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RESEARCH

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Effects of seed age, inoculum density, and culture conditions on growth and hydrocarbon accumulation of *Botryococcus braunii* SAG807-1 with attached culture

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Abstract

Background: *Botryococcus braunii* is difficult to cultivate and has a limited amount of substantive scale-up and productivity assessments with conventionally suspended cultivation systems, such as open pond or closed photobioreactors. The biomass concentrations of cultivated microalgal biofilms are much higher than those of suspension cultures, and the attached microalgal cells are easily separated from cultivation media. However, studies on the attached cultivation conditions for *B. braunii* have been rarely performed.

Results: Herein, an attached cultivation method for *B. braunii* SAG 807-1 incubation was introduced. The effects of primary culture conditions on growth and hydrocarbon accumulation were investigated. Seed age influenced the biomass and hydrocarbon accumulation in *B. braunii*, and the highest values were 5.97 and 2.99 g m^{-2} day^{-1}, respectively, when seed age was 14 days. The appropriate range of initial inoculation density was 7.9–10.1 g m^{-2}. Light intensity was a dominating factor influencing *B. braunii*'s growth in the attached culture, and the light saturation point was 100–150 μmol m^{-2} s^{-1}. Periodic illumination in 8:16 light: dark cycle had the highest utilisation of photons at approximately 1.0 g of biomass per mole of photons. The increasing CO_2 concentration in aerated gas improved the growth rate, but its concentration should be 1%.

Conclusions: Attached algal cultivation systems have been widely explored. However, the optimised values for aqueous suspension methods may be unnecessary for the attached system. Optimised seed age, inoculum density, CO_2 concentration, light intensity and photoperiod can improve the growth and hydrocarbon accumulation of *B. braunii* SAG807-1 with the attached culture.

Keywords: *Botryococcus braunii*, Attached culture, Culture conditions, Biomass, Hydrocarbon

Background

Microalgae are important resources for the conversion of CO_2 and sunlight into usable energies because they show the potential for higher lipid production than other biomass sources (Service 2011; Fan et al. 2014; Su et al. 2016). Bioregenerative methods involving photosynthesis by microalgal cells have been applied to reduce the amount of atmospheric CO_2 to ensure a safe and reliable living environment. Among microalgal species, *Botryococcus braunii* has a high lipid content, which mostly consists of hydrocarbons (Banerjee et al. 2012). Hydrocarbons are easily transformed into fuels; thus, the effective utilisation of *B. braunii* will lead to the development of a method for biofuel production, thereby reducing CO_2 emissions and building a sustainable society (Tasić et al. 2016; Yoo et al. 2010).

Several strategies, including optimisation of medium composition, physical parameters and type of metabolism (Mata et al. 2010), have been adopted to improve

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microalgal growth, lipid production and biochemical composition. However, a cost-effective algal cultivation technology for large-scale applications has yet to be established to considerably reduce dependency on foreign oil (Chisti 2007). The major challenges in large-scale *B. braunii*'s cultivation, especially traditional open ponds and closed photobioreactors, are the slow growth rate and negative effects of this species (Ruangsomboon 2012; Baba et al. 2012). These photobioreactors are used by cultivating algae in liquid nutrient media, and algae need excessive amounts of water to produce lipid; energy-intensive dewatering and biomass concentration processes are also required for the downstream processing of algae in biorefinery (Berner et al. 2015).

Attached algal cultivation systems have been widely explored. We previously introduced a novel biofilm cultivation system called attached cultivation. The biofilm-attached cultivation of algae involves algal cells that are generally immobilised in high density and fed with nutrient solutions and differs from conventional aqueous suspended cultures. In this system, a high-density microalgal paste was attached to a supporting structure, which consisted of a glass plate, a filter paper and a cellulose acetate/cellulose nitrate membrane, to form an artificial 'leaf'; several pieces of these leaves are vertically inserted into a glass chamber (Liu et al. 2013; Cheng et al. 2013, 2014).

Studies have been conducted to identify the potential of biofilm growth and the overall lipid productivity of *B. braunii*; however, current knowledge on *B. braunii* biofilm growth and lipid productivity is limited (Wijihastuti et al. 2017; Ozkana et al. 2012). The growth rates of *B. braunii* in a culture system are also affected by a combination of environmental parameters, such as CO_2, light intensity and photoperiod (Yoshimura et al. 2013). Light intensity and photoperiod play an important role in algal growth and distribution, but requirements vary significantly in different species, culture conditions and algal culture density. Light cycle, including fluctuations in intensity and photoperiod, is among the main factors influencing the growth and biochemical composition of microalgae (Wahidin et al. 2013).

