as horizontal tubular and vertical flat panel PBRs have many drawbacks, including (1) high cost for manufacturing; the tubular PBR cost at small scale was 2400 €/m², and even if reduce this to 750 €/m² (or 5 €/L) at large scale, it would still account for 94% of total major equipment cost, and result in algal biomass production cost of

12.6 €/kg (Acien et al. 2012); (2) high energy consumption for mixing; the energy consumption for blower in flat panel PBR was 49 w/m³, which accounted for 68% of total operating energy input (Tredici et al. 2015), and tubular PBR driven with centrifuge pump has even higher energy consumption rate (Jorquera et al. 2010); (3) high labor cost for installation and maintenance (Acien et al. 2012; Norsker et al. 2011); (4) difficulties to be scaled up (Acien Fernandez et al. 2013). These drawbacks directly or indirectly lead to microalgae's high production cost. This is corresponding to economical analysis on commercial scale microalgae culture, which revealed that major production cost come from photobioreactor (PBR) manufacturing, power consumption, CO₂ supply, and labor cost (Acien et al. 2012; Norsker et al. 2011; Tredici et al.

*Correspondence: chizhy@dlut.edu.cn
School of Life Science and Biotechnology, Dalian University of Technology, Dalian 116024, China

2015). To reduce these costs, innovative algal culture systems need to be developed, and it should have low cost for both PBR manufacturing and energy consumption for mixing. Besides, labor utilization for PBR installation and maintenance should be reduced to minimum, and inorganic carbon should be supplied with cost-effective way.

To explore a better approach for supplying carbon to microalgae culture, bicarbonate-based integrated carbon capture and algae production system (BICCAPS) has been developed in our previous study (Chi et al. 2011, 2013). This system uses high concentration of bicarbonate derived from carbon capture to provide sufficient inorganic carbon, and no continuous CO₂ sparging in algae culture process is necessary. This can avoid a series of problems, including high cost of CO₂ purification and transportation, difficulty of CO₂ storage, and low utilization rate due to outgassing. Another advantage of this system is that configuration of photobioreactor for BICCAPS can be very simple, since no sparging system is required. For example, a tissue culture flask (T-flask) was used as a simple PBR, and it well supported growth of alkalihalophilic cyanobacterium *Eubhalothece* sp. in our previous study (Chisti 2013). If this T-flask is scaled up, it is actually a horizontal PBR, which is a hybrid design of PBR and open pond, and shares advantages from both, including low manufacturing cost, closed system, short light path, and easy scaling-up (Dogaris et al. 2015). Due to these merits, horizontal PBR has great potential application in future algae culture, and it is an excellent candidate for BICCAPS at large scale.

To develop an efficient algal culture system, sufficient mixing is obligatory in that it not only enhances mass transfer, but also improves frequency of algal cells' shifting between dark and light, which is crucial for improving photosynthesis efficiency (Abu-Ghosh et al. 2016). However, it consumes intensive energy to provide sufficient mixing. In traditional PBR systems, mixing is the largest portion of energy consumption, and it contributes significantly to total operating cost. For example, power consumption in horizontal tubular PBR and vertical flat panel PBR was estimated as 2500 and 53 w/m³, respectively (Jorquera et al. 2010). Although raceway pond has a low estimated energy cost of 3.7 w/m³ (Jorquera et al. 2010), this is actually not enough to provide sufficient mixing, which results in poor mass transfer, as well as limited cell movement in vertical direction (Mendoza et al. 2013a, b; de Godos et al. 2014). Intensive energy consumption for mixing also results in negative energy balance of algal biomass production, which is a great hurdle in commercialization of algal biofuel. Using electricity generated from renewable energy sources, such as solar or wind, may address this problem. However, it will

result in biofuel with even higher cost than using electricity from fossil fuel, since electricity generated from solar energy or wind is still more expensive than that of fossil fuel combustion by far (Davis and Martín 2014). Thus, it is obligatory to develop PBR systems driven by renewable energy with a cost-effective way.

In our previous study with T-flask as the PBR for BICCAPS, mixing was provided with an orbital shaker, and it well supported algae growth. This is obvious not practical for culture at large scale. However, it may be feasible to drive rocking movement of this simple PBR at large scale with nature forces such as falling water, wave, or wind (Kim et al. 2016). To investigate if this rocking movement can provide sufficient mixing, the effect of rocking cycle and culture depth was studied at different light intensities with small-scale horizontal PBR placed on a see-saw rocker. Biomass production, oxygen accumulation, and pH change were measured at these conditions. In addition, mass transfer coefficient ($k_L a$) in this horizontal PBR was measured. At 1.0 m² scale, a plastic bag was used as low-cost PBR, which was placed on a rocking platform driven by water power. This system supported algae growth, indicating that sufficient mixing may be provided by horizontal PBR's rocking movement. This proved feasibility of a novel system with low cost of both PBR manufacturing and energy consumption.

Methods

Strain and medium

The alkalihalophilic cyanobacterium strain *Eubhalothece* sp. ZM001 was purchased from Culture Collection of Autotrophic Organisms (CCALA), Academy of Sciences of the Czech Republic. The seed was cultured in M-medium, which contains Na₂CO₃, 53 g/L; NaHCO₃, 42 g/L; NaCl, 50 g/L; KCl, 2 g/L; Na₂SO₄, 1.4 g/L; KNO₃, 2.5 g/L; K₂HPO₄·3H₂O, 0.5 g/L; FeCl₃, 0.0003 g/L; EDTA, 0.0005 g/L; and 1 mL of the A5 trace element solution (Mikhodyuk et al. 2008). The pH of M-medium was 10.5. It was inoculated into concentrated medium (C-medium) for cultivation, which contains Na₂CO₃, 53 g/L; NaHCO₃, 42 g/L; KCl, 10 g/L; Na₂SO₄, 7 g/L; KNO₃, 12.5 g/L; K₂HPO₄·3H₂O, 2.5 g/L; FeCl₃, 0.0015 g/L; EDTA, 0.0025 g/L; and 5 mL of the A5 trace element solution. The pH of C-medium was 9.5.

