Shi-Kai Wang^{2,3}Chen Guo³Wei Wu¹Kun-Yan Sui¹Chun-Zhao Liu^{1,3}

Research Article

Effects of incident light intensity and light path length on cell growth and oil accumulation in *Botryococcus braunii* (Chlorophyta)

¹State Key Laboratory of Bio-fibers and Eco-textiles, Institute of Biochemical Engineering, Shandong Collaborative Innovation Center of Marine Biobased Fibers and Ecological textiles, College of Materials Science and Engineering, Qingdao University, Qingdao, P. R. China

²College of Bioscience and Biotechnology, Yangzhou University, Yangzhou, P. R. China

³State Key Laboratory of Biochemical Engineering & Key Laboratory of Green Process and Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, P. R. China

Botryococcus braunii was cultured in different light path length under different incident light intensity to investigate the effect of light on alga growth as well as hydrocarbon and fatty acid accumulation. Results indicated that longer light path length required higher incident light intensity in order to meet the light requirement of algal growth and hydrocarbon accumulation during the course of cultivation. However, hydrocarbon profile was only affected by the incident light intensity and not influenced by the light path length. High incident light intensity enhanced the accumulation of hydrocarbons with longer carbon chains. Besides, the fatty acid content and profiles were significantly influenced by both incident light intensity and light path. Higher fatty acid content and higher percentage of C18 and monounsaturated fatty acid components were achieved at the higher incident light intensity and lower light path length. Taken together, these results are benefit to improve its biomass and oil productivity through the optimization of light and photobioreactor design.

Keywords: *Botryococcus braunii* / Cell growth / Fatty acid / Hydrocarbon / Light

Received: August 10, 2018; **revised:** October 26, 2018; **accepted:** November 8, 2018

DOI: 10.1002/elsc.201800128

1 Introduction

Botryococcus braunii is regarded as a promising renewable feedstock for the production of biofuels, due to its ability to accumulate high amount of hydrocarbons [1, 2]. It is widespread in freshwater and brackish waters, and it has a higher CO₂ fixation rate compared with other algal species [3, 4]. In addition, following catalytic cracking, the long-chain hydrocarbons produced by *B. braunii* can be easily converted to liquid fuels [5].

Numerous studies on the growth and hydrocarbon production of *B. braunii* have been conducted. The effects of various nutritional and environmental factors such as nitrate, phosphate, micronutrients, carbon source, temperature, salinity, and pH on the growth and metabolism of *B. braunii* have been investigated, as well as the optimization of photobioreactor types [2, 6–11]. When cultivated in photoautotrophic conditions, light is one of the most important factors as it is the essential energy source

for the synthesis of algal biomass and metabolites. Baba et al. reported that red and blue light was more effective than green light for photosynthetic CO₂ fixation, algal growth, and hydrocarbon accumulation [1]. And Okumura et al. also found that high intensity blue light was most efficient for the growth of *B. braunii* (strain NIES-836) [12]. However, studies investigating the effects of light intensity on the cultivation of *B. braunii* indicated that different strains had specific optimum light intensities for algal growth, and the optimal light intensity for cell growth is often different from the optimal light intensity for the synthesis of specific products [2, 9, 10, 13, 14].

Due to the light absorption by intracellular pigments, light scattering by the algal cells and other suspended particles, and light shading between algal cells result in light attenuation, which means the inhomogeneous distribution of the light intensity [15]. The available light is high at section of short light path length and it exponentially decreases with the increase of light path length. For this reason, higher incident light intensity is necessary to keep better illumination at section with longer light path length, but this may cause photoinhibition and photooxidation for cells at section with short light path length. Oppositely, suitable incident light intensity for section with short light path length may limit the algal growth at section with high light path length [16]. Therefore, the choice of incident light intensity should integrate with specific light path length. Meanwhile,

Correspondence: Dr. Chun-Zhao Liu & Dr. Chen Guo (czliu@ipe.ac.cn, cguo@ipe.ac.cn), State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, P. R. China

Abbreviations: DCW, dry cell weight; FA, fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

studies about the effects of light on cell growth and metabolism should simultaneously consider the incident light intensity with the light path length. However, current researches on the light intensity requirement were usually investigated at a constant light path length [17, 18].

