



The growth, lipid and hydrocarbon production of *Botryococcus braunii* with attached cultivation



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HIGHLIGHTS

- Attached cultivation + light dilution boosts the biomass production of *Botryococcus braunii*.
- Biomass productivity of 61 g m⁻² d⁻¹ was obtained with a multi-layers arrayed photobioreactor.
- Photosynthetic efficiency of 14.9% (visible light) for *B. braunii* was achieved.
- Fatty acid and hydrocarbon of *B. braunii* with attached cultivation were analyzed.

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ABSTRACT

The green alga *Botryococcus braunii* is regarded as a potential source of renewable fuel due to its high lipid and hydrocarbon contents. However, the slow growth rate damaged its feasibility for biofuel production. In this study, a novel method of 'attached cultivation' was introduced to incubate *B. braunii* FACHB 357 (B race). A high biomass productivity of 6.5 g m⁻² d⁻¹ was achieved in single layer attached system at early stage of cultivation. At day 10, the biomass, lipid and hydrocarbon productivities were 5.5, 2.34 and 1.06 g m⁻² d⁻¹, respectively. Under nitrogen starvation condition, both of the contents of lipid and hydrocarbon were increased, whereas the profile of hydrocarbon kept almost unchanged, while the content for oleic acid (18:1) increased and linolenic acid (18:3) decreased. With a multi-layer photobioreactor, a biomass productivity of 49.1 g m⁻² d⁻¹ or a photosynthetic efficiency of 14.9% (visible light) were obtained under continuous illumination of 500 μmol m⁻² s⁻¹.

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1. Introduction

Microalgae, a group of tiny photosynthetic organisms, has been attracting much focus during the past decades due to its potentials in CO₂ mitigation and producing sustainable biofuels and the production of high value products (Chisti, 2007; Mata et al., 2010; Sostaric et al., 2012). Among these huge diversity of species (~40,000) (Hu et al., 2008), the green alga *Botryococcus braunii* is a notable one that secreting hydrocarbons under different conditions (Metzger and Largeau, 2005), which is more similar to fossil oil to be converted into oxygen-free fuels (Banerjee et al., 2002; Hillen et al., 1982). Large amounts of studies have been performed to optimize the growth of *B. braunii* (Ruangsomboon, 2012; Baba et al., 2012), however, a frustrating fact was the application of *B. braunii* for biofuel production was seriously hampered by its very slow-growing rate (Metzger and Largeau, 2005; Banerjee et al., 2002).

Thus far, the prevailing microalgae cultivation devices are open ponds and closed photobioreactors of various designs in which microalgae are maintained in liquid suspensions. The highest biomass productivities of ca. 40 g m⁻² d⁻¹ were reported from such culture systems (Mata et al., 2010; Brennan and Owende, 2010), which was far less than the theoretical maximum of ca. 150 g m⁻² d⁻¹, or ca. 13% photosynthetic efficiency (PE, based on total solar radiation) (Boyer, 1982; Zhu et al., 2008; Tredici, 2010). The irregular energy dissipation and poor mass transfer due to the aqueous suspended environment, which were regarded as the main reasons for the restrained growth (Jung et al., 2012). Research on immobilized algae cultivation systems have been attracting attention as these systems, which could offer the potentials to remove the pollutants (nitrogen and phosphate, especially) in waste water (Nowack et al., 2005; Shi et al., 2007). Ozkan et al. (2012) reported a biofilm photobioreactor for the cultivation of *B. braunii*. This study gave encouraging results for the water and energy savings, however, the low biomass productivity and photosynthetic efficiency are extremely low, indicating this cultivation system is still far from actual application.