Small-scale horizontal PBR for algae culture

Simple square boxes made from Acryline at the size of 12 cm × 12 cm were used as horizontal PBRs in this experiment, which were placed on a see-saw rocker to provide mixing. To investigate different mixing intensities, the see-saw rocker was controlled to finish one up/down rocking cycle every 1 s or every 2 s. Culture depth

of 2.5, 5.0, 7.5, and 10 cm were investigated with this system. To prevent evaporation, the reactor was sealed with transparent membrane on the top, with two holes left to release oxygen. LED panel with 70% red and 30% blue light was placed above the PBRs. Light intensities investigated were 70, 135, and 340 $\mu\text{mol}/\text{m}^2/\text{s}$, which were measured at the surface of culture medium. The whole culture system was placed in an incubator, which was controlled at 35 °C (Chi et al. 2013).

Dry cell weight measurement

To measure biomass concentration, 40 mL of sampled cell suspension was centrifuged at 10,000 rpm for 15 min. The cell pellets were then washed twice with 1% (w/v) salt water, to avoid osmotic pressure change caused by fresh water. After that, this salt water was added to the cell pellets to make a final volume of 5 mL, and placed in a petri dish for drying at 105 °C for 4 h. Then, dry cell weight was calculated by subtracting salt weight from the total dry weight (Chi et al. 2014).

Dissolved oxygen (DO) measurement

A non-invasive Fibox 4 fiber optic oxygen transmitter (PreSens Precision Sensing GmbH, Germany) was used to measure DO in PBRs. Since DO value in some experiments were read as over saturation (higher than 34 mg/L), the accuracy of this instrument was verified by following procedure: the culture medium used in this experiment was bubbled with pure oxygen for half an hour, and the reading DO value was 32.7 mg/L. Thus, this measure error was 4.0%, and acceptable.

To measure DO at different depths in the PBR, oxygen sensor spots were placed on the side wall at different distances from PBR bottom. For the culture depth of 2.5 cm, one sensor spot was placed 1.25 cm above the bottom. For culture depth of 5.0, 7.5, and 10 cm, two sensor spots were placed: one was 1.0 cm below the medium surface, and the other was 1.0 cm above PBR bottom.

$k_L a$ measurement

$k_L a$ was determined with a dynamic method. PBR was filled with culture medium, and oxygen was bubbled through a diffuser at the bottom of PBR. It kept bubbling until DO reached 27.3 mg/L. Change of DO over time was measured with a probe at the liquid surface until DO reached 13.6 mg/L. Change of DO over time was assumed to be a function of $k_L a$ and driving force ($[\text{O}_2^*] - [\text{O}_2]$) in Eq. 1.

$$\frac{d[\text{O}_2]}{dt} = k_L a ([\text{O}_2^*] - [\text{O}_2]). \quad (1)$$

By integrating this equation between time zero and time t , the mass transfer coefficient $k_L a$ can be obtained by Eq. 2.

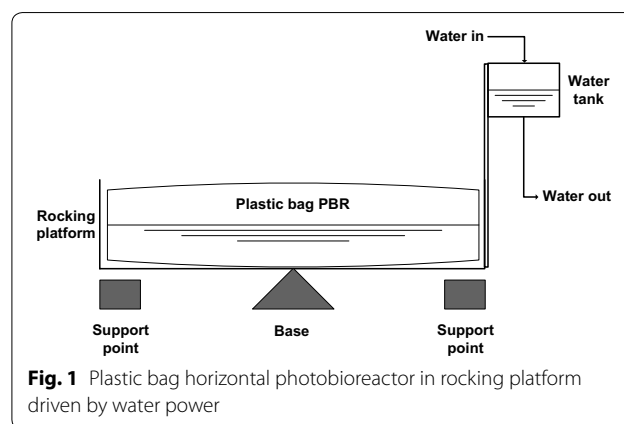
$$\ln \left(\frac{[\text{O}_2^*] - [\text{O}_2]_{t=0}}{[\text{O}_2^*] - [\text{O}_2]} \right) = k_L a \cdot t. \quad (2)$$

Test of oxygen tolerance capability of *Euhalothece* sp.

Euhalothece sp. ZM001 was cultured in bubble column reactors with diameter of 5 cm, and the total volume was 500 mL. The room temperature was maintained at 25 °C with air conditioner. The cultures were placed in front of a LED panel with white light, and the light intensity was 135 $\mu\text{mol}/\text{m}^2/\text{s}$. Three reactors bubbled with pure oxygen, and the other three bubbled with air. Biomass production, DO, and pH were measured during the culture process.

Culture with plastic bag as horizontal PBR at 1 m² scale

A plastic bag with a size of 1 m \times 1 m was used as the horizontal PBR. It was placed on a rocking platform driven with water power (Fig. 1). This platform has a size of 1 m \times 1 m \times 0.3 m ($L: W: H$), with steel as frame and thick PVC membrane as bottom and side walls. A water tank was attached to one side of its frame, and water keeps flowing into the tank. When this water tank is half-full, this side of platform was pushed down by the weight of water. Once water tank is full, the water was discharged until empty, which is controlled by a pressure-sensing valve. Then, this side of rocking platform was lifted up. In this way, this platform generates a continuous rocking movement. The rocking cycle of this platform was measured as 60 s. Culture depth of 5.0 cm was used in the outdoor culture with plastic bag, and this culture was conducted from September 6 to September 26, 2016 at Dalian City, China.



Results

Mixing driven by rocking movement of horizontal PBR is quite different from traditional way such as paddle wheel or circulation pump. Rocking cycle should be a decisive factor to mixing. Thus, the effect of rocking cycle to biomass production and DO accumulation was investigated with small-scale horizontal PBRs at different light intensities. Culture depth of 2.5, 5.0, 7.5, and 10.0 cm were used in these experiments since it provides light path at the same level as traditional PBRs.