In this study, *B. braunii* cells were cultivated at different incident light intensity in culture systems with different light path length. The cell growth under different light conditions was determined. Besides, the influence of light on hydrocarbon and fatty acid accumulation and its profiles was also identified. The results are helpful for the optimization of light conditions for efficient oil accumulation using *B. braunii*.

2 Materials and methods

2.1 Microalgal strain and culture methods

B. braunii IPE 001 (race B, stored in the Institute of Process Engineering, Chinese Academy of Sciences) was cultivated in modified Chu 13 medium for all experiments [19]. Algal cells grown in agar slants were first cultivated in 100 mL medium in 250 mL Erlenmeyer flasks for 3 weeks and then transferred to 2 L airlift bioreactor for 2 weeks [11]. In the Erlenmeyer flasks, the cultures were kept on an orbital shaker at 100 rpm, while for the 2 L bioreactors cultivation, CO₂-enriched air (1% v/v) was bubbled into the reactor through a 0.22 µm filter (Millipore, MA, USA) at a flow rate of 0.1 v/v/min. All cultures were photoautotrophically grown in sterile conditions at 25°C under a light intensity of 35 µmol/m²/s with a light/dark cycle of 16/8 h per day. The 2 L bioreactor-cultured cells were used as the inoculum for the flat-plate bioreactor experiments.

The experiments were conducted in 15 cm *L* × 10 cm *H* flat-plate photobioreactors with the wide of 2.5, 5, 7.5, 10, and 15 cm, respectively, constructed of clear float glass. Using a loading height of 8 cm, the working volume was 300, 600, 900, 1200, and 1800 mL, respectively. During cultivation, the photobioreactor was enclosed in a dark box with a lid constructed from a dark acrylic board. An array of white LED lights was attached to the inner side of the box.

The cultured cells in the 2 L bioreactor were collected by filtration using a 500 mesh nylon sheet, and then transferred to the culture systems with an inoculum concentration of 0.27 g DCW /L. CO₂-enriched air (1%, v/v) was directly bubbled into the reactor at a flow rate of 0.2 v/v/min through a flexible porous air diffuser uniformly arranged at the bottom of the photobioreactor (Fig 1A). The cultures were kept at 25°C. The incident light intensity was set at 17, 170, 480, 690, and 800 µmol/m²/s, respectively to cover photo-inhibition region and light-limited region. The light intensity was controlled by adjusting the output of the voltage and measured using a QSPL 2101 Quantum Scalar Laboratory PAR Irradiance Sensors equipped with a LOGGER 2100 data acquisition software (Biospherical, San Diego, USA).

2.2 Analytical methods

The algal biomass values were determined by first calculating the relationship between the optical density at 680 nm (OD₆₈₀) and

the corresponding dry biomass. To determine the relationship, the culture was concentrated or diluted to various OD₆₈₀ values using a UV-2100 spectrophotometer (Unico, Shanghai, China), and then filtered through a 500 mesh nylon sheet. The algal pellets were washed three times with distilled water and lyophilized using a lyophilizer (ALPHA1–2, CHRIST, Germany). The dry weight (g DCW/L) of the algal biomass was determined gravimetrically, and the relationship between the OD₆₈₀ and the *B. braunii* biomass was calculated using Eq. (1) as follows:

$$DW(gDCW/L) = 1.9732 \times OD_{680} - 0.0016 (R^2 = 0.9997) (1)$$

The specific growth rate (μ_{max} , day⁻¹) was calculated according to equation reported by Wang et al. [20]. To determine the content and composition of the hydrocarbons, the collected cells were lyophilized and then measured according to the method described by Wang et al. [20]. The lipid content and composition was analyzed according to the method described by Wang et al. [20].

2.3 Statistical analysis

Data was presented as means ± standard error of the mean based on three parallel experiments. The statistical significances were analyzed by one-way analysis of variance (ANOVA) ($p < 0.05$) using SPSS statistics 17.0 (IBM, USA).