The optimised values for aqueous suspension methods may be unnecessary for the attached system because inherent differences exist between these systems in the following aspects: (1) In illumination, for the suspended methods, algal cells continuously switch between light and dark cycles. However, the attached algal cells were immobilised to ensure that light condition was relatively stable when the natural fluctuation of light sources was not considered. (2) In CO_2 transfer, in suspended cultivation, the carbon source supplied by CO_2 should be dissolved firstly in the aqueous medium to form diluted HCO_3^-, CO_3^{2-} and CO_2 solutions, and these gases were then absorbed by algal cells (Van Den Hende et al. 2012). However, a 'water wall' segregating the carbon source and algal cells is eliminated in the attached system, and the concentrated CO_2 gas easily reaches algal cells (Wang et al. 2015; Cheng et al. 2017). These differences in the supply patterns of light and CO_2 between suspended and attached methods likely alter the key metabolism pathways in algal cells (e.g. photosynthesis and lipid synthesis) and mass cultivation biotechnology.

Given the specificity for attached photobioreactors, algal cells must adhere onto a filter paper or other materials. Therefore, inoculum density of microalgal cells was crucial in biomass production to achieve high photosynthetic efficiency. Microalgal cells undergo changes in morphological characteristics, cell wall structure and composition in different growth phases. Thus, the seed age of algal cells may affect the growth or lipid production of microalgae in this attached culture.

This study investigated the effects of seed age, inoculum density, CO_2, light intensity and photoperiod on growth and hydrocarbon accumulation in *B. braunii* SAG807-1 grown in the attached culture. The results revealed the optimal conditions for *B. braunii* SAG807-1 cultivation in the attached culture.

Methods

Algal strain and broth seed culture for inoculum preparation

Botryococcus braunii SAG 807-1 (SAG Culture Collection, University of Gottingen, Germany) was grown in a modified Chu 13 medium (Largeau et al. 1980). For the preparation of the inocula, which were used in the attached bioreactors, algae were firstly cultivated in glass bubbling columns (diameter = 0.05 m) for approximately 2 weeks (in the middle of exponential phase) and then harvested through centrifugation at 5000×*g*. The column containing 0.7 L of algal broth was continuously illuminated with cold white fluorescent lamps (NFL28-T5, NVC, China) under a light intensity of 100 ± 10 μmol m^{-2} s^{-1}. The algal broth temperature during cultivation was 25 °C ± 2 °C. Air bubble containing 1% CO_2 (v/v) was continuously injected into the bottom of the columns at a speed of 1 vvm to agitate the algal broth and to supply carbon.

Attached cultivation system

The attached cultivation system used in this research was similar to that described by Liu et al. (2013) and Cheng et al. (2013). Single-layer vertical plates were attached to the photobioreactors. In brief, a glass chamber comprising a glass plate and an attached algal disc was placed on an iron rack and tilted at a certain angle

against the horizontal plane. The medium was propelled (~10 mL min^{-1}) using a peristaltic pump (TP12DC 12V, Guangzhou JU PlasFitting Technology Co., Ltd., China) to facilitate the circulation of the medium inside the system. The light intensity inside the chamber at the position of the attached algal cells was 100 ± 10 μmol m^{-2} s^{-1}. Continuous airflow containing 1% CO_2 (v/v) was injected into the glass chamber at a speed of 0.1 vvm to supply carbon, and the temperature inside the glass chamber was 25 °C ± 2 °C during the experiments. For accurate measurement, each culture period was maintained for 8 days in all of the attached cultivations (Cheng et al. 2013).

Experimental design

Effects of seed age on algal cell growth and hydrocarbon accumulation

Different growth phases are defined in the growth curve. Algal cells at different growth phases were cultivated in glass bubbling columns for 7, 14, 21 and 28 days, harvested and placed in the attached photobioreactors. The initial inoculum density was approximately 8–10 g m^{-2}, and the glass chamber was bubbled with 1% CO_2 (air/CO_2) for the growth of the attached algal cells. The temperature and light intensity were 25 °C ± 2 °C and 100 μmol photons m^{-2} s^{-1}, respectively.

Effect of inoculum density on algal growth and hydrocarbon accumulation

A stock culture of *B. braunii* SAG807-1 was cultured in a 1 L Erlenmeyer flask containing 0.7 L of medium. After 14 days, the microalgal cells in the exponential growth phase were obtained and washed thrice with sterilised water. The algal cells were subsequently inoculated in different volumes to prepare seven levels of cell density (1.9, 4.1, 5.7, 7.9, 10.1, 15.2 and 24.9 g m^{-2}) and bubbled with 1% CO_2 (air/CO_2). The temperature and light intensity were 25 °C ± 2 °C and 100 μmol photons m^{-2} s^{-1}, respectively.

Effects of CO_2 concentrations on algal growth and hydrocarbon accumulation

The growth of *B. braunii* SAG807-1 under different CO_2 concentrations was measured. The initial inoculum density was approximately 8–10 g m^{-2}. The growth and hydrocarbon accumulation under bubbling with ambient air (0.038% CO_2) and air containing 0.5, 1, 5 and 10% CO_2 were investigated. Air-CO_2 mixtures, purchased from a commercial gas supply company, were bubbled directly into the attached photobioreactors at a speed of 0.1 vvm to supply carbon throughout the culture period. The temperature and light intensity were 25 °C ± 2 °C and 100 μmol photons m^{-2} s^{-1}, respectively.