Culture at high light intensity and short rocking cycle

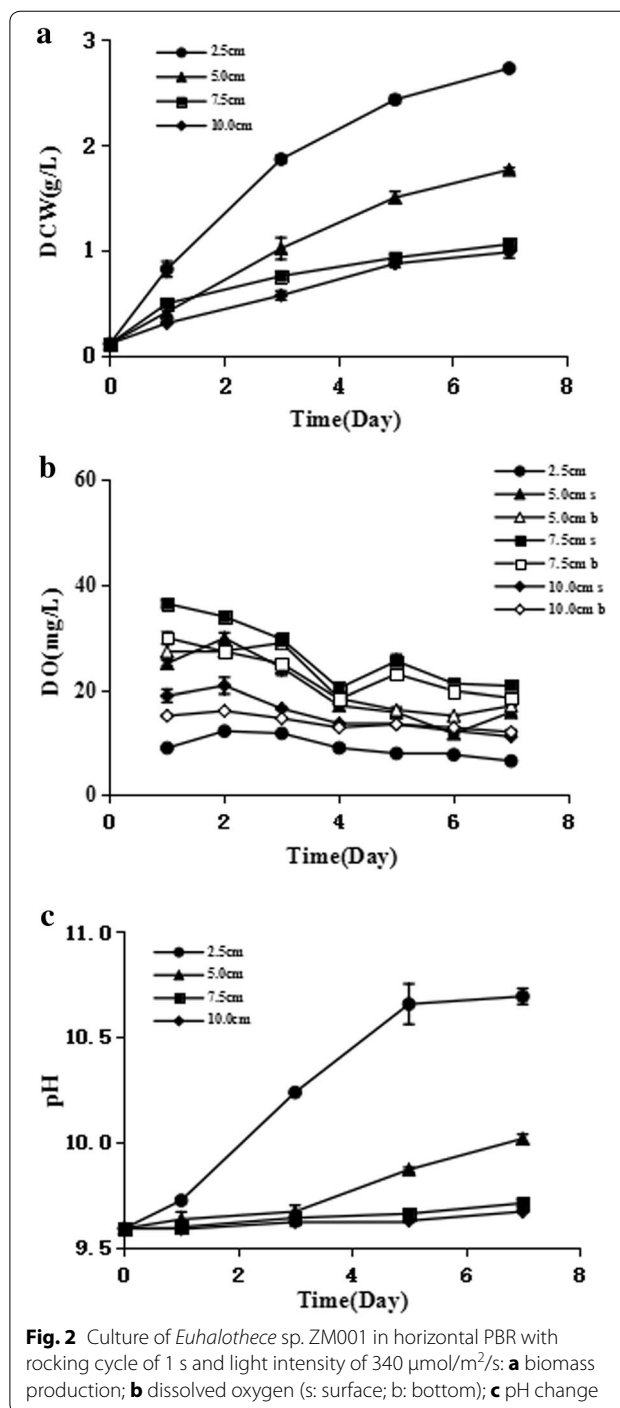
High light intensity of $340 \mu\text{mol}/\text{m}^2/\text{s}$ and short rocking cycle of 1 s was tested at first. With 7-day culture, the final biomass concentration reached 2.73, 1.76, 1.06, and 0.98 g/L for 2.5, 5.0, 7.5, and 10 cm culture depths, respectively (Fig. 2a). There were pH drifting during the culture, but pH was no more than 11.0 in all experiments. As an extremely alkaliphilic cyanobacterium, *Euhalotheca* sp. ZM001 grow well in this pH range (Acien Fernandez et al. 2013).

DO in culture with 2.5 cm depth was kept below 12.1 mg/L during the whole culture process, indicating that it has excellent oxygen mass transfer. Compared with this, 5.0 cm culture accumulated higher DO level than this, which was from 15.0 to 29.8 mg/L. DO in 7.5 cm culture accumulated to an even higher level, from 18.2 to 36.4 mg/L. It is interesting that DO in 10 cm culture accumulated to a level only a little higher than that of 2.5 cm, but much lower than that of 7.5 and 5.0 cm. The maximum DO observed during the culture process was 12.1, 29.8, 36.4, and 20.8 mg/L for 2.5, 5.0, 7.5, and 10 cm of culture depth, respectively (Fig. 2b).

There was no much difference between DO at surface and bottom for 5.0 cm culture. However, the DO at surface was obviously higher than bottom for cultures with 7.5 and 10 cm depth. For 10 cm, this difference was significant when cell growth was in exponential phase ($p = 0.057$). This should attribute to the fact that cells closer to surface receive more light and produce more oxygen. This is actually a non-negligible character of horizontal PBR, since oxygen has to be released from water surface. In this viewpoint, intensive mixing making homogeneous condition may not be necessary, since it brings oxygen from surface to bottom, which is actually more difficult to be released.

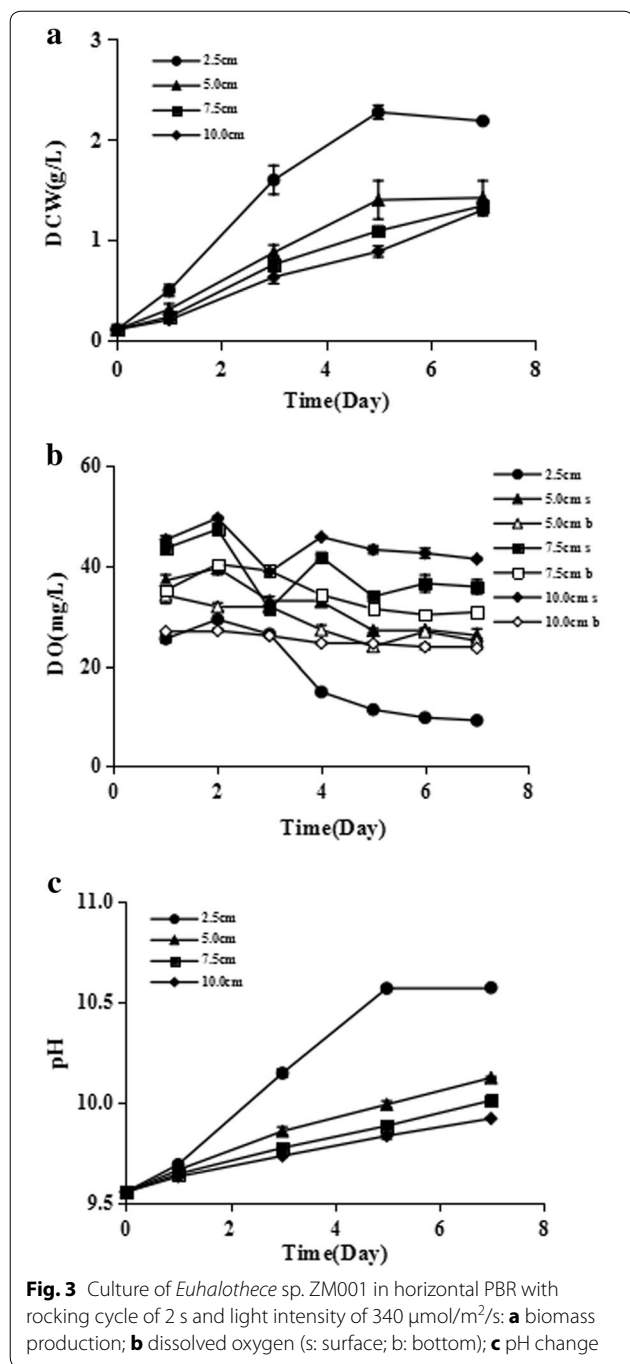
Culture at high light intensity and long rocking cycle

High light intensity of $340 \mu\text{mol}/\text{m}^2/\text{s}$ and longer rocking cycle of 2 s was tested in this experiment. The results show that maximum DO observed during the whole culture process was 29.3, 39.6, 47.3, and 49.6 mg/L for 2.5, 5.0, 7.5, and 10 cm of culture depth, respectively (Fig. 3b).



