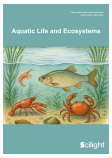
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Hydrocarbon Production of *Botryococcus braunii* in a Photobioreactor: Optimization of Irradiance and Mixing Regimes

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Abstract: The slow growth of *Botryococcus braunii* impedes its large-scale cultivation as a feedstock for biofuel production despite its high hydrocarbon content. This study investigates how light and mixing regimes affect the growth performance of this green alga in a newly designed flat-plate photobioreactor. Three light intensities (75, 150 and 300 μmol s^{-1} m^{-2}) and three mixing rates (0.95, 1.10 and 1.25 L L^{-1} min^{-1}) were used to evaluate algal biomass and hydrocarbon production. The algal performance was then compared with a static culture under the same light and mixing conditions as in the photobioreactor. The results showed that light and mixing regimes significantly affected both biomass and hydrocarbon production in *B. braunii*. The best algal performance was obtained under high irradiance (300 μmol s^{-1} m^{-2}) and medium mixing (1.10 L L^{-1} min^{-1}). Mixing had no significant effect on biomass and hydrocarbon production under low light intensity (75 μmol s^{-1} m^{-2}), while the high mixing rate (1.25 L L^{-1} min^{-1}) reduced algal biomass at all light intensities. We again compared algal performance between the photobioreactor and the static culture system under the same light and mixing conditions to evaluate system efficacy. The photobioreactor produced 4.04- and 1.97-fold higher algal biomass and hydrocarbon yields, respectively, than the static culture. An independent study validated these results, showing that biomass growth and hydrocarbon production of *B. braunii* in the photobioreactor were 3.97-fold and 1.91-fold higher than in static culture, respectively. Therefore, a photobioreactor can increase biomass and hydrocarbon production in this slow-growing *B. braunii* under laboratory conditions, offering a promising strategy for future exploration of oil-producing algal species towards commercial production.

Keywords: algae; photobioreactor; light; mixing; biomass; hydrocarbon

1. Introduction

Researchers have investigated the feasibility of using the green microalga *Botryococcus braunii* as an alternative renewable energy source for decades [1–3]. The lipid content of this alga ranges from 20% to over 50% of dry weight, depending on environmental conditions and *B. braunii* strains [4]. Despite its high hydrocarbon content, slow growth makes the biomass production of *B. braunii* too expensive to be economically feasible for

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energy production. Recent interest in microalgae farming has shifted toward developing open-air mass cultures, leading to the construction of large-scale production facilities worldwide [4]. Open-culture systems are inexpensive to operate, but they are prone to contamination by other species and to unpredictable patterns of algal succession. As the growth of the desired species can be competitively suppressed by unwanted algal species, it is recommended that highly desired algal species be maintained in closed photobioreactors [5].

The design of a closed photobioreactor needs to consider the unique biological requirements and environmental conditions of the targeted algal species [6]. *Botryococcus braunii* is a colonial, non-flagellated green microalga that requires mixing to keep the alga in suspension when the algal oil content is insufficient to keep the alga floating [7]. Thin, flat photobioreactors are commonly used to grow non-flagellated microalgae because of their ready access to light. The construction of flat-plate photobioreactors dates back to the early 1950s [8]. The flat-plate photobioreactor has important advantages for algal growth because its high surface area-to-volume ratio allows efficient light penetration. The front side receives direct light, and the other sides are illuminated by a homogeneous, low-energy photon flux via diffuse and reflected light for algal photosynthesis.

One major concern in microalgal cultivation is efficiently using light to produce algal biomass and secondary metabolites. In a model simulation, algal growth depends on light intensity when nutrients are not limited [9] and light can regulate algal growth and production of secondary products [10,11]. Zhang and Kojima [12] firstly examined the influence of light intensity on the productivity of *B. braunii*. They found that a low light intensity of 47 μmol s^{-1} m^{-2} limited growth, whereas a high light intensity of 740 μmol s^{-1} m^{-2} inhibited growth and reduced chloroplast size. In a photobioreactor culture, Zhang and Kojima [12] found that *B. braunii* colony size decreased with reduced light illumination, but the exact range of light intensity that caused this effect has not been further studied.

In a photobioreactor culture system, optimizing mixing conditions is also essential for algal production efficacy because mixing ensures the homogeneous distribution of microalgal cells, prevents thermal stratification of the medium, and breaks down nutrient gradients [13]. Mixing can also remove photosynthetically generated oxygen that inhibits algal growth and reproduction [14]. In outdoor microalgal cultures, physical mixing can markedly increase *Spirulina* sp. productivity [15] and *Chlorella* culture [16]. However, the proliferation of other algae, such as dinoflagellates, is sensitive to turbulence and mechanical stress, which can disrupt phases of the cell reproductive cycle [17,18]. Similarly, the growth of a multicellular green alga *Scenedesmus quadricauda* is also inhibited when the level of turbulence in the water column increases [19].

Although the scientific literature highlights that light and mixing can regulate algal growth and productivity, the interactive effects of light and mixing on algal growth and hydrocarbon production have rarely been tested in photobioreactors. In this study, we used the slow-growing green alga *B. braunii* to test its response to light and mixing and to identify optimal regimes for producing this oily alga in a newly designed flat photobioreactor. Algal performance was then compared between a cylindrical bottle and the photobioreactor in terms of algal biomass and hydrocarbon production under the same light and mixing regime. This study illustrates a practical method to increase the production of slow-growing algae by using a photobioreactor under optimal light and mixing conditions.

2. Materials and Methods

2.1. Algal Stock Culture

The *Botryococcus braunii* (UTEX 572) was obtained from the University of Texas Culture Collection (Austin, TX, USA) of Algae. The microalgal stock was cultured in Schott® bottles (Schott AG, Hattenbergstrasse 10, Mainz, Germany) at 24 ± 1 °C in an incubator with a 12 h light/12 h dark photoperiod, illuminated by fluorescent lights at a photon flux density of 150 μmol s^{-1} m^{-2}. Air was filtered through 0.45-μm pores into the Schott® bottle to maintain a homogeneous algal distribution. The growth medium for algal culture was Bold 3N [20], which has been shown to be suitable for culturing this alga [21]. Algae were separately introduced into the flat plate photobioreactor and static bottle cultures when algal growth in the stock culture reached the exponential phase. The inoculating algal density in both the photobioreactor and the Schott® bottle was 0.505 ± 0.02 g L^{-1}.