Effects of light intensity and photoperiod on algal growth and hydrocarbon accumulation

A wide range of light intensities (10, 20, 40, 60, 80, 100, 150, 200 and 250 μmol m^{-2} s^{-1}) produced by cold white fluorescent lamps was set to study the effects of different light intensities. Six different photoperiods, namely, 24:0, 20:4, 16:8, 12:12, 8:16 and 4:20 h light:dark (L:D) cycles, with 100 μmol m^{-2} s^{-1} light intensity were used to determine the effect of photoperiod on growth and hydrocarbon accumulation in *B. braunii* SAG807-1. The initial inoculum density was approximately 8–10 g m^{-2}. The temperature and CO_2 concentrations were 25 °C ± 2 °C and 1%, respectively.

Growth analysis

The biomass concentration of an algal disc (DW, g m^{-2}), which was a wet paste of algal cells attached onto a supporting material to form a thin layer of algal population, was determined using gravimetric method (Liu et al. 2013). The cells of the algal disc were rinsed, resuspended in deionised water and filtered through a preweighted 0.45-μm GF/C filter membrane (DW_0; Whatman, England). The membrane was oven dried at 105 °C for 12 h and then cooled to room temperature for dry weight (DW_1) measurement. DW was calculated as follows:

$$DW = (DW_1 - DW_0)/0.001, \quad (1)$$

where 0.001 represents the footprint area of algal disc (m^2).

Hydrocarbon analysis

Hydrocarbon analysis was conducted in accordance with previously described methods (Cheng et al. 2013; Sawayama et al. 1992). In brief, attached algal cells were harvested by washing with deionised water and centrifuging at 3800×*g* for 10 min. The algal pellets were rinsed thrice with deionised water to remove the attached salt. After the algal pellets were freeze dried, 50 mg of dried algal biomass was homogenised and then extracted with *n*-hexane thrice. The supernatants were combined in a preweighted glass vial, and the solvent was blown away with nitrogen gas (> 99%). The residue remaining in the glass vial was considered 'crude hydrocarbon' (Largeau et al. 1980; Singh and Kumar 1992; Dayananda et al. 2005).

Statistical analysis

Experiments were performed in triplicates. Data were presented as mean of three independent replicates and further analysed through one-way analysis of variance (ANOVA) using Microsoft Office Excel 2010 (Microsoft, USA). $P < 0.05$ indicated significant difference.

Results and discussion

Effects of seed age on biomass productivity and hydrocarbon accumulation

In this study, *B. braunii* SAG 807-1 seed liquid was cultured for 7, 14, 21 and 28 days in a traditional liquid column reactor. Subsequently, the attached culture was prepared in Chu 13 culture medium at 25 °C ± 2 °C under a continuous illumination intensity of 100 ± 10 μmol m^{-2} s^{-1} and 1% CO_2. After 8 days, the biomass productivity, hydrocarbon content and hydrocarbon yield of the culture were determined (Fig. 1). Figure 1a shows that the seed liquid in the attached culture for 14 days displayed the highest biomass productivity of approximately 5.97 g m^{-2} day^{-1}, followed by the culture with a seed age of 7 days (5.62 g m^{-2} $days^{-1}$). The seed liquid in the attached culture for 21 and 28 days supported the lowest biomass productivity at 3.24 and 2.35 g m^{-2} $days^{-1}$, respectively. Figure 1b illustrates that the highest hydrocarbon content of approximately 58.9% was observed in the attached culture system with a seed age of 28 days. The highest hydrocarbon productivity (approximately 2.99 g m^{-2} $days^{-1}$) was observed in an attached culture with a seed age of 14 days. The lowest hydrocarbon yield of approximately 1.38 g m^{-2} $days^{-1}$ was obtained in the seed age of 28 days. The hydrocarbon yields of seed ages 7 and 21 days were 2.71 and 1.83 g m^{-2} $days^{-1}$, respectively.