3 Results and discussion

3.1 Effects of light on the growth of *B. braunii* cells

As shown in Fig. 1, the growth of *B. braunii* was greatly depended on light. Cell growth was poor at extremely low incident light intensity (17 µmol/m²/s) and increased with the increase of incident light intensity. However, extremely high incident light intensity, 800 µmol/m²/s, inhibited algal growth in these culture systems with short light path length (2.5 and 5 cm). The highest biomass concentration of 2.54 g/L was achieved at incident light intensity of 690 µmol/m²/s with a light path length of 2.5 cm. The specific growth rate at different conditions was shown in Fig. 2. It was observed that the cell growth was simultaneously influenced by both incident light intensity and light path length. Light attenuation caused the inhomogeneous distribution of the light intensity in algal cultures. As that microalgal cells continuously moved between light zones and dark zones, the available light for each microalgal cell was usually represented as the average light intensity. At a constant incident light intensity, a longer light path length resulted in a lower average light intensity [21]. It was identified that the algal growth was markedly influenced by the average light intensity, which means that the light attenuation has direct effect on cell growth.

When incident light intensity was relatively low, photosynthetic activity was limited by the light supply, resulting in a low specific growth rate. When the incident light intensity was increased to more than 170 µmol/m²/s, the growth rate at all light path length was significantly improved. In addition, cells cultured at a shorter light path length kept a higher average light intensity and obtain a higher specific growth rate. When the

Figure 1. The growth curves of *B. braunii* under different incident light conditions and light path length. (Incident light intensity: (A) 17 $\mu\text{mol}/\text{m}^2/\text{s}$; (B) 170 $\mu\text{mol}/\text{m}^2/\text{s}$; (C) 480 $\mu\text{mol}/\text{m}^2/\text{s}$; (D) 690 $\mu\text{mol}/\text{m}^2/\text{s}$; E: 800 $\mu\text{mol}/\text{m}^2/\text{s}$).

incident light intensity was increased to as high as 800 $\mu\text{mol}/\text{m}^2/\text{s}$, average light intensity was remarkably high at short light path length (2.5 cm and 5 cm), which might result in the damaged PS II (photosystem II) complex of algal cells and thus photoinhibition occurred. Carvalho et al. [22] reported that in the circumstance of high illumination, chlorophyll molecules may be excited to the triplet state and then react with oxygen,

which transfers energy from chlorophyll molecules to oxygen. Once the excited oxygen reacts with fatty acids, the formed lipid peroxides are detrimental to the cell membrane and even lead to cell death. Similar results were detected by Sakamoto et al., It was found that when cultured *B. braunii* BOT-22 in a flat-oblong glass vessel with the light path length of 3.5 cm, the optimal light intensity for the cell growth was 100 $\mu\text{mol}/\text{m}^2/\text{s}$. Further increase

Figure 2. Specific growth rate of *B. braunii* cultured under different incident light conditions and light path length.

of light intensity inhibited the growth of cells [14]. Due to the influence of light attenuation, the optimal incident light intensity was related to the light path. A higher incident light intensity was necessary for algal growth at the culture system with a relatively long light path length. For *Chlorella* sp., when it was cultured in the vertical tubular type photo-bioreactor with a light path length of 4 cm, a 500 mL flask with the diameter of about 10 cm, and a transparent polyethylene bag with the diameter of 10 cm, the optimal incident light intensity was 105.41–175.68, 200, and 350 $\mu\text{mol}/\text{m}^2/\text{s}$, respectively [23–25]. Taken together, these results indicated that the choice of incident light intensity should integrate with specific light path length. In addition, with regard to the design of photobioreactor for outdoor cultivation, the choice of light path length should consider the local illumination conditions.

3.2 Effects of light on the hydrocarbon content and profiles in *B. braunii* cells

The hydrocarbon content of *B. braunii* cells grown at different light conditions was shown in Fig 3A. It was detected that the hydrocarbon content was influenced by both incident light intensity and the light path length. The optimal incident light intensity for hydrocarbon production became higher along with the increase of light path length. However, this corresponding optimal value at different light path length was lower than the counterpart for cell growth. This was contrasted with the results derived by Sakamoto et al., who detected that the saturated light intensity (200 $\mu\text{mol}/\text{m}^2/\text{s}$) for hydrocarbon production in *B. braunii* BOT-22 was higher than the light intensity necessary to achieve maximum cell growth (100 $\mu\text{mol}/\text{m}^2/\text{s}$) [14].

However, Yoshimura et al. [2] found that the hydrocarbon content of *B. braunii* strain Showa did not correlate with the irradiance level. These differences were most likely due to the different requirement characteristics for light among the different *B. braunii* strains.