2.2. Photobioreactor Design

Figure 1 (left) shows a schematic diagram of the newly designed flat photobioreactor and its dimensions. The flat-plate photobioreactor, measuring 72 cm high, 20 cm long, and 3 cm internally wide, with a culture volume of 3200 mL (surface area of 1160 cm^2, yielding a surface/volume ratio of 0.363 cm^{-2} mL^{-1}), was used to culture *B. braunii*. The lid had ports for adding inoculum (A), fresh media (B), sampling (C), and an air outlet (D). The base had two gas spargers (E and F) on each side to achieve a relatively uniform bubble-size distribution (Figure S1 and Figure S2). The Schott® bottle with a 1800-mL culture volume, a surface area of 187.5 cm^2, and a

surface/volume ratio of 0.104 cm^2 mL^{-1} was sketched in Figure 1 (right) and used in parallel with the photobioreactor as a system control. The aeration port (G) and air outlet port (H) were in the middle of the stopper.

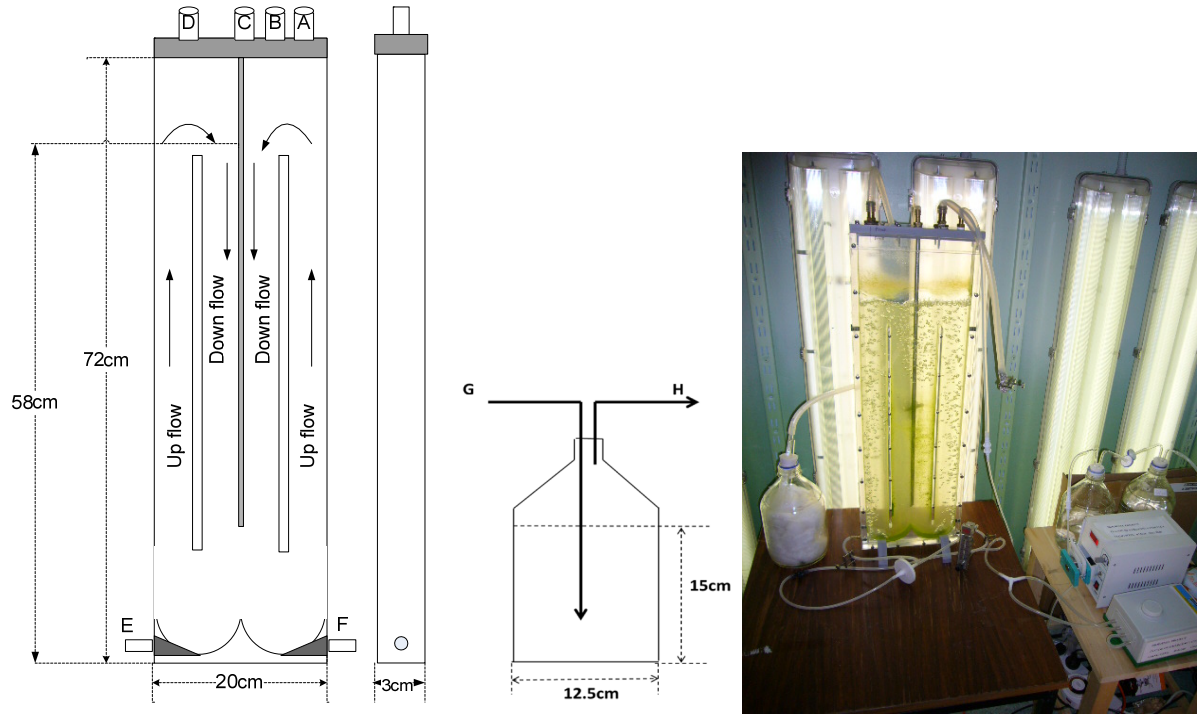


Figure 1. Left (photobioreactor): The schematic diagram of the flat photobioreactor showing the passages for inoculum (A), fresh media (B) and water sampling (C), and air (D), and two gas spargers (E and F); **Right** (static bottle): The schematic diagram of a cylindrical bottle showing the in (G) and out (H) air passages. The lighting and photobioreactor are shown on the right.

2.3. Experimental Design

This study used a factorial design with nine combinations of three irradiance levels (75, 150, and 300 μmol s^{-1} m^{-2}) and three mixing rates (0.95, 1.10, and 1.25 L L^{-1} min^{-1}). Fluorescent light tubes (Philips Fluro Tube White 40 W 600 mm, Lincoln, NE, USA) illuminated the reactor, and we achieved three light-intensity levels by varying the distance between the light source and the reactor to reach the target irradiance levels. We used a Li-190SA quantum light meter (LI-COR Biosciences, Lincoln, NE, USA) to measure irradiance levels. We obtained three mixing rates by adjusting the volume of air injected into the experimental systems, which indicated water mixing. To measure the liquid mixing rate within the culture vessels at various airflow rates, we used a colored tracer to estimate the rate of liquid dispersal in the system. After preliminary trials, mixing rates of 0.95, 1.10, and 1.25 litres of air injected per min into a litre of culture vessel (L L^{-1} min^{-1}) were chosen for the mixing-manipulation levels.

To compare the microalgal performance of this flat photobioreactor with a conventional culture system, we inoculated a 1.8 L cylindrical bottle with the same algal culture and ran identical experiments in parallel as a reference. Because we constructed and used only one photobioreactor in this study, we sequentially tested its efficacy in growing algae under nine light and mixing regimes for 15 days each, for a total study duration of 135 days. For each run, we took three parallel samples from the system on each sampling day. This testing protocol for evaluating photobioreactor performance for algal cultivation has been widely used in similar studies [22,23].

After identifying the optimal light and mixing conditions as 300 μmol s^{-1} m^{-2} and 1.10 L L^{-1} min^{-1}, we validated these conditions by conducting an independent study in the flat-plate photobioreactor and the Schott® bottle. In both culture systems, *B. braunii* was inoculated separately to compare algal growth and hydrocarbon production at the same algal density as described above. This independent study was conducted for 15 days using the same experimental protocols.

2.4. Determination of Algal Biomass

For each experimental run with different treatment combinations, the algal cell suspension was sampled at 3-day intervals over 15 days, and the algal biomass was collected by vacuum filtration onto pre-weighed Whatman®

GF/C filters [24]. The filters with algal cells were freeze-dried, measured gravimetrically and expressed as biomass production per litre of algal culture (g L^{-1}).

2.5. Determination of Hydrocarbon Production

Hydrocarbons in the dry microalgal biomass were extracted on glass filters with *η*-hexane [24]. Solvents were removed from the extracts using a rotary evaporator, and the residue was rinsed with a small amount of *η*-hexane. Hydrocarbon fractions were purified by passing the samples through an alumina gel plug and eluting with *η*-hexane. Solvents were evaporated to dryness under a nitrogen stream and pure hydrocarbon fractions were measured gravimetrically and expressed as hydrocarbon production (g L^{-1}).