Compared with experiment above, DO was accumulated to a much higher level and it was often over-saturated, indicating that 2-s rocking cycle was not enough to provide sufficient mixing.

Final biomass concentration in cultures with 2.5 and 5.0 cm depth was 2.18 and 1.42 g/L, respectively, which was lower than its counterpart with 1 s rocking cycle.



However, in culture depth of 7.5 and 10 cm, the final biomass concentration was 1.34 and 1.29 g/L, respectively, which was higher than its counterpart with 1-s rocking cycle. It was observed that there was no good circulation flow in 7.5 and 10.0 cm culture when rocking cycle of 1 s was applied. This may result in limited cell movement between light and dark zone, and probably be the reason

why they resulted in lower biomass production than that of longer rocking cycle in this experiment.

Similar to above experiment with short rocking cycle, there was significant difference between DO at surface and bottom ($p < 0.05$) for the all culture days in culture with 10 cm depth. The maximum difference was 22.6 mg/L in day 2, and this difference is even greater than that in above experiment with rocking cycle of 1 s (Fig. 3b). Compared with 10 cm culture, the maximum difference of DO between surface and bottom in 5.0 and 7.5 cm cultures was much less (Fig. 3b).

Culture at medium light intensity and long rocking cycle

Medium light intensity of 135 $\mu\text{mol}/\text{m}^2/\text{s}$ and 2-s rocking cycle was tested in this experiment. At this condition, final biomass concentration reached 2.12, 1.15, 0.75, and 0.70 g/L for 2.5, 5.0, 7.5, and 10 cm culture depth, respectively (Fig. 4a). Final biomass concentration was obviously lower than that with high light intensity for 5.0, 7.5, and 10 cm cultures, but not 2.5 cm.

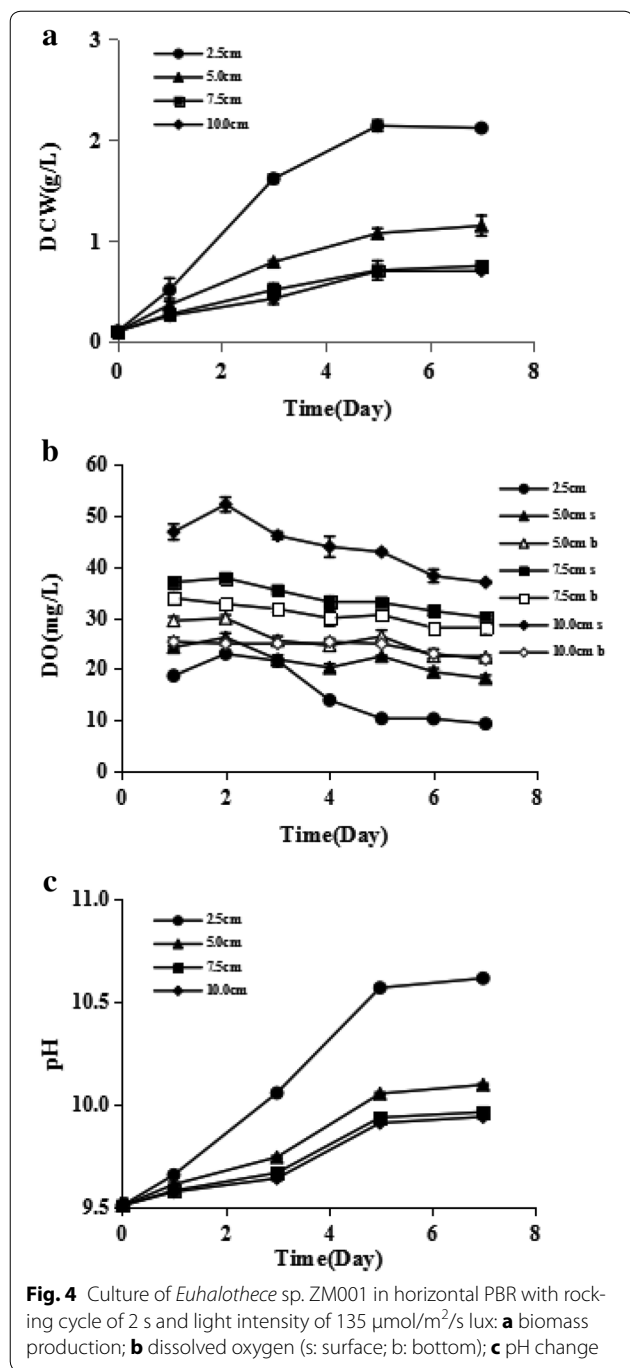
The maximum DO observed in this experiment was 23.0, 30.0, 37.9, and 52.2 mg/L for 2.5, 5.0, 7.5, and 10 cm cultures, respectively. The DO difference between surface and bottom in 5.0 and 7.5 cm cultures was small, which was no more than 5.2 and 5.1 mg/L, respectively. However, this difference in 10 cm culture was still high (Fig. 4b).

Culture at low light intensity and long rocking cycle

In this experiment, culture was tested with low light intensity of 70 $\mu\text{mol}/\text{m}^2/\text{s}$ and rocking cycle of 2 s. The microalgae final biomass concentration was 1.47, 0.99, 0.62, and 0.62 g/L for 2.5, 5.0, 7.5, and 10 cm culture depth, respectively (Fig. 5a). These final biomass concentrations were much lower than their counterpart with high light intensity, since low light intensity input less energy to the culture. The maximum DO was 23.9, 26.5, 32.1, and 23.4 mg/L for 2.5, 5.0, 7.5, and 10 cm cultures (Fig. 5b), indicating less oxygen is accumulated. Also, there was no significant difference between DO of surface and bottom for all the culture depths. DO was at the level of 20–30 mg/L during the whole culture process for all culture depths.