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These negative effects might be relieved dramatically in biofilm photobioreactors, in which the immobilized algal cells attached on supporting surfaces and, by and large, separated with the aqueous medium. Recently, we reported a significant progress in PE with an improved biofilm reactor, named “attached cultivation system”. With this system, the high dense microalgae cells were attached on artificial supporting surfaces and multiple of which were then arranged in arrays in a chamber to dilute the high light. The biomass productivity of oleaginous microalgae *Scenedesmus obliquus* could reach 50–80 g m⁻² d⁻¹ outdoors with this attached cultivation methods, which is 400–700% higher than that of open pond under same climate and light conditions (Liu et al., 2013).

In this research, the potential of ‘attached cultivation’ method was applied on *B. braunii*. The growth rate, lipid accumulation and hydrocarbon profile under nitrogen sufficient and deficient conditions were investigated. Results indicated that this attached cultivation method might be an efficient technology to boost the growth of *B. braunii*.

2. Methods

2.1. Algal strain and inocula preparation

The algal strain *B. braunii* FACHB 357 was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, PR China. The inoculum was cultivated in the autotrophic nutrient medium Chu 13 due to its superiority over BG11, BBM in terms of biomass productivity and lipid content (data not shown). The medium contained (mg L⁻¹) (Largeau et al., 1980): KNO₃, 200; K₂HPO₄, 40; MgSO₄·7H₂O, 100; CaCl₂·6H₂O, 80; Ferric citrate, 10; Citric acid, 20; micro elements: B and Mn, 0.5; Zn, 0.05; Cu, Co and Mo, 0.02 respectively. The alga was firstly cultivated with glass bubbling columns (diameter = 0.05 m) for 2 weeks to prepare the inocula for attached bioreactors. Each of these columns contains 0.7 L of algal broth and was continuously illuminated by cold-white fluorescent lamps (NFL28-T5, NVC, China) with light intensity of 60 μmol m⁻² s⁻¹. The temperature for algal broth was 25 ± 2 °C during the cultivation. Air bubble that contained 1% CO₂ (v/v) was continuously injected into the bottom of the columns with a speed of 1 vvm to agitate the algal broth as well as supply carbon resource.

2.2. Attached cultivation bioreactors

The type 1 (Fig. 1A) and type 2 (Fig. 1B) attached photobioreactors as described in detail in the authors’ previous study (Liu et al., 2013), which were used in this study to cultivate *B. braunii* FACHB 357. Type 1 photobioreactor, in which a 0.2 m × 0.4 m glass plate (0.003 m in thickness) placed in the center of a 0.5 m × 0.3 m × 0.05 m glass chamber, was used to investigate the growth feasibility of algae cells at attachment. One surface of the inserted glass plate, which would be illuminated in the following cultivation, was covered by a layer of filter paper. For the experiments with type1 photobioreactor, the light intensity measured inside the chamber at the position of attached algal cells was 100 ± 10 μmol m⁻² s⁻¹.

Type 2 photobioreactor, which was used to evaluate the potential of area biomass output, consists of a glass chamber and multiple glass plates other than single layer of type 1. In briefly, a size of 0.3 m × 0.1 m glass plates inserted the glass chamber which the dimensions were 0.4 m × 0.1 m × 0.3 m. The inserted glass plates where the algal cells grow on both sides were placed in array style. A face of the glass chamber was uncovered and served as to receive light illumination of 500 ± 20 μmol m⁻² s⁻¹, while the other five faces were covered by aluminum foil to isolate the un-wanted illumination. For the experiments of type 2 photobioreactors, the incident light penetrated into the glass chamber was

diluted and light dilution rate (R_L) was 10×. The light dilution rate (R_L) for this type 2 photobioreactor was defined as:

$$R_L = A_C/A_L \quad (1)$$

where the A_C represented the total cultivation surface and A_L represented the light incident area.