2.6. Data Analysis

All values reported in the tables and figures were the mean of three replicate treatments with standard error. Two-way ANOVA was separately used at the end of the exponential phase (day 9) and the end of the stationary phase (day 15) to analyze the effects of light and mixing levels and their interactive effect on algal biomass and hydrocarbon (SPSS ver. 18.0). To compare the microalgal growth pattern over time, data on each sampling day between nine treatment combinations were analyzed using a one-way ANOVA and Tukey test to identify the best light and mixing combination supporting the algal growth and hydrocarbon production. To evaluate the system efficacy, a *t*-test was used to compare microalgal performance between the photobioreactor and bottle cultures in an independent study, in which the optimal light and mixing regime was identified from early trials conducted in the photobioreactor. The statistical significance was set at $p < 0.05$.

3. Results

The biomass of *B. braunii* under different light and mixing conditions was harvested from the photobioreactor and recorded on each sampling day throughout the experimental period (Table 1). On the day of inoculation, no significant differences were observed across all treatments ($p > 0.05$). However, light, mixing, and their interaction significantly affected microalgal biomass from day 3 to the end of the experiment ($p < 0.05$). The highest biomass was obtained in the treatment combination of high irradiance (300 μmol s^{-1} m^{-2}) and medium mixing rate (1.10 L L^{-1} min^{-1}), whereas the lowest biomass occurred in the treatment combination of low irradiance (75 μmol s^{-1} m^{-2}) and high mixing (1.25 L L^{-1} min^{-1}) throughout the experimental period after the day of algal inoculation (Table 1). The growth of *B. braunii* exhibited two distinct growth phases, namely an exponential phase (0–9 days) and a stationary phase (9–15 days; Table 1). By the end of the exponential phase (day 9, Figure 2a) or the end of the stationary phase (day 15, Figure 2b), microalgal growth was significantly affected by mixing ($p < 0.05$) only at medium or higher light intensities (150 or 300 μmol s^{-1} m^{-2}), whereas the mixing rate did not affect algal biomass ($p > 0.05$, Figure 2) at the low light intensity (75 μmol s^{-1} m^{-2}). The high mixing rate (1.25 L L^{-1} min^{-1}) resulted in the lowest biomass across all light intensities ($p < 0.05$).

Table 1. Algal biomass in the photobioreactor under three light intensities and three mixing rates. Different letters in the same column represent significant differences ($p < 0.05$) between treatments. The ANOVA results (bottom) show the probability of difference in the impact of light, mixing and their interactions on each sampling day.

Treatment		Biomass (g L^{-1})					
Light (μmol s^{-1} m^{-2})	Mixing (L L^{-1} min^{-1})	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
75	0.95	0.503 ± 0.006 a	1.300 ± 0.001 de	1.967 ± 0.058 d	2.433 ± 0.115 f	2.133 ± 0.115 e	2.000 ± 0.100 e
75	1.10	0.503 ± 0.031 a	1.367 ± 0.058 de	2.133 ± 0.058 d	2.333 ± 0.058 f	2.367 ± 0.058 e	2.133 ± 0.058 e
75	1.25	0.507 ± 0.021 a	1.033 ± 0.058 e	1.867 ± 0.153 d	2.233 ± 0.208 f	1.900 ± 0.100 e	1.833 ± 0.058 e
150	0.95	0.510 ± 0.010 a	1.700 ± 0.100 cd	2.867 ± 0.153 c	4.300 ± 0.200 d	5.400 ± 0.200 c	5.567 ± 0.208 c
150	1.10	0.507 ± 0.025 a	1.933 ± 0.153 bc	3.633 ± 0.252 b	6.367 ± 0.208 b	7.100 ± 0.100 b	7.567 ± 0.208 b
150	1.25	0.507 ± 0.006 a	1.600 ± 0.100 d	2.800 ± 0.200 c	3.333 ± 0.058 e	3.467 ± 0.058 d	3.267 ± 0.153 d
300	0.95	0.510 ± 0.020 a	2.033 ± 0.153 b	3.900 ± 0.200 b	5.000 ± 0.300 c	7.100 ± 0.300 b	7.367 ± 0.306 b
300	1.10	0.507 ± 0.006 a	2.400 ± 0.200 a	4.700 ± 0.200 a	8.033 ± 0.208 a	8.633 ± 0.321 a	8.867 ± 0.651 a
300	1.25	0.510 ± 0.001 a	1.467 ± 0.115 d	2.733 ± 0.153 c	2.967 ± 0.058 e	3.200 ± 0.100 d	2.933 ± 0.058 d
ANOVA results (*p* value)							
Light		0.848	0.001	0.001	0.001	0.001	0.001
Mixing		0.950	0.001	0.001	0.001	0.001	0.001
Light × mixing		0.998	0.001	0.001	0.001	0.001	0.001