Oxygen tolerance of *Euhalothece* sp. ZM001

It is interesting that *Euhalothece* sp. ZM001 resulted in growth even when DO was over-saturated in the above experiments. To verify its tolerance to oxygen, it was cultured in bubble column with pure oxygen sparging. As the result show in Fig. 6, the final biomass concentration was 1.61 and 3.16 g/L for cultures bubbled with pure oxygen and air, respectively. When continuous bubbling



with oxygen, DO in the whole culture process was kept from 32.6 to 39.7 mg/L. The pH of these two cultures was always kept below 10.5, indicating that the difference between the two cultures' biomass production was not caused by pH. These results showed that high DO does affect growth of *Euhalotheca* sp. ZM001, but it still grew to a density of 1.69 g/L (13 days), indicating it as an excellent species tolerant to high oxygen level.

k_La measurement at different culture conditions

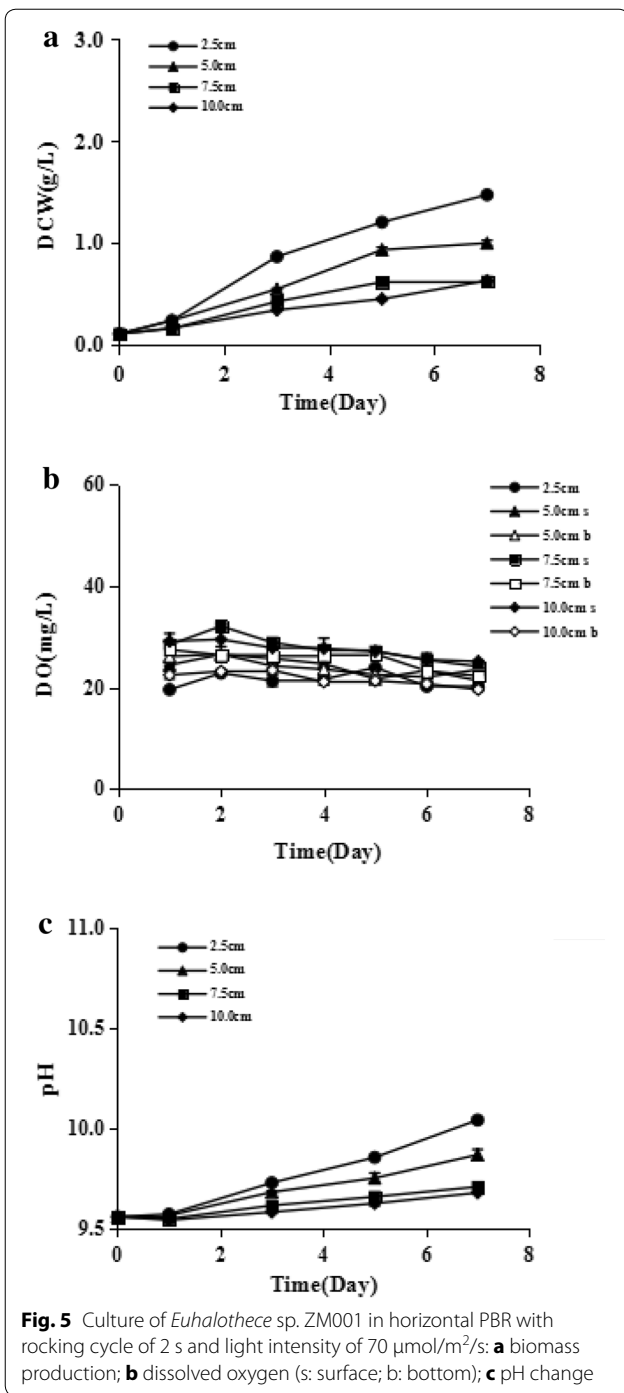
To investigate mass transfer rate at different culture depths and rocking cycles, the k_La in each condition is measured and shown in Table 1. With 1-s rocking cycle, the 2.5 cm culture depth had the highest k_La of 33.49 h^{-1} . This result is corresponding to DO accumulation, which was always below 12.1 mg/L in culture at light intensity of 340 $\mu\text{mol}/\text{m}^2/\text{s}$. Compared with this, k_La of 5.0 and 10 cm cultures was 5.76 and 3.86 h^{-1} , and the maximum DO was 29.8, and 20.8 mg/L, respectively (Fig. 1b). 7.5 cm culture had lowest k_La of 1.04 h^{-1} , and this is the direct reason why it resulted in high DO level, and the maximum observed DO was 36.4 mg/L (Fig. 1b).

For the rocking cycle of 2 s, 2.5 cm depth had a low k_La of 1.50 h^{-1} , and the maximum DO observed at 340 $\mu\text{mol}/\text{m}^2/\text{s}$ light intensity was 29.3 mg/L. Compared with this, 5.0, 7.5, and 10 cm cultures with lower k_La all resulted in high level of DO accumulation, and the maximum observed DO was 39.6, 47.3, and 49.6 mg/L, respectively. The high DO directly affect algae growth, as indicated in oxygen tolerance experiment, and led to lower biomass concentration.