In the preliminary experiment, it was proved that the cells of *B. braunii* could attach and grow on the surface of the filter paper or other hydrophilic materials (data not shown). In this study, for the sake of easy sampling and precise measuring of the biomass, the algal cells were evenly filtered on nitrate cellulose/cellulose acetate filter membranes (Motimo Co., Tianjin, China, pore size = 0.45 μm) to form an ‘algal disk’. Multiple of these ‘algal disks’ were put on a filter paper which covered on a glass plate (Fig. 1A and B). The foot print area of the ‘algal disk’ was 10 ± 0.5 cm². Culture medium flowed through the filter paper to provide water and nutrient for the membranes to support the growth of the algal cells. The flow rate of the culture medium was gently controlled to maintain the well attachment of the algal cells with minimum wash-off. For the two types of photobioreactors, continuous airflow that contained 1% CO₂ (v/v) was injected into the glass chamber with a speed of 0.1 vvm to supply carbon source and the temperature inside the glass chamber was 25 ± 2 °C during the experiments. The whole cultivation set was illuminated continuously with cold-white fluorescent lamps. In order to study the growth and lipid production under nitrogen replete and depleted conditions conveniently, culture medium of the type 1 bioreactor (single layer) with a flow rate of 0.55 ml/min in this study was disposable without circulation. However, culture medium of the type 2 bioreactor (multiple-layer) was circulated inside the system with a speed of ~10 ml/min.

2.3. Growth analysis

The biomass concentration of an ‘algal disk’ (DW , g m⁻²) was measured with gravimetric method. The cells of ‘algal disk’ were washed down and re-suspended with de-ionized water and then filtered to pre-weighted 0.45 μm GF/C filter membrane (Whatman, England; DW_0). The membrane was oven dried at 105 °C for 12 h and then cooled down to room temperature to measure dry weight (DW_1). The DW was calculated as follows:

$$DW = (DW_1 - DW_0)/0.001 \quad (2)$$

in which the 0.001 represented the footprint area of the ‘algal disk’ (m²). For the experiment with type 1 bioreactor, the biomass density of ‘algal disk’ was considered as identical to that of the cultivation surface. For the experiment with type 2 bioreactor, the light to biomass energy conversion efficiency, η_b , was calculated as:

$$\eta_b = (W_{net} \times E_b)/(G_{in} \times A_s \times \Delta t) \quad (3)$$

due to the light dilution effect, the biomass concentration of the illuminating area (W_{net}) was calculated as $DW \times R_L$, of which R_L is 10× in this research. E_b is the heating value of the dry biomass equal to 28.3 MJ/kg dry weight for *B. braunii* according to Ozkan et al. (2012), G_{in} is the irradiation (μmol m⁻² s⁻¹), A_s is the cultivation surface area and Δt is the total duration of the experiment. η_b was calculated on the basis of the fact that 48% of the solar radiation is visible light (400–700 nm).

2.4. Lipid and hydrocarbon analysis

The attached algal cells were harvested by washing down with de-ionized water and centrifugation at 3800g for 10 min (Allegra X-22R, Beckman coulter, America). The algal pellets were washed three times with de-ionized water to remove the attached salt. Then the total lipid was measured according to Bligh and Dyer’s method (Bligh and Dyer, 1959). This “total lipid” contains all of