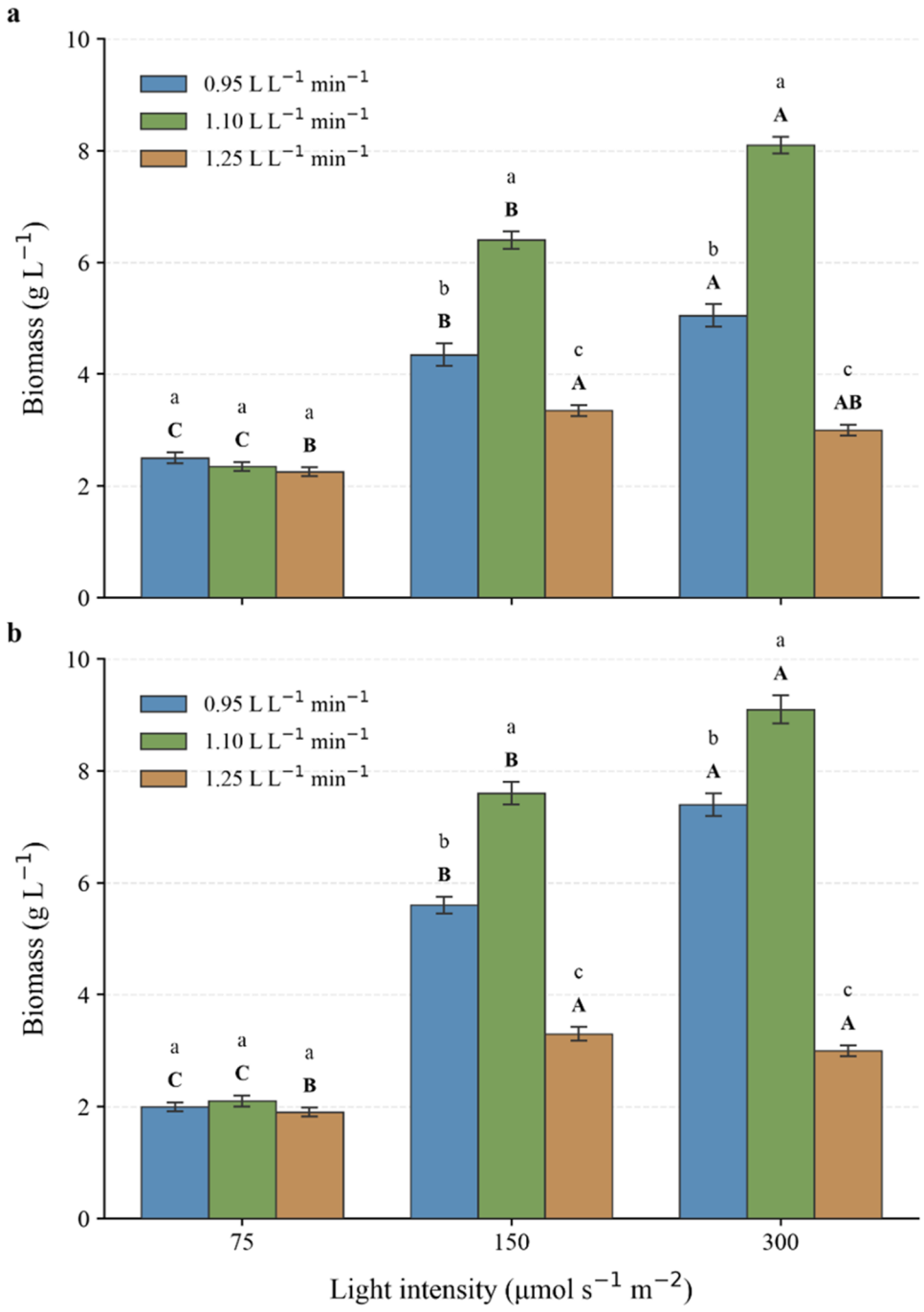


Figure 2. Impact of light and mixing regimes on the biomass of *B. braunii* at the end of (**a**) exponential phase (day 9) and (**b**) stationary phase (day 15) in the photobioreactor. The lowercase letters (a, b, and c) indicate significant differences ($p < 0.05$) between mixing regimes within the same light level, and the uppercase letters (A, B, and C) indicate significant differences ($p < 0.05$) between light regimes within the same mixing level.

The hydrocarbon production pattern of *B. braunii* exposed to different light and mixing regimes in the photobioreactor was similar to the microalgal biomass production response (Table 2). Differences among treatments in algal hydrocarbon production were not significant on the day of inoculation ($p > 0.05$), whereas light, mixing, and their interaction considerably affected hydrocarbon production from the third day to the end of the experiments ($p < 0.05$). The highest hydrocarbon production consistently occurred in the treatment combination of high irradiance (300 μmol s^{-1} m^{-2}) and medium mixing rate (1.10 L L^{-1} min^{-1}), but the lowest hydrocarbon production occurred in the treatment of low irradiance (75 μmol s^{-1} m^{-2}) and high mixing (1.25 L L^{-1} min^{-1}) throughout the experimental period after the day of algal inoculation. By the end of the exponential phase (day 9, Figure 3a) or the end of the stationary phase (day 15, Figure 3b), the production of hydrocarbon was significantly affected by the irradiance and mixing regimes ($p < 0.05$) at the medium to high irradiance (i.e., 150–300 μmol s^{-1} m^{-2}). However,

at the low irradiance level (75 μmol s^{-1} m^{-2}), the evaluated mixing rate did not affect hydrocarbon production ($p > 0.05$). The lowest hydrocarbon production occurred at the high mixing rate (1.25 L L^{-1} min^{-1}), regardless of irradiance level ($p < 0.05$).

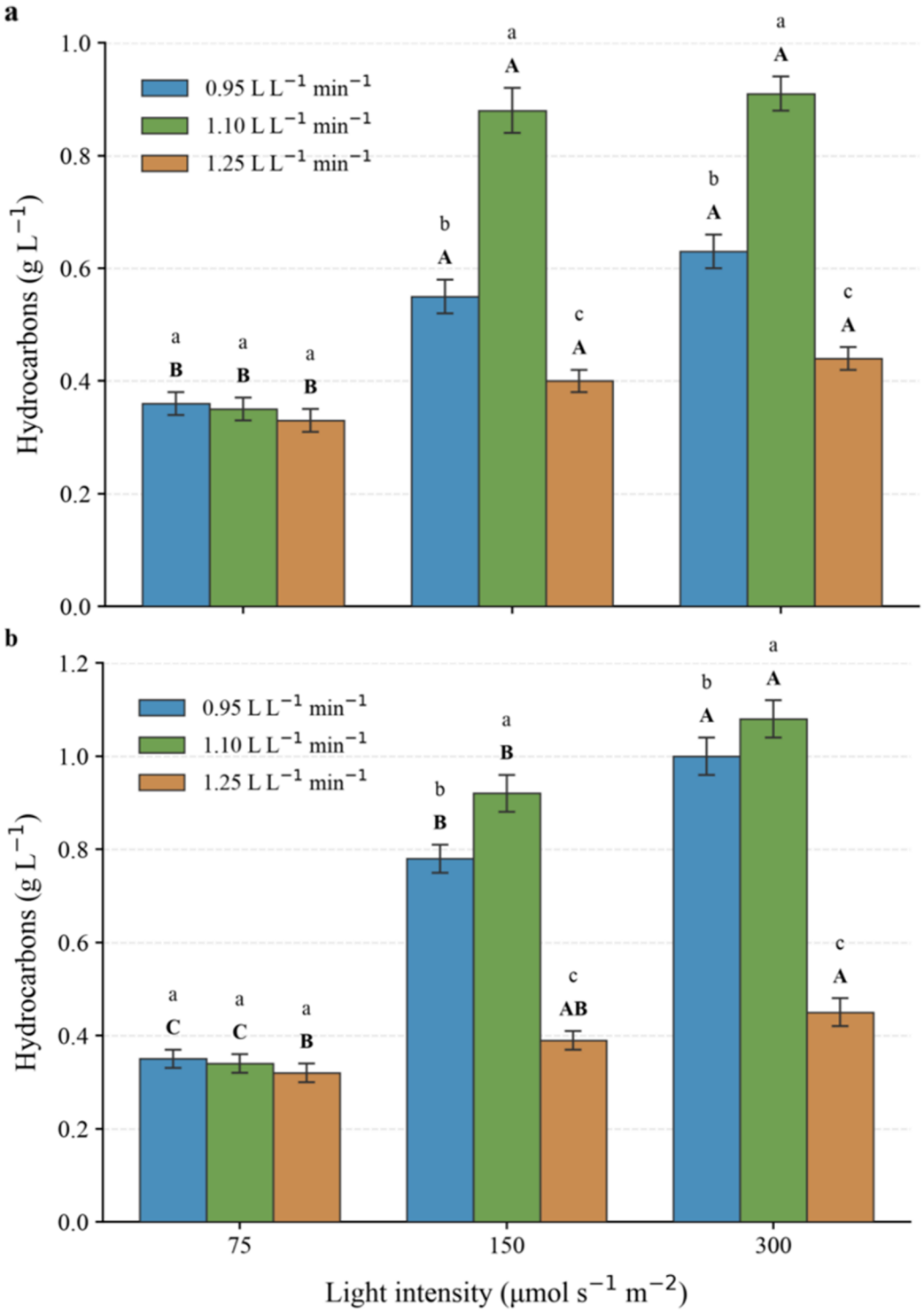


Figure 3. Hydrocarbon production of *B. braunii* at the end of the exponential phase on day 9 (**a**) and the end of the stationary phase on day 15 (**b**) in the photobioreactor as a function of light and mixing. The lowercase letters (a, b and c) indicate significant differences ($p < 0.05$) between the different mixing regimes within each light level and the uppercase letters (A, B and C) indicate significant differences ($p < 0.05$) between the light regimes within each mixing level.