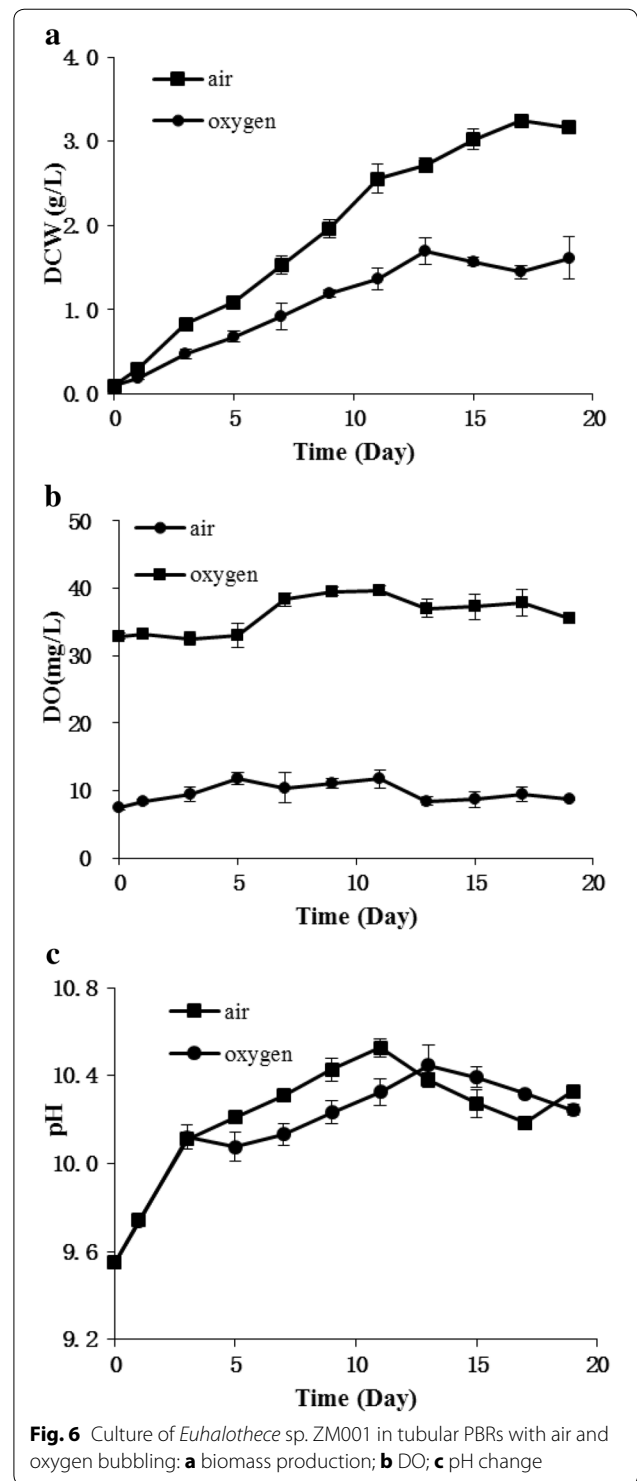
It can be learned from Table 1 that k_La increases with the decrease of culture depth. This should be attributed to two reasons. On one hand, lower culture depth has a larger surface/volume ratio, which increases the value of "a" in k_La . On the other hand, thinner water in horizontal PBR has better circulation flow, and it increase the value of " k_L " in k_La . However, 7.5 cm depth has a much lower k_La than 10 cm at both rocking cycles, and this should be attributing to its poor circulation flow, which was observed during this k_La measurement test.

Outdoor culture at 1 m^2 scale

At the scale of 1 m^2 , rocking cycle of the platform driven by water power was measured as 60 s, which is quite different from that of small-scale PBR. This cycle is dependent to time spent for water flow into the tank and discharge. *Euhalotheca* sp. ZM001 was inoculated into the plastic bag PBR at 0.1 g/L. After experiencing a long lag phase, this culture resulted in a final biomass concentration of 1.88 g/L (Fig. 7). The average daily biomass productivity can be calculated as 0.08 g/L/day or 4.2 $\text{g}/\text{m}^2/\text{day}$. During the culture process, the high temperature was from 24 to 36 $^\circ\text{C}$, and the low temperature during the night was from 14 to 22 $^\circ\text{C}$. *Euhalotheca* sp. ZM001 has optimal growth between 35 and 40 $^\circ\text{C}$. This temperature range experienced in outdoor culture was actually not ideal and resulted in low average productivity. However, it is notable that the maximum daily biomass productivity was 0.4 g/L/day or 20 $\text{g}/\text{m}^2/\text{day}$, which is obtained from day 13 to 15 (Fig. 7).



pH change during this culture was from 9.56 to 9.89. With rocking cycle of 60 s, the $k_L a$ was measured as 0.89 h^{-1} . Due to this low mass transfer rate, the DO accumulated to a high level during the day time, and the recorded high was 35.6 mg/L. As an oxygen-tolerant strain, it still resulted in growth to 1.88 g/L final biomass concentration at this high DO level. This indicates that



horizontal PBR mixed with rocking movement driven by water power is feasible to support growth of alkalihalophilic cyanobacterium of *Euzhalotheca* sp. ZM001, but the oxygen transfer rate should be improved to obtain higher productivity.

Table 1 $k_L a$ at different culture depths and rocking cycles in small-scale horizontal PBR

Culture depth (cm)	$k_L a$ (h^{-1})	
	1-s rocking cycle	2-s rocking cycle
2.5	33.49	1.50
5.0	5.76	1.15
7.5	1.04	0.57
10	3.86	0.84

Discussion

This study aims to develop a horizontal PBR system mixed with rocking movement driven by nature force, and these preliminary results showed the feasibility of it. Plastic bag can be used as horizontal PBR for this system, and culture depth no more than 10 cm will not face high water pressure problems as experienced in vertical PBRs. Plastic bag PBR has the advantage of low cost, and it can be simply disposed when facing fouling problem. The shape of plastic bag horizontal PBR culture system developed in this study is similar to closed pond. However, it is difficult to construct a pond with culture depth of only 10 cm or less, and it is difficult to use paddle wheel to drive the mixing in such shallow water. Instead, mixing in horizontal PBR can be driven by rocking movement, and much less culture depth can be used. This short light path resulted in much higher biomass concentration than closed pond, as shown in this study.

Since high concentration of bicarbonate supplied sufficient carbon “once for all” at beginning of the culture, it is not necessary to continuously bubbling CO₂ during algae culture process. This not only allows a simpler PBR design, but also reduces the cost of gas pipeline construction in massive culture, as well as the related cost for maintenance and labor utilization. It should be noted that using bicarbonate as the carbon source would not have a higher cost than bubbling CO₂, since bicarbonate only work as a carrier of CO₂ and there is no net consumption of it. When algal culture process finished, part of bicarbonate is converted into carbonate, and it can be recycled to absorb more CO₂ and supply into the next batch of algal culture (Chi et al. 2011; Chisti 2013). Thus, this culture system would have a low algal biomass production cost on the whole, since it systematically reduced the PBR manufacturing cost, mixing energy cost, carbon supplying cost, and labor utilization cost.

As a preliminary study, the algal biomass productivity of this system is still low. The average areal productivities for the cultures at small scale are summarized and shown in Table 2. In the indoor culture, the highest areal productivity was 17.06 g/m²/day, which is obtained in the culture with 10 cm depth, 340 μmol/m²/s light intensity, and 2-s rocking cycle. In the outdoor culture at the scale of 1 m², the average productivity was 4.2 g/m²/day. However, the maximum daily biomass productivity was 0.4 g/L/day or 20 g/m²/day (Fig. 7), indicating that the productivity could be great improved with further study.