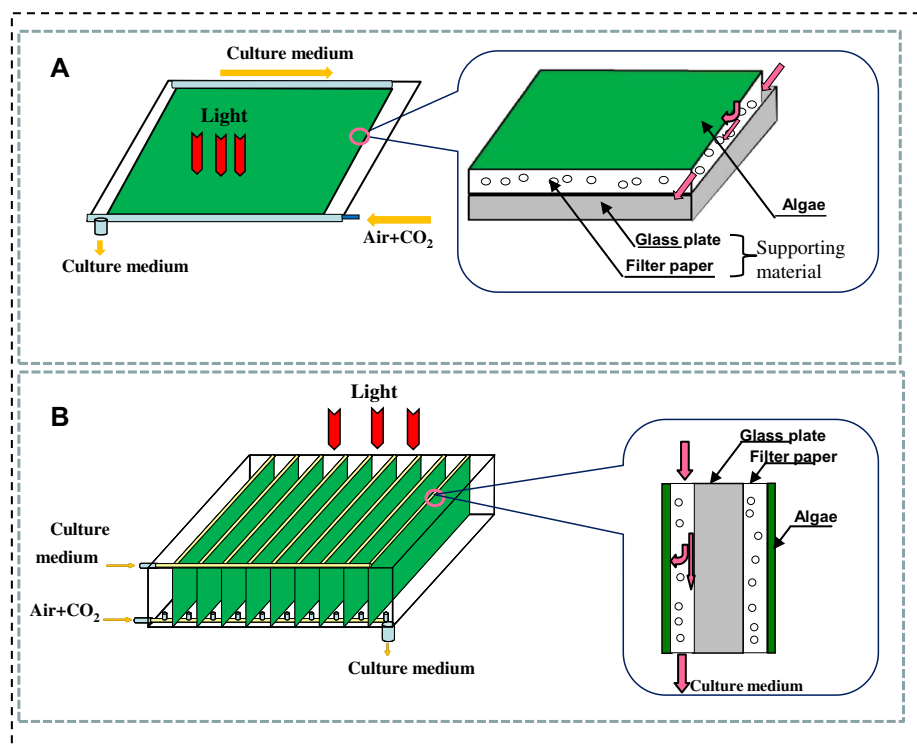


Fig. 1. The schematic diagrams of attached cultivation devices. (A) Attached cultivation module of the type 1 photobioreactor (single layer), the residual medium collected was discarded without any recycling. (B) The schematic diagram of the multiple-plates photobioreactors (type 2) that used indoors in this research. Multiple cultivation modules were inserted inside the glass chamber to dilute the light. The algal cells were attached on both sides of the inserted cultivation module. The light impinged on the top surface of the bioreactor. The R_L was 10 \times . The medium was propelled by a peristaltic pump to flow through the system during the cultivation.

the compounds that dissolved in chloroform, including polar lipids (chlorophyll), non-polar lipids (triacylglycerol), hydrocarbon, etc. For fatty acid composition was analyzed by gas chromatography (Varian 450 GC, USA) and Agilent HP-5 GC Capillary Column (30 m \times 0.25 mm \times 0.25 μ m) according to the method of Chen et al. (2012).

The content and composition of hydrocarbon were determined according to Sawayama et al. (1992). Some 50 mg of dried algal biomass was homogenized and then extracted with *n*-hexane. The extraction process was repeated three times and finally the supernatant was combined in a pre-weighed glass vial and the solvent was blown away by nitrogen gas (>99%). The residue remained in the glass vial was considered as the crude hydrocarbon.

The sample of the crude hydrocarbon was then purified by column chromatography on silica gel with *n*-hexane as an eluent. The residual extracts were fractionated on a same column using chloroform and methanol. As a result, pure hydrocarbon, non-polar lipids and polar lipids were well isolated with reference to their elution with that of the retention times of the internal standard (Singh and Kumar, 1992; Largeau et al., 1980; Dayananda et al., 2005). According to the length of the carbon chain, the hydrocarbons were grouped into three categories, viz. <C₂₀, C₂₀–C₃₀ and >C₃₀. The eluent was finally concentrated and analyzed by GC–MS using Agilent HP-5MS column (length = 30 m, inner diameter = 0.25 mm, film thickness = 0.25 μ m). The composition of pure hydrocarbon sample was analyzed as described by Dayananda et al. (2005).

3. Results and discussion

3.1. The accumulation of biomass, lipid and hydrocarbon of *B. braunii* under attached cultivation

The algal cells of the attached *B. braunii* FACHB 357 under nitrogen sufficient condition (not shown here) were yellow–green in

color in the early stage and then turned to moss green after 10 days. The algal colony perched on membranes became thicker and thicker during the cultivation. The cells were attached by stretches itself between two or three distinct clumps of cell which was similar to the typical cultures in aqueous-suspended condition (Metzger and Largeau, 2005). Some colorless droplets were observed in outer walls of the algal cells after 10 days of cultivation, which might indicate the accumulation of hydrocarbons (Largeau et al., 1980).