Table 2. Hydrocarbon production in the photobioreactor under three intensities and three mixing rates. Different letters within the same column indicate significant differences ($p < 0.05$) between treatments. The ANOVA results (bottom) show the probability of difference in the impact of light, mixing and their interactions on each sampling day.

Treatment		Hydrocarbon Production (g L^{-1})					
Light (μmol s^{-1} m^{-2})	Mixing (L L^{-1} min^{-1})	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
75	0.95	0.092 ± 0.001 a	0.190 ± 0.008 d	0.281 ± 0.004 d	0.336 ± 0.022 d	0.278 ± 0.010 d	0.283 ± 0.013 e
75	1.10	0.091 ± 0.006 a	0.198 ± 0.005 d	0.275 ± 0.002 d	0.329 ± 0.013 d	0.286 ± 0.008 d	0.279 ± 0.008 e
75	1.25	0.091 ± 0.002 a	0.164 ± 0.003 d	0.243 ± 0.006 d	0.297 ± 0.004 d	0.258 ± 0.008 d	0.254 ± 0.004 e
150	0.95	0.092 ± 0.001 a	0.254 ± 0.014 bc	0.401 ± 0.025 c	0.605 ± 0.027 b	0.698 ± 0.024 b	0.729 ± 0.032 c
150	1.10	0.091 ± 0.001 a	0.272 ± 0.024 bc	0.512 ± 0.038 b	0.832 ± 0.033 a	0.859 ± 0.023 a	0.838 ± 0.041 b
150	1.25	0.092 ± 0.001 a	0.242 ± 0.016 c	0.405 ± 0.008 c	0.402 ± 0.012 c	0.419 ± 0.016 c	0.329 ± 0.020 de
300	0.95	0.092 ± 0.003 a	0.304 ± 0.023 b	0.583 ± 0.032 a	0.655 ± 0.042 b	0.833 ± 0.015 a	0.891 ± 0.030 b
300	1.10	0.093 ± 0.001 a	0.361 ± 0.036 a	0.614 ± 0.031 a	0.876 ± 0.054 a	0.869 ± 0.025 a	0.972 ± 0.064 a
300	1.25	0.093 ± 0.001 a	0.252 ± 0.022 bc	0.397 ± 0.015 c	0.440 ± 0.008 c	0.413 ± 0.011 c	0.384 ± 0.004 d
		ANOVA results (p value)					
Light		0.458	0.001	0.001	0.001	0.001	0.001
Mixing		0.994	0.001	0.001	0.001	0.001	0.001
Light × mixing		0.975	0.017	0.001	0.001	0.001	0.001

To compare system efficacy between the photobioreactor and conventional bottle cultures, algal growth was evaluated under optimal light and mixing conditions (i.e., 300 μmol s^{-1} m^{-2} and 1.10 L L^{-1} min^{-1}). Starting on day 3 after algal inoculation, algal biomass and hydrocarbon production in the photobioreactor were significantly greater than in the bottle ($p < 0.05$; Figure 4a,b). When dry algal weight was calculated, the maximal biomass productivity in the photobioreactor was 1.11 ± 0.107 g L^{-1} day^{-1}, which was significantly higher than that of the bottle culture (0.27 ± 0.096 g L^{-1} day^{-1}), yielding a 4.04-fold increase in the photobioreactor (Figure 5a). When expressed in hydrocarbon, the maximal productivity in photobioreactor culture was 0.089 ± 0.012 g L^{-1} day^{-1}, while that in the bottle culture was 0.045 ± 0.016 g L^{-1} day^{-1}, resulting in a 1.97-fold increase in the photobioreactor (Figure 5b).

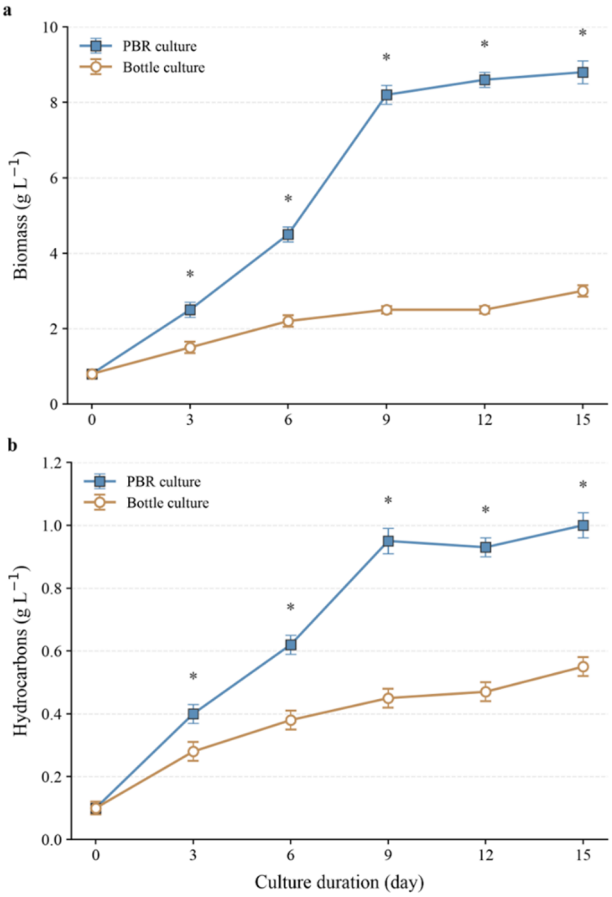


Figure 4. Algal biomass (**a**) and hydrocarbon production (**b**) in the photobioreactor (PBR) and the bottle at the optimal light intensity (300 μmol s^{-1} m^{-2}) and the optimal mixing (1.10 L L^{-1} min^{-1}) regime. Asterisks (*) indicate significant differences between the two culture systems ($p < 0.05$).