Since high concentration of bicarbonate supplied plenty of inorganic carbon, the mass transfer of carbon is no longer a problem for this system. The problem for oxygen, however, is still to be studied, since it may cause photo-inhibition when accumulated to high level (Peng et al. 2013). In this study, there was great variation on

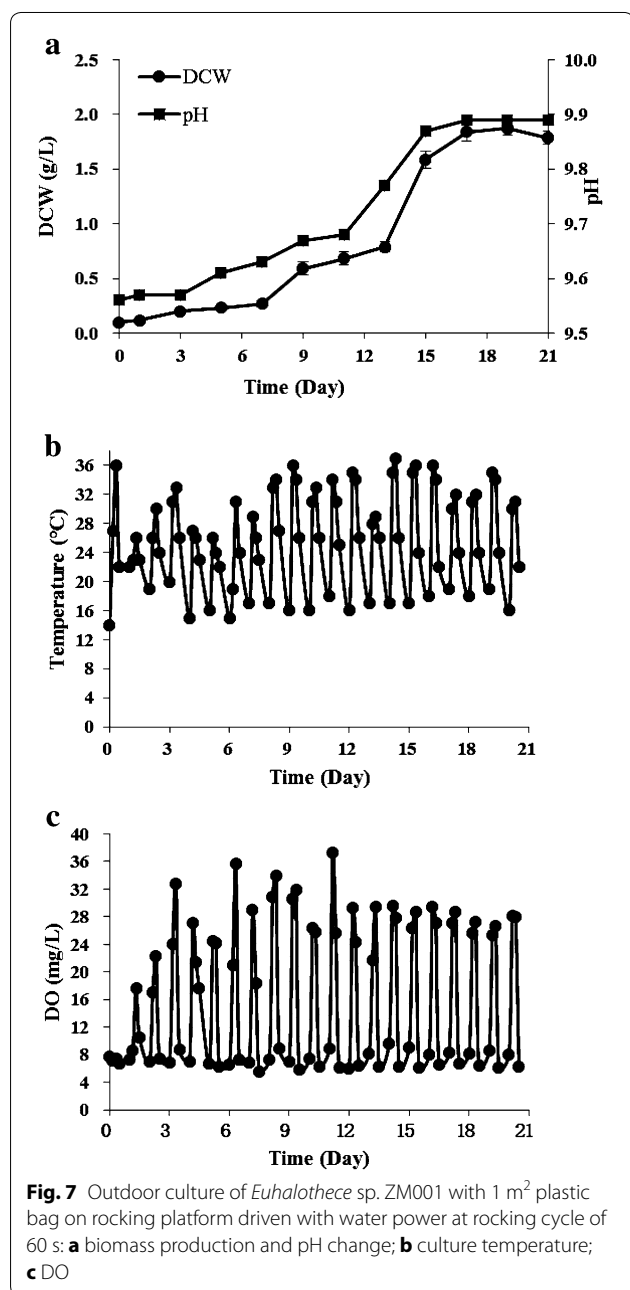


Table 2 Average productivity of different culture conditions (g/m²/day)

	2.5 cm	5.0 cm	7.5 cm	10 cm
340 $\mu\text{mol}/\text{m}^2/\text{s}$, 1 s	9.39	11.89	10.24	12.58
340 $\mu\text{mol}/\text{m}^2/\text{s}$, 2 s	7.44	9.40	13.24	17.06
135 $\mu\text{mol}/\text{m}^2/\text{s}$, 2 s	7.20	7.48	6.96	8.62
70 $\mu\text{mol}/\text{m}^2/\text{s}$, 2 s	4.89	6.39	5.54	7.50

k_La values in small-scale horizontal PBRs. The maximum k_La measured was 33.49 h^{-1} in culture with 2.5 cm depth and rocking cycle of 1 s. This value is at the same level as bubble columns, which was from 16.2 to 50.4 h^{-1} (Peng et al. 2013). With the same rocking cycle, the k_La in 5 and 10 cm cultures was 5.76 and 3.86 h^{-1} . These values are comparable with horizontal tubular PBR, which was reported as from 3.6 to 10.8 h^{-1} (Peng et al. 2013). However, with still the same rocking cycle of 1 s, k_La in 7.5 cm culture was only 1.04 h^{-1} . This is at the same level as raceway pond, which is reported as 0.87 and 0.94 h^{-1} in the straight and curved channel part (Mendoza et al. 2013a, b). When longer rocking cycle of 2 s was used, the ranges of k_La were from 0.57 to 1.50 h^{-1} , and the DO often accumulated to very high level, indicating that it actually did not provide sufficient mixing. This is also true for the culture at 1.0 m² scale, which had a k_La of only 0.89 h^{-1} at the rocking cycle of 60 s.

Although high biomass concentration was obtained in culture of oxygen-tolerant *Euhalothece* sp., it is actually not practical to culture oxygen-sensitive algal strains if mixing is not sufficient. Thus, the capability of oxygen mass transfer in horizontal PBR should be improved in future study. This may be realized by optimizing the angle and cycle of horizontal PBR's rocking movement. Also, the length of PBR would be a significant factor to mixing condition, since it can significantly change the flow field. In addition, baffles in horizontal PBR or open pond can change vertical flow, which was reported to improve mass transfer and frequency of light/dark cycle, and resulted in significant higher biomass concentration than its control (Zhang et al. 2015). Thus, mixing and mass transfer in horizontal PBR driven by rocking movement deserve intensive study in future. An interesting phenomenon observed in this study is that there was great difference between DO at the culture surface and bottom when culture depth was 7.5 cm or greater. This may also occur in open pond system, and special attention should be paid to this issue in study on mixing in horizontal PBR, as well as its design and operation.

Different algae species have different capabilities of tolerance to oxygen. For instance, when DO was elevated to a level higher than 13 mg/L, the final biomass

concentration was substantially reduced in culture of *Chlorella sorokiniana* (Ugwu et al. 2007), while cell death occurred only when DO reached 36 mg/L for *Spirulina platensis* Vonshak et al. (1996). It is interesting that *Euhalothece* sp. ZM001 has extraordinary tolerance capability to high DO. It resulted in growth to 1.69 g/L even with pure oxygen bubbling. Due to this character, it may be an excellent algae strain to be applied in life support system for oxygen production in spacecraft or submarine. Besides, this algae strain also has excellent capability of tolerant to high temperature, high pH, and high salt concentration (Chi et al. 2011; Chisti 2013). The tolerance mechanism is a great research interest, which is worthy of studying in depth.