The areal biomass density of the *B. braunii* FACHB 357 cultivated with type 1 photobioreactor (single layer) increased from 7.1 g m⁻² to 62.0 g m⁻² in 10 days of cultivation (Fig. 2), resulted in a biomass productivity of 5.5–6.5 g m⁻² d⁻¹. A high biomass productivity of 6.5 g m⁻² d⁻¹ was achieved at early stage of cultivation and then decreased, which was much higher than the reported value of 0.71 g m⁻² d⁻¹ with ‘biofilm’ photobioreactor under continuous illumination of 55 μ mol m⁻² s⁻¹ (Ozkan et al., 2012). The increased biomass productivity might because the gas form carbon source (CO₂) was added in our system, and according to Van Den Hende et al., (2012) CO₂ is absorbed by green algae much easier than HCO₃⁻ and CO₃²⁻.

After 10 days of attached cultivation under nitrogen sufficient condition, the contents for total lipid and hydrocarbon were 42.6% and 19.4%, respectively, and the total lipid and hydrocarbon productivities were 2.34 and 1.06 g m⁻² d⁻¹, respectively (Fig. 3). The hydrocarbon contents were consistent with previous results of Metzger et al. (1985), whereas total lipid content were much higher than that by Ge et al. (2011) with different CO₂ aeration.

The total lipid and hydrocarbon contents were increased from 26.8% and 15.6% to 51.6% and 34.3%, respectively in 8 days of attached cultivation with nitrogen-free medium (Fig. 4), while the biomass productivity reduced only half of that with standard Chu 13 medium (control). In conventional suspended cultivation, two strategies were usually adopted to achieve nutrient depletion.

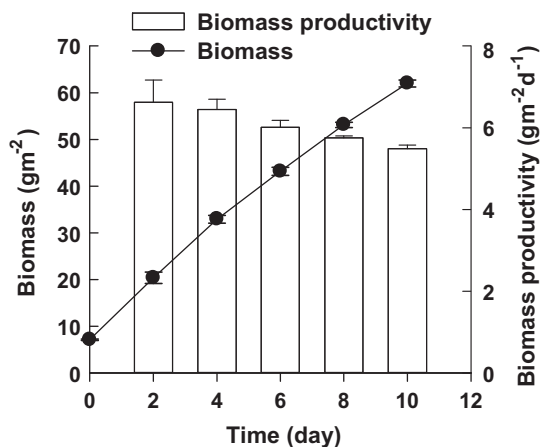


Fig. 2. The growth of *B. braunii* FACHB 357 with attached cultivation under nitrogen sufficient condition. The algal cells were cultivated in type 1 photobioreactor under continuous illumination of $100 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$. The medium was Chu 13 with 1.98 mM of nitrate. The medium was disposable when it flowed through the chamber to keep the nutrient environment constant for algal growth. Data are means \pm standard deviations of three replicates.

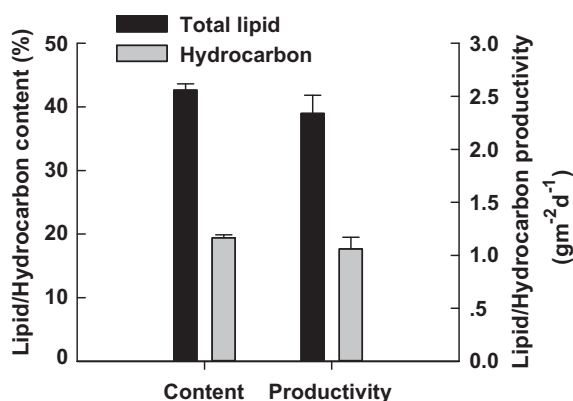


Fig. 3. Contents and productivities of lipid and hydrocarbon of *B. braunii* FACHB 357 with attached cultivation under nitrogen sufficient condition. The algal cells were cultivated in type 1 photobioreactor under continuous illumination of $100 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$. The medium was Chu 13 with 1.98 mM of nitrate. The medium was disposable when it flowed through the chamber to keep the nutrient environment constant for algal growth. Data are means \pm standard deviations of three replicates.