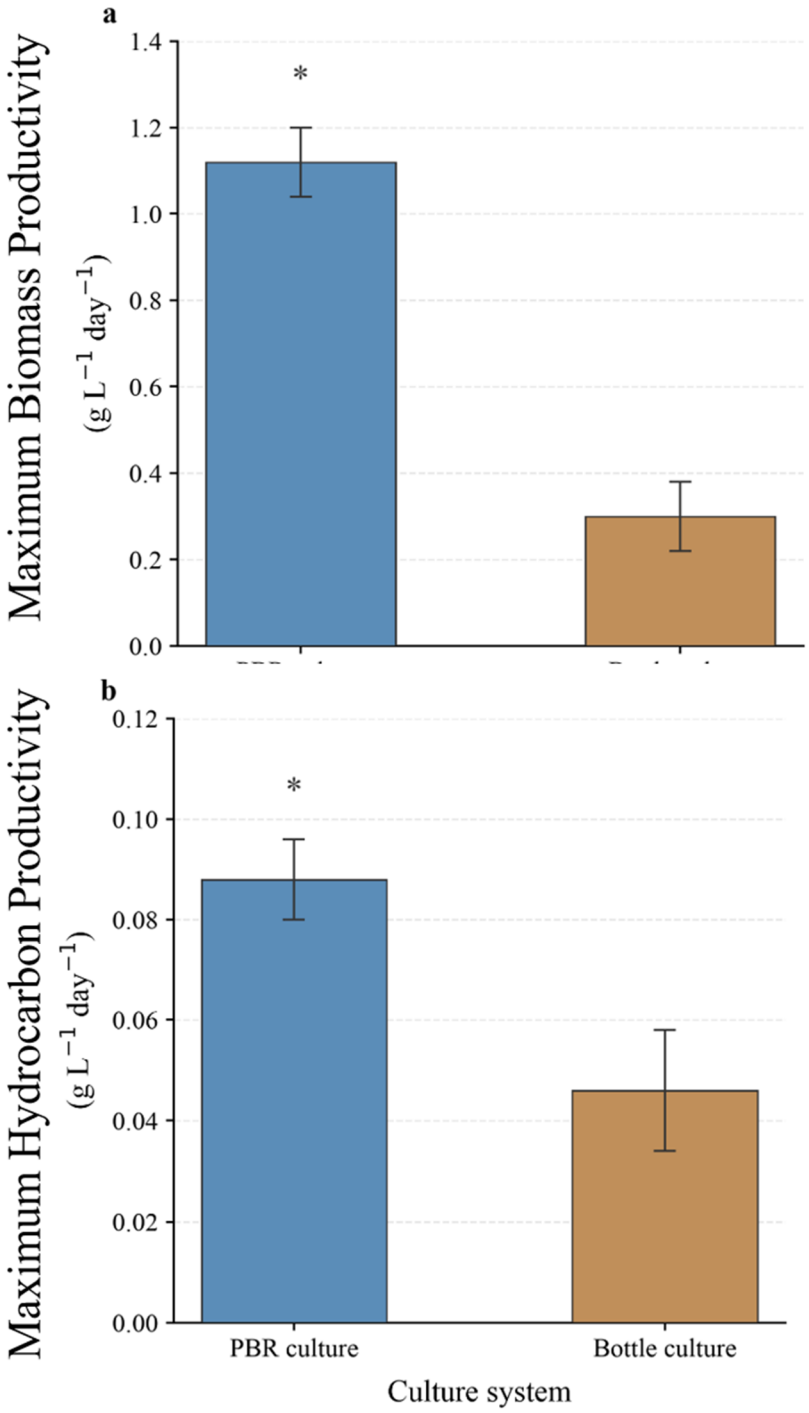


Figure 5. The maximal biomass (**a**) and hydrocarbon productivity (**b**) of *B. braunii* in the photobioreactor (PBR) and the bottle culture system under optimal culture conditions. Asterisks (*) indicate significant differences between the two culture systems ($p < 0.05$).

In the validation study, algal biomass production in the photobioreactor was significantly higher than in the bottle from day 3 onward ($p < 0.05$; Figure 6a,b). The maximal biomass productivity in the photobioreactor was 0.967 ± 0.033 g L^{-1} day^{-1}, while that in the bottle culture was 0.246 ± 0.022 g L^{-1} day^{-1} (Figure 7a), indicating a 3.97-fold difference. Likewise, maximal hydrocarbon productivity in the photobioreactor was 0.087 ± 0.002 g L^{-1} day^{-1}, 1.91 times higher than in the bottle (0.045 ± 0.004 g L^{-1} day^{-1}; Figure 7b).