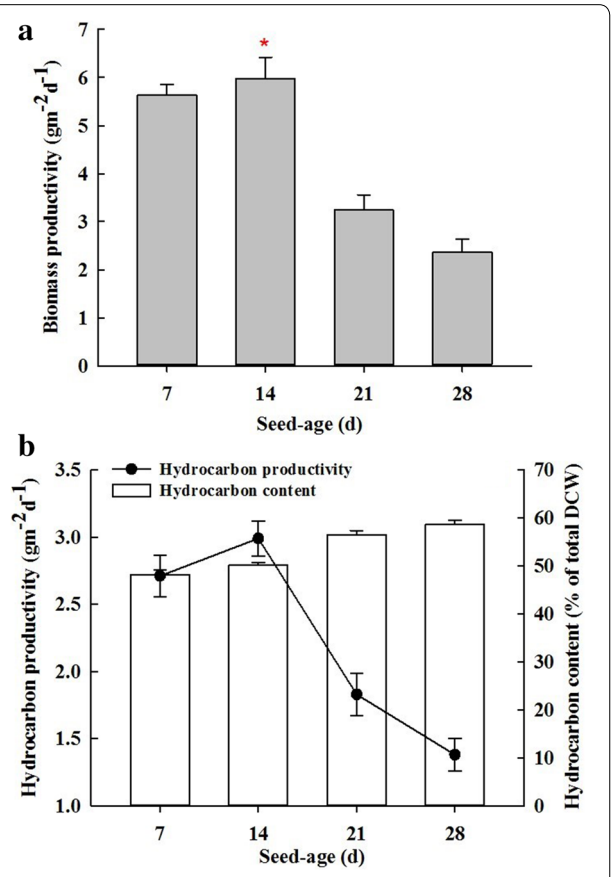

Fig. 1 Effect of seed age on growth and hydrocarbon accumulation of *B. braunii* SAG807-1 under attached cultivation: **a** Biomass productivity. **b** Hydrocarbon content and productivity. Data are means ± standard deviations of three replicates

The prolonged culture time of *B. braunii* SAG 807-1 seed liquid increased the hydrocarbon content with the attached culture. However, the higher the seed age was, the lower the biomass productivity would be. Thus, the corresponding hydrocarbon yield decreased. These results (Fig. 1) indicated that 14 days was the optimal seed age of *B. braunii* SAG 807-1 for seed liquid cultivation through the attached culture. Hydrocarbons were the secondary metabolites in the microalgal culture, and seed age significantly influenced the growth of algae and the synthesis of their metabolites. Seed age considerably influenced the efficiency and economic viability of attached culture method for algal cultivation. In general, the logarithmic phase of seed age in liquid cultivation is exuberant. In the logarithmic phase, cells grow rapidly, and their number increases exponentially. Given the specificity of the attached photobioreactor, a well-cultured seed solution should be initially inoculated onto an adherence membrane, and a culture may be subsequently prepared.

Effects of inoculum density on growth and hydrocarbon accumulation

In this study, *B. braunii* SAG 807-1 was cultured in a liquid column reactor for 14 days, and the algal cells were then cultured in the attached reactors at different initial inoculum densities, namely, 1.9, 4.1, 5.7, 7.9, 10.1, 15.2 and 24.9 g m^{-2} for 8 days. In Fig. 2, the biomass productivity of *B. braunii* SAG 807-1 gradually increased as the initial inoculum density increased. Conversely, the biomass productivity gradually decreased when the initial inoculum density increased to a certain degree. The lowest biomass productivity (approximately 2.14 g m^{-2} $days^{-1}$) was obtained at an initial inoculum density of 1.9 g m^{-2}. The biomass productivity of *B. braunii* SAG 807-1 slightly changed at an initial inoculum density of 7.9–24.9 g m^{-2}.

The effects of different initial inoculum densities on the hydrocarbon content of *B. braunii* SAG807-1 did not vary significantly (Table 1). At a high initial inoculum density, the hydrocarbon content slightly decreased. When the initial inoculum density was 1.9 g m^{-2}, the hydrocarbon content was 51.6% higher than those under the initial inoculum densities of 15.2 and 24.9 g m^{-2} (47.6 and 44.2%, respectively) possibly because microalgae

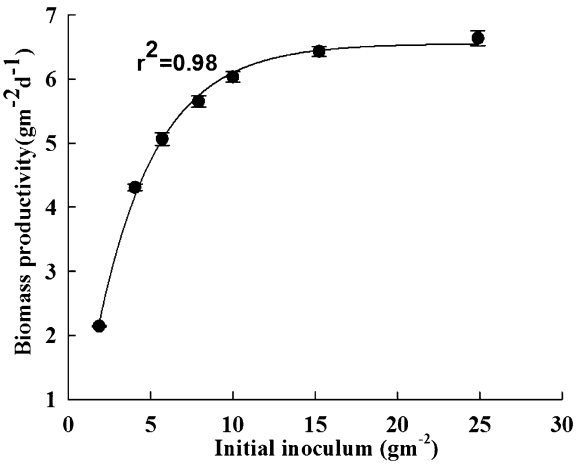

Fig. 2 Effect of inoculum density on growth of *B. braunii* SAG807-1 under attached cultivation. Data are means ± standard deviations of three replicates

exposed to illumination, CO_2 and other factors were not uniform at a high inoculum density.

Our experimental results showed that the optimal initial inoculum density for the attached culture of *B. braunii* SAG807-1 was approximately 7.9–10.1 g m^{-2} (Fig. 2 and Table 1). Too high or too low inoculum density possibly causes photoinhibition or optical limitation (Wang et al. 2013). Khatri et al. (2013) reported that algal cell concentration decreases as initial inoculum densities increase because of light-limited growth conditions. Moreover, seed cost should be considered in a culture with a high inoculum density. In traditional microbial/fungal fermentation, high inoculum density can shorten the time of cell proliferation and increase the production of metabolites. However, excessive inoculum may promote the rapid growth of cells, increase the viscosity of culture media and lead to a lack of matrix or dissolved oxygen, consequently affecting metabolite synthesis. A considerably small amount of inoculum will prolong the fermentation cycle, increase the chance of bacterial contamination and cause mycelial agglomeration and other fermentation abnormalities. Algal cultures similarly face these problems. Attached culture requires the inoculation of microalgal seed liquid onto membrane materials for a certain period. Therefore, the control of initial inoculum density was a key to ensuring normal algal culture and metabolite generation.