The first was natural consumption by microalgae in a prolonged culture time. Such process required long cultivation time and as a result, the cultivation efficiency was compromised. The other method was harvesting the algal cells first and then transferring to nutrient-depleted medium. However, harvesting algal cells from a much diluted broth was very costive and had the risks of contamination. Considering the fact the water requirement for this attached cultivation was greatly reduced (Liu et al., 2013). The way to promote lipid induction by replacing the sufficient nitrogen medium with depleted nitrogen medium became feasible and affordable.

The fatty acid composition of the attached *B. braunii* FACHB 357 was showed in Table 1. According to previous report (Knothe, 2008), palmitic, stearic, oleic and linolenic acid were recognized as the most common fatty acids contained in biodiesel. In this work, algal strain *B. braunii* FACHB 357, which was confirmed as B race by GC-MS spectra of hydrocarbons, also produced mainly oleic acid (C18:1, 52.25%), linolenic acid (C18:3, 15.81%) and palmitic (C16:0, 11.32%). Oleic acid (18:1) was the dominant

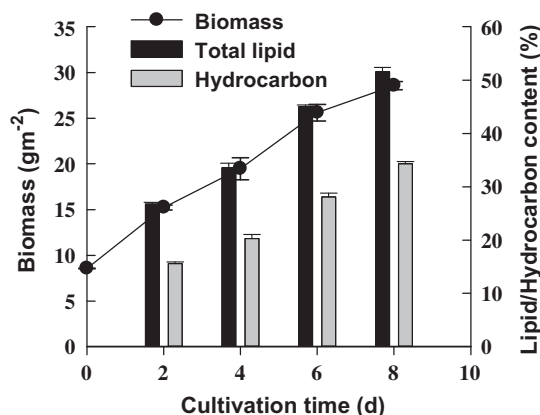


Fig. 4. Biomass, lipid and hydrocarbon accumulation of *B. braunii* FACHB 357 with attached cultivation under nitrogen deficient condition. The algal cells were cultivated in type 1 photobioreactor under continuous illumination of $100 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$. The medium was Chu 13 contained no nitrogen source. The medium was disposable when it flowed through the chamber to keep the nutrient constant for algal growth. Data are means \pm standard deviations of three replicates.

component in the total fatty acid of *B. braunii* FACHB 357 cultivated with attached methods under both of nitrogen sufficient and deficient conditions (Table 1). The nitrogen concentration in culture medium significantly affected the content of oleic acid (18:1) and linolenic acid (C18:3). Under nitrogen deficient condition, the content for oleic acid (18:1) increased and linolenic acid (18:3) decreased. It was agreed with those cultivated in suspended medium by Xu et al. (2001). Oils with high oleic acid content were better to balance the fuel properties including oxidative stability for longer storage (Rashid et al., 2008) and cold filter plugging point (CFPP) for use in cold regions (Stournas et al., 1995).

The nitrogen content had less effects on the hydrocarbon profile of attached *B. braunii* FACHB 357 as indicated by the results of GC analyze (Table 2). The hydrocarbon portions of C₂₀–C₃₀ and >C₃₀ decreased slightly for nitrogen deficiency treated, whereas the lower than C₂₀ hydrocarbon increased. The variation in the hydrocarbon profile could be attributed to the difference of the strains and culture stages (Ranga Rao et al., 2012; Metzger and Largaueu, 2005).

Under nitrogen starvation condition, the contents of hydrocarbons (crude and pure) and non-polar lipids were increased and the polar lipids were decreased, whereas the lipid content (polar + non-polar) were changed marginally (Table 3). It appeared from the results that nitrogen starvation promoted the biosynthesis of hydrocarbons (Singh and Kumar, 1992) and high nitrate

Table 1

Fatty acid composition of attached *B. braunii* FACHB 357 under nitrogen sufficient (control) and deficient conditions.