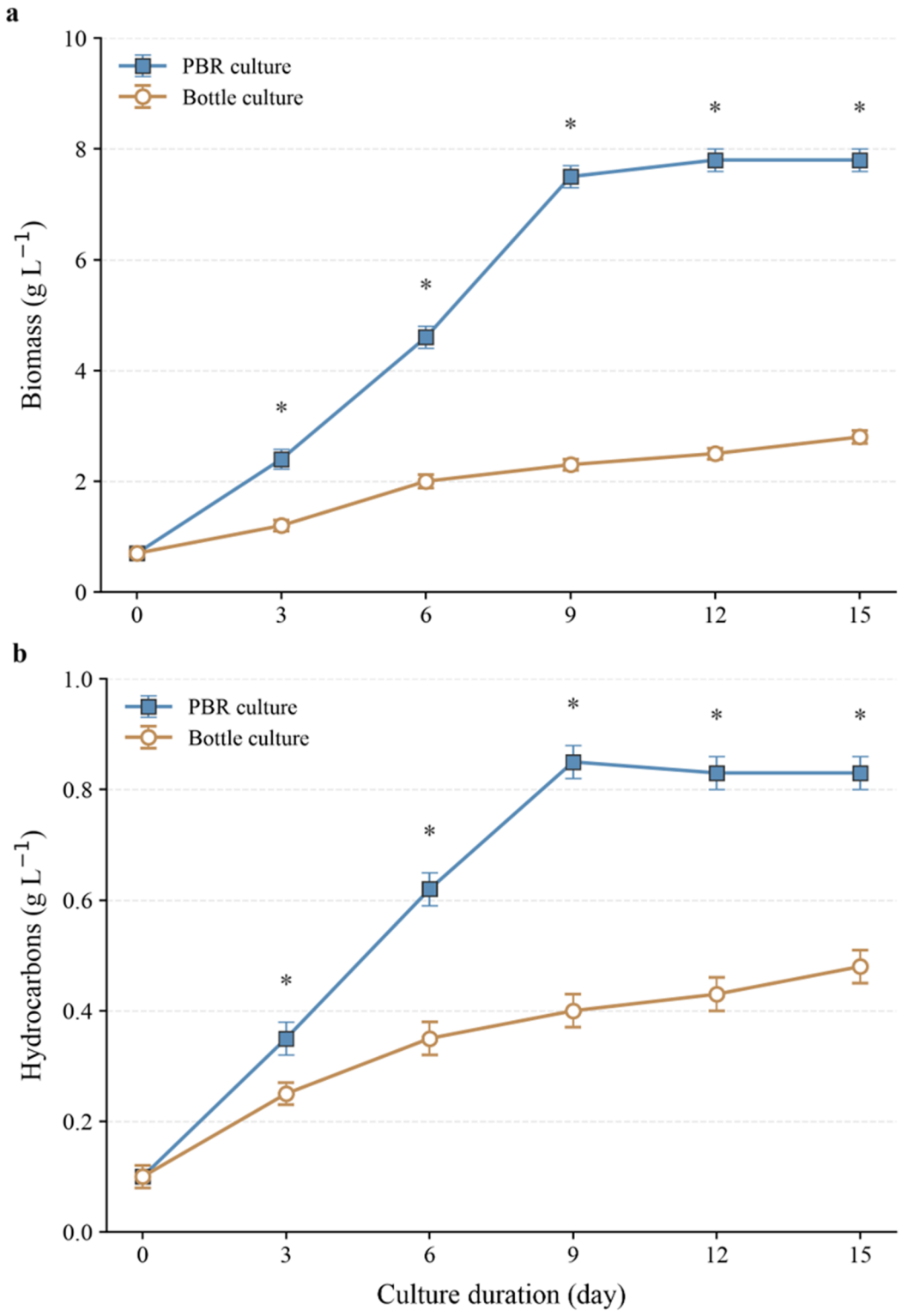


Figure 6. Independent validations of the algal biomass (**a**) and hydrocarbon production (**b**) in the photobioreactor (PBR) and the bottle culture system at the optimal light intensity (300 μmol s^{-1} m^{-2}) and mixing (1.10 L L^{-1} min^{-1}) regime. Asterisks (*) indicate significant differences between the two culture systems ($p < 0.05$).

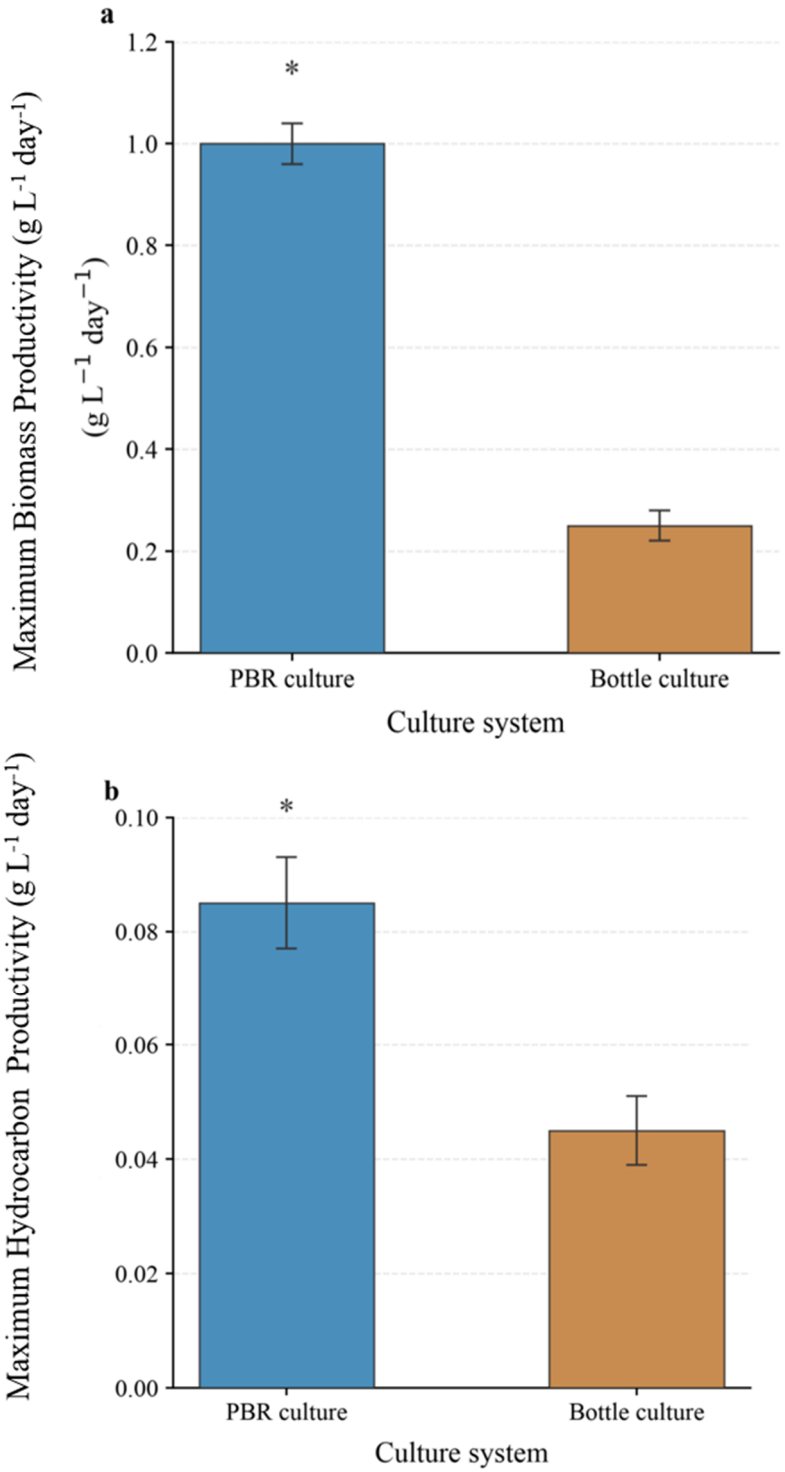


Figure 7. Independent validations of the maximal biomass (**a**) and hydrocarbon productivity (**b**) of *B. braunii* in the photobioreactor (PBR) and bottle culture system at the optimal culture conditions. Asterisks (*) indicate significant differences between the two culture systems ($p < 0.05$).