It was demonstrated previously that $\text{C}_{33}\text{H}_{56}$ and three isomers of $\text{C}_{34}\text{H}_{58}$ were the major hydrocarbons synthesized by the algal strain used in this study [26]. As showed in Fig. 3B and C, interestingly, light path had no obvious influence on hydrocarbon profile, whereas incident light intensity significantly influenced the hydrocarbon profile. With the increase of incident light intensity, the percentage of $\text{C}_{34}\text{H}_{58}$ increased from 55 to 66% while the percentage of $\text{C}_{33}\text{H}_{56}$ decreased from 45 to 34%. It has been previously established that the synthesis of botryococcene in race-B results from the reductive rearrangement of presqualene diphosphate [27]. Higher homologues of botryococcenes are synthesized using C30 botryococcene as the precursor and S-adenosylmethionine as the methylating agent [3]. In addition, the alkylation of botryococcene requires additional energy. In algal suspension, algal cells randomly moved between the light zones and the dark zones. The derived energy level for the alkylation of botryococcene may depend on the light level received in the light zones, in this case, the incident light intensity. As the only energy source under the autotrophic conditions, a higher light intensity may provide energy for the alkylation process and allow for the accumulation of higher homologues. These results indicated that hydrocarbon composition in race-B was not only dependent on genetic characteristics, but also depends on culture conditions [28]. Moreover, the relationship between light and hydrocarbon synthesis related genes and enzymes should be further investigated, which is helpful to enhance the accumulation of hydrocarbon in the cultivation of *B. braunii*.

Figure 3. Effects of incident light conditions and light path length on the hydrocarbon content (A) and composition (B, C) of *B. braunii*.

3.3 Effects of light on the FA content and profile in *B. braunii* cells

As shown in Fig 4A, both incident light intensity and light path had significant influence on FA content and composition. The FA content increased with increasing of incident light intensity from $17 \mu\text{mol}/\text{m}^2/\text{s}$ to $690 \mu\text{mol}/\text{m}^2/\text{s}$ at all given light path lengths. At a fixed incident light intensity, the FA content increased with decreasing of light path length. Nevertheless, when light intensity was further increased, photoinhibition and photooxidation

occurred, which go against to the accumulation of both biomass and lipid. It is well known that under high light intensity lipid accumulation is intensified [29, 30]. The improvement of FA content at high light intensities is due to the storage of excess photoassimilates, where excess light energy gets converted to chemical energy to overcome the photooxidative damage to the cell [29]. In addition, Xiao et al. [31] found that high light intensity decreased the contents of osmoprotectants (e.g., mannitol, proline and trehalose) accompanied by the accumulation of triacylglycerols in the culture of *Nannochloropsis oceanica* IMET1.

Figure 4. Effects of incident light conditions and light path length on the FA content (A) and composition (B, C, D, E, F) of *B. braunii*.

Besides FA content, FA profiles were also greatly influenced by light supply. As shown in Fig. 4 B and C, FAs with 16 and 18 carbon atoms were the main FA components in *B. braunii* cells, both of which reached up to 95% of the total FAs. Besides, continuous decreases of C16 proportion and increase of

C18 proportion occurred with increasing of light intensity from $17 \mu\text{mol/m}^2/\text{s}$ to $690 \mu\text{mol/m}^2/\text{s}$. Same tendency was observed for the effect of light path length on FA profile at a fixed incident light intensity. Krzemińska et al. [32] detected that the percentage of C16 decreased from 18.11 to 15.24% and the per-

centage of C18 increased from 61.71 to 73.7% with the of light intensity from 130 $\mu\text{mol}/\text{m}^2/\text{s}$ to 420 $\mu\text{mol}/\text{m}^2/\text{s}$ in the culture of *C. protothecoides*. This result indicated that higher light intensity is benefit for the accumulation of FA with longer carbon chains. The percentage of total SFA, MUFA, and PUFA were also affected by different light conditions (Fig. 4D, E, F). With increasing of incident light intensity, the percentage of MUFA was significantly increased while both SFA and PUFA were decreased. Many researchers reported that PUFA are important for acclimation to low light intensities [33, 34]. However, the influence of the accumulation of SFA was quite different in different alga. Krzemińska et al. [32] reported that the amount of SFA was not affected by changes in light intensity while Hu et al. [35] reported that high light induced the major formation of SFA and MUFA. Sukenik and Livne speculated that the effect of light on FA profiles was due to that light induces the changes of pH, Mg^{2+} , and NADPH in the stroma, which finally influences and modulates the activity of key enzymes responsible for FA synthesis [36]. Due to the superior oxidative stability and higher cetane number of SFA and the instability of PUFA [35], FA derived at a higher light was preferred for biodiesel production.