Fatty acids composition	Control	Nitrogen deficiency
16:0 (Palmitic acid)	11.32 \pm 1.22	13.90 \pm 0.86
16:1 (Palmitonic acid)	2.73 \pm 0.43	1.68 \pm 0.32
16:2 (Hexadecadienoic acid)	0.32 \pm 0.12	Trace
16:3 (Hexadecatrienoic acid)	2.20 \pm 0.34	0.82 \pm 0.13
18:0 (Stearic acid)	2.15 \pm 0.31	4.01 \pm 0.43
18:1 (Oleic acid)	52.25 \pm 1.73	65.53 \pm 1.82
18:2 (Linoleic acid)	6.69 \pm 0.55	1.78 \pm 0.34
18:3 (Linolenic acid)	15.81 \pm 0.63	6.43 \pm 0.47
19:0 (Nonadecanoic acid)	2.78 \pm 0.36	3.11 \pm 0.41
20:1 (Eicosaenoic acid)	0.54 \pm 0.08	0.95 \pm 0.14
20:5 (Eicosapentaenoic acid)	2.72 \pm 0.28	1.80 \pm 0.14
21:5 (Heneicosapentaenoic acid)	0.49 \pm 0.07	Trace

The data were means \pm standard deviations of three replicates.

Table 2
Hydrocarbon profile of *B. braunii* FACHB357 under nitrogen sufficient (control) and deficient conditions.

<i>B. braunii</i> FACHB 357	Less than C ₂₀ (%)	Between C ₂₀ –C ₃₀ (%)	Higher than C ₃₀ (%)
Control	24.28 ± 2.68	36.47 ± 7.14	39.05 ± 3.97
Nitrogen deficiency	28.32 ± 3.18	34.47 ± 4.26	37.21 ± 2.62

The data were means ± standard deviations of three replicates.

Table 3
Contents of crude hydrocarbon, pure hydrocarbon and lipids of *B. braunii* FACHB357 under nitrogen sufficient (control) and deficient conditions.

Compositions of crude hydrocarbon	Control (% of total biomass)	Nitrogen deficiency (% of total biomass)
Crude hydrocarbon	38.09	48.91
Pure hydrocarbon	19.43	34.29
Non-polar lipids	8.21	9.16
Polar lipids	4.38	2.34
Chlorophyll and other impurity	5.07	3.12

concentration may interfere with hydrocarbon production. The mechanism that polar lipids converted to non-polar lipids might be activated by nitrogen starvation Tredici, 2010; Yoon et al., 2012), however, the *de novo* synthesis of lipid might be surpassed.

3.2. Effect of light intensity on biomass productivity of *B. braunii* FACHB 357 in the attached photobioreactor

The relationship between light intensity and biomass productivity of *B. braunii* FACHB 357 was summarized in Fig. 5. In the light range of 10–60 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the biomass productivity increased from 1.06 to 4.42 $\text{g m}^{-2} \text{d}^{-1}$ in a linear mode. With the further increase of the light intensity, the increase of biomass productivity slowed down and leveled off at 7.42 $\text{g m}^{-2} \text{d}^{-1}$ when the light intensity beyond the 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Accordingly, the light intensity of 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ could be considered as the light saturation point (*LSP*). The *LSP* is critical reference for determining the light dilution rate (R_L) of type 2 photobioreactors. To