This study tested horizontal PBR at the scale of 1 m², and its scaling-up requires rocking platform with much larger size. A possible solution is to make rocking platform with the size of raceway pond, which is usually several meters in width and several tens of meters in length. This would result in rocking platform at the size of several hundred square meters. Driving rocking movement of this large-scale platform would not be a big problem, since falling water can provide intensive mechanical power and was used to drive heavy machines before electricity is popularized. Using hundreds of these rocking platforms would realize massive cultures at the scale of hectares. Construction of rocking platforms at this scale may be costly, but it would not be greater than horizontal tubular or vertical flat plate PBR systems at the same scale, in authors' opinion. If hydraulic power is used to drive this system, the cultivation field should be built at the site with plenty of water resources. Also, this horizontal PBR can be placed on water surface of ocean, river, or lake, driving rocking movement with wave, which is actually an ongoing research conducted by the authors. In addition, its rocking movement may be driven with many other nature forces, such as tide or wind, as long as they can provide mechanical power to drive a back/forth movement. There were actually many mature technologies to realize this before electricity power is popularized. Using nature force to drive the mixing would significantly improve energy balance for algal biomass production, which is a great hurdle in the commercialization of algal biofuel.

Conclusion

The algal culture system developed in this study proved feasibility of using nature force to drive movement of rocking platform to realize mixing in horizontal PBR. Low-cost plastic bag can be used in this system to reduce the manufacturing cost of horizontal PBR. This PBR well supported growth of alkalihalophilic microalgae *Euhalothece* sp. ZM001 without CO₂ gas bubbling.

Abbreviations

BICCAPS: bicarbonate-based integrated carbon capture and algae production system; DO: dissolved oxygen; $k_L a$: mass transfer coefficient; PBR: photobioreactor.

Authors' contributions

HZ conducted most microalgae culture work; CZ conducted analysis work on mass transfer coefficient measuring; LC participated in making photobioreactor and conducted part of microalgae culture work. ZC is the corresponding author. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data obtained in this study are presented in this manuscript. Raw data are available in the authors' group, which can be provided if requested.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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References

- Abu-Ghosh S, Fixler D, Dubinsky Z, Iluz D (2016) Flashing light in microalgae biotechnology. *Bioresour Technol* 203:357–363
- Acién Fernández FG, Fernández Sevilla JM, Molina Grima E (2013) Photobioreactors for the production of microalgae. *Rev Environ Sci Bio-Technol* 12(2):131–151
- Acién FG, Fernández JM, Magan JJ, Molina E (2012) Production cost of a real microalgae production plant and strategies to reduce it. *Biotechnol Adv* 30(6):1344–1353
- Chi Z, O'Fallon JV, Chen S (2011) Bicarbonate produced from carbon capture for algae culture. *Trends Biotechnol* 29(11):537–541
- Chi Z, Xie Y, Elloy F, Zheng Y, Hu Y, Chen S (2013) Bicarbonate-based Integrated Carbon Capture and Algae Production System with alkalihalophilic cyanobacterium. *Bioresour Technol* 133:513–521
- Chi Z, Elloy F, Xie Y, Hu Y, Chen S (2014) Selection of microalgae and cyanobacteria strains for bicarbonate-based integrated carbon capture and algae production system. *Appl Biochem Biotechnol* 172(1):447–457
- Chisti Y (2013) The problems with algal fuels. *Biotechnol Bioeng* 110(9):2318–2319
- Davis W, Martín M (2014) Optimal year-round operation for methane production from CO₂ and water using wind and/or solar energy. *J Clean Prod* 80:252–261
- de Godos I, Mendoza JL, Acién FG, Molina E, Banks CJ, Heaven S, Rogalla F (2014) Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases. *Bioresour Technol* 153:307–314
- Dogaris I, Welch M, Meiser A, Walmsley L, Philippidis G (2015) A novel horizontal photobioreactor for high-density cultivation of microalgae. *Bioresour Technol* 198:316–324
- Jorquera O, Kiperstok A, Sales EA, Embirucu M, Ghirardi ML (2010) Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresour Technol* 101(4):1406–1413
- Kim ZH, Park H, Hong SJ, Lim SM, Lee CG (2016) Development of a floating photobioreactor with internal partitions for efficient utilization of ocean wave into improved mass transfer and algal culture mixing. *Bioprocess Biosyst Eng* 39(5):713–723
- Mendoza JL, Granados MR, de Godos I, Acién FG, Molina E, Banks C, Heaven S (2013a) Fluid-dynamic characterization of real-scale raceway reactors for microalgae production. *Biomass Bioenergy* 54:267–275
- Mendoza JL, Granados MR, de Godos I, Acién FG, Molina E, Heaven S, Banks CJ (2013b) Oxygen transfer and evolution in microalgal culture in open raceways. *Bioresour Technol* 137:188–195
- Mikhodyuk OS, Gerasimenko LM, Akimov VN, Ivanovsky RN, Zavarzin GA (2008) Ecophysiology and polymorphism of the unicellular extremely natronophilic cyanobacterium *Eubhalothece* sp. Z-M001 from Lake Magadi. *Microbiology* 77(6):717–725
- Norsker N-H, Barbosa MJ, Vermue MH, Wijffels RH (2011) Microalgal production—a close look at the economics. *Biotechnol Adv* 29(1):24–27
- Peng L, Lan CQ, Zhang Z (2013) Evolution, detrimental effects, and removal of oxygen in microalga cultures: a review. *Environ Prog Sustain Energy* 32(4):982–988
- Tredici MR, Bassi N, Prussi M, Biondi N, Rodolfi L, Chini Zittelli G, Sampietro G (2015) Energy balance of algal biomass production in a 1-ha "Green Wall Panel" plant: how to produce algal biomass in a closed reactor achieving a high Net Energy Ratio. *Appl Energy* 154:1103–1111
- Ugwu CU, Aoyagi H, Uchiyama H (2007) Influence of irradiance, dissolved oxygen concentration, and temperature on the growth of *Chlorella sorokiniana*. *Photosynthetica* 45(2):309–311
- Vonshak A, Torzillo G, Accolla P, Tomaselli L (1996) Light and oxygen stress in *Spirulina platensis* (cyanobacteria) grown outdoors in tubular reactors. *Physiol Plant* 97:175–179
- Zhang Q, Xue S, Yan C, Wu X, Wen S, Cong W (2015) Installation of flow deflectors and wing baffles to reduce dead zone and enhance flashing light effect in an open raceway pond. *Bioresour Technol* 198:150–156

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