Effects of CO_2 concentrations on growth and hydrocarbon accumulation

This study investigated the effect of 0.038% air and 0.5, 1, 5 and 10% CO_2 on growth and hydrocarbon accumulation in *B. braunii* SAG 807-1 with the attached culture. In Fig. 3, *B. braunii* SAG 807-1 could grow under different CO_2 concentrations with the attached culture. However, different CO_2 concentrations elicited various effects on *B. braunii*'s growth. In the attached culture, the increasing CO_2 concentration resulted in a rapid growth and a corresponding increase in biomass productivity. When air was introduced to the attached culture (0.038%), the biomass productivity of *B. braunii* SAG 807-1 was approximately 2.56 g m^{-2} day^{-1}. As the CO_2 concentration increased, biomass productivity also increased. The highest biomass productivity was observed at approximately 5.11 g m^{-2} day^{-1} at 1% CO_2. At 5 and 10% CO_2, the corresponding biomass productivities were 4.88 and 4.71 g m^{-2} day^{-1}, respectively. The biomass productivity likely stabilised and did not further increase.

In terms of the effects of different CO_2 concentrations on hydrocarbon accumulation in *B. braunii* SAG 807-1 grown in the attached culture, high CO_2 concentration could promote hydrocarbon synthesis (Fig. 4). In Fig. 4, when air (0.038%) was introduced to the culture medium, the hydrocarbon content of *B. braunii* was approximately 36.7%, which was relatively low. When the CO_2 concentration in the culture medium increased to 0.5 and 1%, the hydrocarbon content also increased to 45.4 and 46.8%. The highest hydrocarbon content of *B. braunii* grown in the attached culture was approximately 60.4% at 5% CO_2. Conversely, the corresponding hydrocarbon content slightly decreased to approximately 55.3% when the CO_2 concentration continuously increased (up to 10%). The influence of different CO_2 concentrations on the hydrocarbon yield of *B. braunii* SAG807-1 was similar. Furthermore, 5, 10 and 1% CO_2 highly affected its hydrocarbon yield.

Our comprehensive analysis showed that the optimal CO_2 concentration for the attached culture of *B.*

Table 1 Effect of inoculum density on growth and hydrocarbon accumulation of *B. braunii* SAG 807-1 under attached cultivation

Initial inoculum densities (g^{-2})	1.9	4.1	5.7	7.9	10.1	15.2	24.9
Biomass productivity (g^{-2} day^{-1})	2.14 ± 0.14	4.32 ± 0.11	5.07 ± 01.9	5.65 ± 0.23	6.03 ± 0.26	6.43 ± 0.32	6.64 ± 0.31
Hydrocarbon content (% of DCW)	51.6 ± 0.42	50.5 ± 0.56	49.8 ± 0.68	49.6 ± 0.46	50.1 ± 0.51	47.6 ± 0.64	44.2 ± 0.38
Hydrocarbon productivity (g^{-2} day^{-1})	1.10 ± 0.06	2.19 ± 0.09	2.52 ± 0.11	2.80 ± 0.08	3.02 ± 0.16	3.06 ± 0.18	2.93 ± 0.14