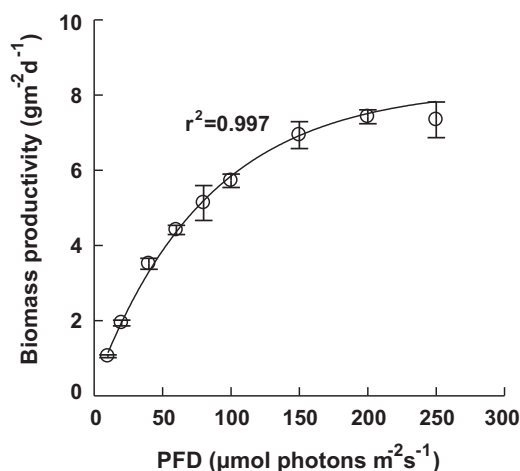


Fig. 5. Effect of light intensity on biomass productivity of *B. braunii* FACHB 357 in the attached photobioreactor. The algal cells were cultivated for 10 days under indoor conditions with type 1 attached photobioreactor and treated by continuous illumination with different light intensities. The biomass productivity was calculated every two days and then the averaged data of the 10 days are presented in the figure. Data are means ± standard deviations of three replicates.

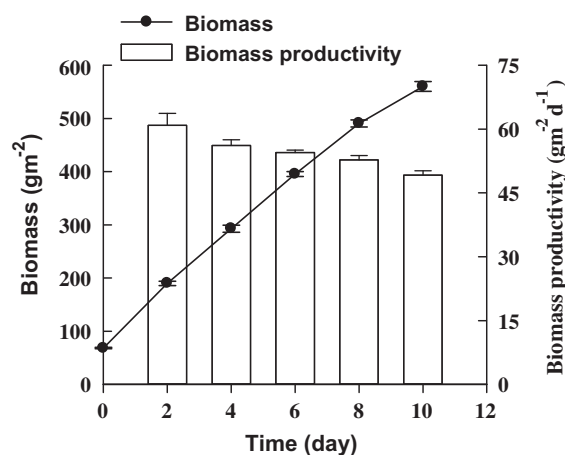


Fig. 6. The growth of *B. braunii* FACHB 357 in insert-plates attached cultivation bioreactors. The algal cells were cultivated in type 2 photobioreactor under continuous illumination of $500 \pm 20 \mu\text{mol m}^{-2} \text{s}^{-1}$ and light dilution rate of $10 \times$. The medium was Chu 13 contained full strength of nitrogen source. Data are means ± standard deviations of three replicates.

achieving the high photosynthetic efficiency, the light intensity received by the algal cells should equal or lower than *LSP* (Chisti, 2007). In this research, the growth potential of *B. braunii* was estimated with type 2 photobioreactor with the averaged light intensity received by cultivation surface was $\sim 50 \mu\text{mol m}^{-2} \text{s}^{-1}$.

3.3. The potential of biomass output for *B. braunii* with attached cultivation methods

With the type 2 photobioreactor (multiple-layers), the areal biomass density was increased from 69g m^{-2} to 560g m^{-2} in 10 days of cultivation, which was corresponded to the biomass productivity of $49.1 \text{g m}^{-2} \text{d}^{-1}$ (Fig. 6.). The averaged photosynthetic efficiency PE (visible light) was also estimated to be $14.9 \pm 0.23\%$, if $2.83 \times 10^4 \text{kJ}$ energy per kilogram of dry biomass of *B. braunii* with each mole of photons contains 217 kJ of energy Tredici, 2010) were adopted in the calculation (Ozkan et al., 2012). This high efficiency should be attributed to the structure of type 2 bioreactor, through which the high light was diluted into an appropriate level (between the light saturation and light compensation point) (Liu et al., 2013) to support algal growth on larger surface than incident surface by light with less light inhibition.

4. Conclusions

With the attached cultivation method, the biomass, lipid and hydrocarbon productivities of the *B. braunii* FACHB 357 reached to 5.5, 2.34 and $1.06 \text{g m}^{-2} \text{d}^{-1}$ after 10 days of cultivation, respectively. Fatty acids distributed in a similar way compared to the traditional algal cultivation technologies. Moreover, the averaged light to biomass conversion efficiency was as higher as 14.9%. Altogether, compared with conventional aqueous suspended cultivation

methods, the attached cultivation might be a better choice for the slow-growing *B. braunii*.

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