4. Discussion

Algal growth can be affected by various biotic and abiotic factors by regulating light energy conversion efficiency during photosynthesis [25]. Therefore, it is necessary to improve irradiation efficiency in the algal culture system to maximize the growth of *B. braunii*. In any algal culture, light gradients mean algal cells experience stochastic light-dark cycles governed by the frequency of water mixing [26]. Although photobioreactors are designed to maximize the efficiency of algal cell exposure to the light, the degree of water mixing can also interfere with the exposure of the algal cells to the ambient light. In this study, at high light intensity (300 μmol s^{-1} m^{-2}), low mixing (0.95 L L^{-1} min^{-1}) did not enhance algal growth. However, at high light

intensity, moderate mixing (1.10 L L^{-1} min^{-1}) increased both algal biomass and hydrocarbon production. This result is consistent with Qiang and Richmond [15], who showed that light intensity determined the growth of *Spirulina platensis*, but mixing strongly compromised algal growth at low light intensity. In another algal study conducted in a photobioreactor, hydrodynamic conditions in the culture medium affected the size distribution of *B. braunii* cells, but the impact of mixing on the growth of this alga was not examined [12]. Li and Qin [27] reported that the range of light irradiance for hydrocarbon production in *B. braunii* was 60–100 W m^{-2}, but light and mixing interactions were not investigated. The present study was the first to suggest that the mixing regime in the photobioreactor contributes to the effective use of light by *B. braunii* especially when the level of light intensity was medium or high (150 or 300 μmol s^{-1} m^{-2}).

When other environmental conditions support algal growth, mixing can be an important factor influencing algal growth [28]. One possible mechanism by which mixing enhances algal productivity is by increasing the frequency of algal cell exposure to light and improving nutrient transfer efficiency between nutrients and algal cells [29]. However, at low light, increased mixing only slightly benefited *B. braunii* growth in this study. This finding is consistent with the result of Richmond [6] who found that the interrelationships between light intensity and mixing were clearly evident in *Spirulina* sp. cultures, where varying the mixing rate significantly affected algal productivity only at high, but not at low, light intensities. Ugwu et al. [30] showed that installing static mixers in a tubular photobioreactor increased light utilization and biomass yields. Another reason mixing must be optimized is that high mixing levels can damage algal cells through shear [25]. Physical damage to algal cells can severely impair the photosynthetic apparatus. Shear could cause more damage to large and colonial algae such as *B. braunii*.

Despite the positive effect of using mixers in algal culture, we found that even at high light intensity, aeration at 1.25 L L^{-1} min^{-1} suppressed the growth and hydrocarbon production of *B. braunii.* The possible reason is that high turbulence may damage the structure of algal colonies. Some algal species are especially sensitive to shear from water turbulence; therefore, mixing can be a critical factor in algal culture [31–33]. From a hydrodynamic perspective, turbulence can impose stress and pressure gradients on algal cells [34]. Algal responses to shear stress can lead to changes in orientation or alterations in cell division cycles [35]. Algal responses to turbulence seem to depend on shear strength and exposure time. Pruvost et al. [36] noted that the effects of shear stress on photosynthetic microorganisms are poorly understood and warrant further study. Evidence from this study suggests that mixing can regulate photosynthetic efficiency in algae, but very high mixing may damage algal cells, especially in colonial algae. Unfortunately, this study did not investigate the impact of mixing on physical damage to *B. braunii*. The speculation about mixing-induced algal cell damage warrants further investigation.

An effective photobioreactor can enhance light penetration and the frequency of cellular exposure to light. Light intensity is critical because, when it is too high, algae exhibit photoinhibition and dissipate excess energy as heat [37,38]. Light saturation can be mitigated by spatially diluting light through mixing, thereby distributing radiation over a greater photosynthetic surface area. Spatial dilution of light can also reduce self-shading among cells and enhance *Spirulina* sp. growth in photobioreactors [39]. Therefore, a guiding principle in photobioreactor design is to maximize the surface area-to-volume ratio to increase light-use efficiency [25]. In this study, the photobioreactor had a surface/volume ratio of 0.363 cm^2 mL^{-1}, while the bottle had 0.104 cm^2 mL^{-1}. Thus, the photobioreactor had a 3.5-fold higher surface/volume ratio than the bottle. Coincidentally, the growth rate of *B. braunii* in the flat-plate photobioreactor was nearly 4-fold higher than that in the bottle, consistent with its higher surface-to-volume ratio. However, when comparing production efficiency, we must consider other differences between the photobioreactor and the static bottle, such as overall geometry, light distribution, and mixing. These factors likely contributed to the performance difference, not just the surface-to-volume ratios.

Beyond the surface area-to-volume ratio, mixing patterns and light/dark cycles may affect biomass and secondary metabolite production [40]. In this study, the fluid dynamics in the photobioreactor differed from those in the bottle. In the photobioreactor, rising bubbles from two bottom corners drove upwelling currents, resulting in strong unidirectional upwelling. In contrast, the upward fluid movement in the bottle occurred in multiple stochastic directions. Krichnavaruk et al. [41] compared the growth of *Chaetoceros calcitrans* in systems with either upwelling or air bubbling and found that the algal biomass in the upwelling system (8.88×10^6 cells mL^{-1}) was nearly twofold higher than that in the bubbling system (4.96×10^6 cells mL^{-1}). In the present study, light could not penetrate through the top of the photobioreactor, resulting in a brief period of dim light as algae passed through it. In comparison, the algae in the bottle did not experience dim light during circulation. Janssen et al. [42] reported that *Chlamydomonas reinhardtii* was not able to maintain a high photosynthetic capacity under a medium duration of light/dark cycles, whereas Richmond [6] found that a high frequency of light/dark cycles enhanced *Spirulina platensis* production. Blocking light with the top lid of the photobioreactor may also contribute to the high algal production, but this needs further investigation.

5. Conclusions

Microalgae cultivation for biofuel production requires cost-effective biomass production. This study demonstrated performance improvements for a slow-growing, high-oil alga in a photobioreactor. Although this study did not specifically explore photobioreactor design, shape, or volume, the results provide useful information for determining the physical conditions for algal growth and hydrocarbon production. In this study, the growth performance of *B. braunii* was efficiently controlled and optimized via light and mixing regimes in the flat photobioreactor evaluated. The results showed that using a photobioreactor could increase algal growth by nearly 4-fold and hydrocarbon production by nearly 2-fold compared with conventional bottle culture. This study demonstrates that photobioreactors can increase the productivity of slow-growing, high-lipid algae. However, the factors contributing to the photobioreactor's superior performance are diverse, including design configuration, surface-to-volume ratios, size, and turbulence. Further studies should focus on scaling up the production system, which may eventually lead to a viable method for producing biofuel from microalgae such as *B. braunii* from laboratories to a commercial setting.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/261113135351412 3/ALE-26090122-SI.pdf. Figure S1: A diagram of the flat plate photobioreactor system; Figure S2: Schematic drawing of a flat plate photobioreactor.

Author Contributions

H.X. contributed to data analysis, writing, and the design of figures and tables. L.S. contributed to data collection, experimental implementation, data analysis, writing, editing, and discussion. J.Q. contributed to concept development, supervision, editing, and discussion. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Conflicts of Interest

Given the role as Editor-in-Chief, Jianguang Qin had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used Grammarly (Version v.1.2.306.1984) for language editing and grammar improvement. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the published article.

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