The data were means ± standard deviations of three replicates

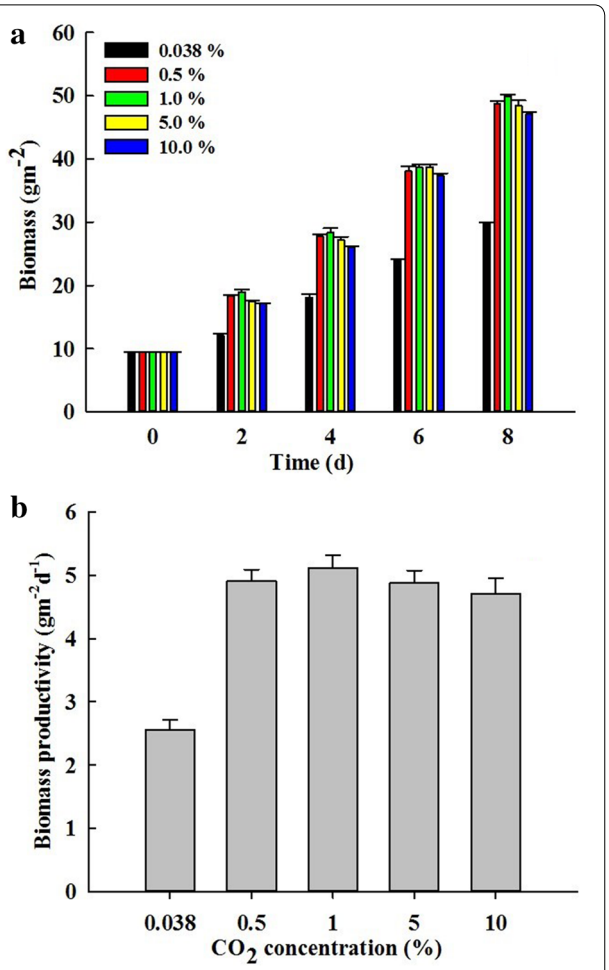

Fig. 3 Effect of CO_2 concentration on growth of *B. braunii* SAG807-1 under attached cultivation. The biomass densities after different days cultivations (**a**) and the average biomass productivity of 8 days cultivations (**b**) at the CO_2 concentrations of 0.038, 0.5, 1.0, 5.0 and 10.0%. Data are means ± standard deviations of three replicates

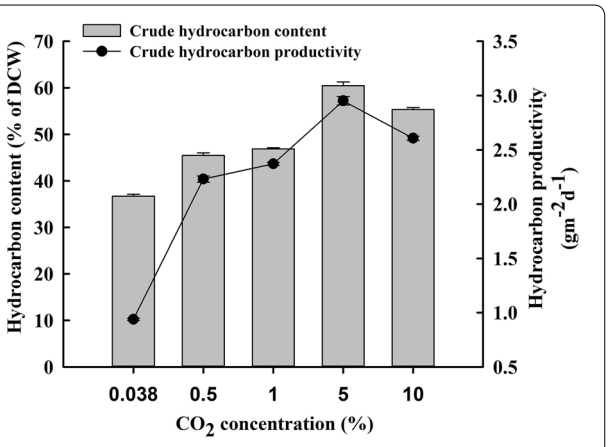

Fig. 4 Effect of CO_2 concentration on hydrocarbon accumulation of *B. braunii* SAG807-1 under attached cultivation. Data are means ± standard deviations of three replicates

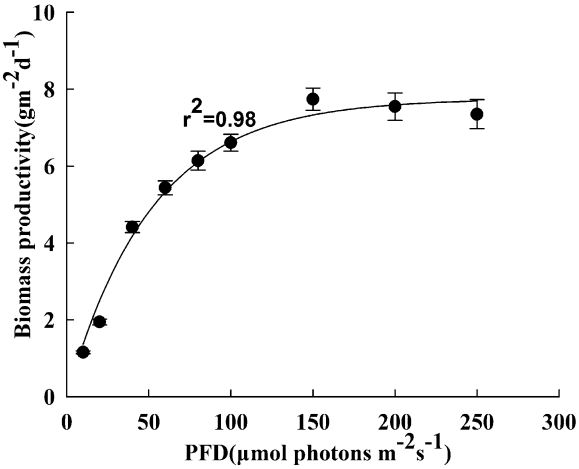

Fig. 5 Effect of light intensity on growth of *B. braunii* SAG807-1 under attached cultivation. Data are means ± standard deviations of three replicates

braunii SAG 807-1 was 1%. High CO_2 concentration could increase the biomass productivity of *B. braunii* and promote hydrocarbon synthesis. CO_2, the main carbon source of autotrophic microalgae, plays an important role in microalgal growth and lipid synthesis. However, high CO_2 concentration considerably increases the cost of algal production and restricts the development of this industry for the commercial production of algal fuel. Current studies on the application of CO_2 in microalgae mainly focus on their CO_2 tolerance, algal growth rate and biomass. An et al. (2003) used high CO_2 to provide carbon for the growth of *B. braunii*. Yoshimura et al. (2013) studied the effect of 0.2–5% CO_2 on *B. braunii* growth and metabolism. However, these studies have focused on culturing *B. braunii* via traditional liquid suspension cultures. In our study, the attached culture was a photoreaction system separating the culture medium from algal cells, and the transfer mode of CO_2 and nutritive salt in the attached culture was different from those in traditional liquid culture (Ji et al. 2014). Therefore, attached culture was significantly essential for research on CO_2 and other factors influencing algal growth.

Effects of light intensity and photoperiod on growth and hydrocarbon accumulation

This study also investigated the effects of different light intensities (10, 20, 40, 60, 80, 100, 150, 200 and 250 μmol m^{-2} s^{-1}) on the growth and hydrocarbon accumulation in *B. braunii* SAG 807-1. Figure 5 shows that the growth of *B. braunii* SAG 807-1 in the attached culture gradually increased as light intensity increased (< 100 μmol m^{-2} s^{-1}). When light intensity was 10 μmol m^{-2} s^{-1}, the biomass productivity was only 1.16 g m^{-2} day^{-1}. Conversely, when light intensity

Table 2 Effect of light intensity on growth and hydrocarbon accumulation of *B. braunii* SAG 807-1 under attached cultivation

Light intensity ($\mu molm^{-2} s^{-1}$)	10	20	40	60	80	100	150	200	250
Biomass productivity ($g^{-2} day^{-1}$)	1.16 ± 0.08	1.94 ± 0.07	4.42 ± 0.14	5.44 ± 0.18	6.14 ± 0.24	6.61 ± 0.22	7.74 ± 0.29	7.55 ± 0.36	7.35 ± 0.58
Hydrocarbon content (%)	24.69 ± 0.54	26.34 ± 0.52	31.68 ± 1.01	36.44 ± 0.49	41.68 ± 0.92	43.74 ± 0.66	49.26 ± 1.21	51.04 ± 0.67	54.1 ± 0.85
Hydrocarbon productivity ($g^{-2} day^{-1}$)	0.29 ± 0.16	0.51 ± 0.04	1.40 ± 0.24	1.98 ± 0.11	2.56 ± 0.46	2.89 ± 0.37	3.81 ± 0.51	3.85 ± 0.39	3.98 ± 0.41

The data were means ± standard deviations of three replicates

increased to 80 μmol m^{-2} s^{-1}, biomass productivity also increased to 6.14 g m^{-2} day^{-1}. As light intensity increased from 100 to 150 μmol m^{-2} s^{-1}, the biomass productivity of *B. braunii* SAG807-1 slightly increased (from 6.61 to 7.74 g m^{-2} day^{-1}). When light intensity continuously increased (> 150 μmol m^{-2} s^{-1}), algal growth was inhibited, and biomass productivity slightly decreased from 7.55 to 7.35 g m^{-2} day^{-1}.

Low light intensity prevented hydrocarbon accumulation in *B. braunii* SAG 807-1 (Table 2). For example, hydrocarbon content at 10 μmol m^{-2} s^{-1} light intensity was 26.69%. As light intensity increased, hydrocarbon content also increased possibly because strong light induced hydrocarbon synthesis and accumulation. However, biomass productivity in *B. braunii* SAG807-1 was low at a low light intensity (Table 2). Hydrocarbon yield was also lower than 2.0 g m^{-2} day^{-1} at a light intensity of lower than 60 μmol m^{-2} s^{-1}. Based on the experimental results, our conclusion was that the optimal light intensity for *B. braunii* SAG 807-1 grown in the attached culture was approximately 100–150 μmol m^{-2} s^{-1}.

Light substantially affects the growth, reproduction, cell morphology and metabolism of microalgae. Appropriate light with a suitable intensity could accelerate the growth and reproduction of microalgae and effectively improve the productivity and quality of algal culture (Fig. 5). High light intensities limit algal growth but favour high lipid content and yield (Ruangsomboon 2012). Light is a complex component with several influencing factors, including light intensity, photoperiod and spectrum. Related studies are currently in the stage of data accumulation. Light intensity and photoperiod are easily regulated. The influencing pattern of light intensity is generally observed in various algae existing within a certain light intensity range suitable for their growth. The light intensity in this range can either enhance or reduce the growth rate (Kuster et al. 2004; Garde and Cailliau 2000; Vervuren et al. 1999). The influencing pattern of photoperiod indicates that the light and dark periods suitable for the growth of different algal species vary (Janssen et al. 2000, 2001).

This study also investigated the effects of different L:D cycles (24:0, 20:4, 16:8, 12:12, 8:16 and 4:20) on the growth of *B. braunii* SAG 807-1 subjected to an optimal light intensity of 100 μmol m^{-2} s^{-1} in the attached culture. In the attached culture system (Fig. 6), *B. braunii*

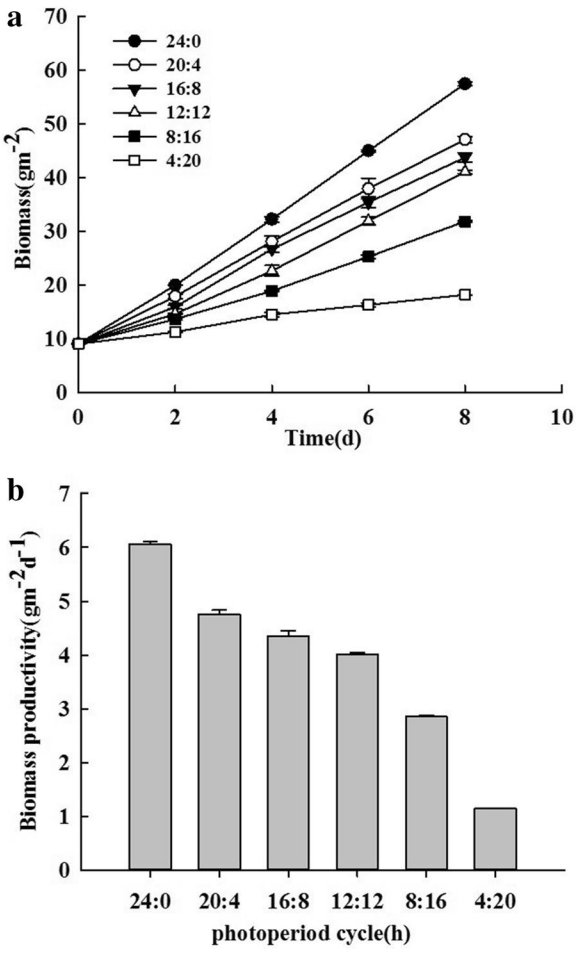

Fig. 6 Effect of illumination cycle on growth of *B. braunii* SAG807-1 under attached cultivation. The biomass densities after different days cultivations (**a**) and the average biomass productivities of 8 days cultivations (**b**) under the illumination cycles of 24:0, 20:0, 16:8, 12:12, 8:16 and 4:20. Data are means ± standard deviations of three replicates

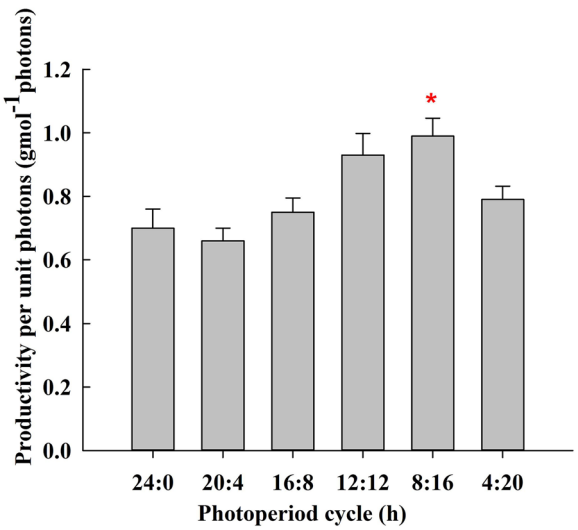

Fig. 7 Effect of illumination cycle on utilisation of unit photons of *B. braunii* SAG807-1 under attached cultivation. Data are means ± standard deviations of three replicates

SAG 807-1 grew rapidly under a prolonged photoperiod, and its biomass productivity increased. Continuous illumination for 24 h yielded the highest biomass productivity of approximately 6.0 g m^{-2} day^{-1}, followed by 20:4 L:D cycle with a biomass productivity of 4.9 g m^{-2} day^{-1}. In a shortened photoperiod, the corresponding biomass productivity also gradually decreased. The lowest biomass productivity was approximately 0.9 g m^{-2} day^{-1} in 4:20 L:D cycle.

In unit photon utilisation, photoperiod was not proportional to photon utilisation. Figure 7 shows that the corresponding unit photon utilisation was 0.7 g mol^{-1} photons, which was lower than that in 4:20 L:D cycle (0.8 g mol^{-1} photons) under continuous illumination for 24 h. The highest unit photon utilisation of *B. braunii* SAG 807-1 grown in the attached culture was approximately 1.0 g mol^{-1} photons in 8:16 L:D cycle. The biomass energy of *B. braunii* was larger than the mean of other algae. The energy contained in 1 kg of *B. braunii* biomass is approximately 28.3MJ (Ozkana et al. 2012). Thus, in 8:16 L:D cycle, the optical energy utilisation of visible light corresponding to unit photon utilisation was approximately 13.0%, which was higher than those under other L:D ratios.

Algal photosynthesis involves light and dark reactions. Continuous illumination rapidly increases the number of algal cells and improves their biomass productivity. This phenomenon was also the reason for the high biomass productivity of *B. braunii* SAG807-1 under continuous illumination (Fig. 6). By contrast, photosynthesis intensity under continuous illumination was low, resulting in low unit photon utilisation and low photosynthesis. This phenomenon was also accounted for the lower number of cells generated from unit photon under 24 h of continuous illumination than that in 4:20 L:D cycle (Fig. 7). In an algal culture, a proper L:D cycle improved the photosynthesis and growth rates of cells. Moreover, dark cycles contributed to the self-repair of damaged cells. However, with a prolonged L:D cycle, the photochemical quantum yield of algal cells, the conversion efficiency of optical energy into chemical energy, the growth rate of algal cells and the optical energy yield decrease (Merchuk et al. 1998).

Conclusions

In this study, seed age influenced the biomass of and hydrocarbon accumulation in *B. braunii* in the attached culture, and the initial inoculation density played important roles in *B. braunii*'s growth. The increasing CO_2 concentration at an appropriate level of 1% in aerated gas improved *B. braunii*'s growth. The suitable light saturation point for algal growth was approximately 100–150 μmol m^{-2} s^{-1}. A 24-h continuous illumination helped obtain the maximum biomass productivity; however, periodical illumination in 8:16 L:D cycle induced the highest utilisation of photons at approximately 1.0 g of biomass per mole of photons.

Authors' contributions

PC and YW conceived and designed the experiments. PC, YW and DOW performed the experiments. DL contributed to analytic tools. TL wrote the paper. All the 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

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Not applicable.

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