4 Concluding remarks

The growth and hydrocarbon accumulation of *B. braunii* cells were obviously affected by both incident irradiance and light path. High light enhanced the accumulation of hydrocarbon with longer chains. Besides, the FA content and profiles were significantly influenced by both incident irradiance and light path. High incident light intensity and low light path length was benefit for the accumulation of total FA and C18, MUFA components. This result indicated that FA derived at a higher light was preferred for biodiesel production.

Practical application

Light is one of the most key factors for the photoautotrophic culture of microalgae. The growth and oil accumulation of *B. braunii* under different incident light intensity in culture systems with different light path length was investigated in this study. These results provide the base for the optimization of light conditions for the production of hydrocarbon and fatty acid using *B. braunii*. In addition, it is also helpful for the design of photobioreactor.

This work was financially supported by the National Natural Science Foundation of China (No. 21476242), the National Key Technology Research and Development Program of China (No. 2015BAK45B01), the National Basic Research Program (973 Program) of China (No. 2011CB200903), and the Key Research and Development Project of Shandong Province (2016GGX102005).

The authors have declared no conflict of interest.

5 References

- [1] Baba, M., Kikuta, F., Suzuki, I., Watanabe, M. M. et al., Wave-length specificity of growth, photosynthesis, and hydrocarbon production in the oil-producing green alga *Botryococcus braunii*. *Bioresour. Technol.* 2012, 109, 266–270.
- [2] Yoshimura, T., Okada, S., Honda, M., Culture of the hydrocarbon producing microalga *Botryococcus braunii* strain Showa: optimal CO_2 , salinity, temperature, and irradiance conditions. *Bioresour. Technol.* 2013, 133, 232–239.
- [3] Metzger, P., Largeau, C., *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Appl. Microbiol. Biotechnol.* 2005, 66, 486–496.
- [4] Sydney, E. B., Sturm, W., de Carvalho, J. C., Thomaz-Soccol, V., et al., Potential carbon dioxide fixation by industrially important microalgae. *Bioresour. Technol.* 2010, 101, 5892–5896.
- [5] Yamamoto, S., Mandokoro, Y., Nagano, S., Nagakubo, M., et al., Catalytic conversion of *Botryococcus braunii* oil to diesel fuel under mild reaction conditions. *J. Appl. Phycol.* 2014, 26, 55–64.
- [6] Pérez-Mora, L. S., Matsudo, M. C., Cezare-Gomes, E. A., Carvalho, J. C. M., An investigation into producing *Botryococcus braunii* in a tubular photobioreactor. *J. Chem. Technol. Biotechnol.* 2016, 91, 3053–3060.
- [7] Zhang, H. F., Wang, W. L., Li, Y. G., Yang, W. J., et al., Mixotrophic cultivation of *Botryococcus braunii*. *Biomass Bioenerg.* 2011, 35, 1710–1715.
- [8] Kalacheva, G. S., Zhila, N. O., Volova, T. G., Gladyshev, M. I., The effect of temperature on the lipid composition of the green alga *Botryococcus*. *Microbiology* 2002, 71, 286–293.
- [9] Yeesang, C., Cheirsilp, B., Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. *Bioresour. Technol.* 2011, 102, 3034–3040.
- [10] Ruangsomboon, S., Effect of light, nutrient, cultivation time and salinity on lipid production of newly isolated strain of the green microalga, *Botryococcus braunii* KMITL 2. *Bioresour. Technol.* 2012, 109, 261–265.
- [11] Xu, L., Liu, R., Wang, F., Liu, C. Z., Development of a draft-tube airlift bioreactor for *Botryococcus braunii* with an optimized inner structure using computational fluid dynamics. *Bioresour. Technol.* 2012, 119, 300–305.
- [12] Okumura, C., Saffreana, N., Rahman, M. A., Hasegawa, H., et al., Economic efficiency of different light wavelengths and intensities using LEDs for the cultivation of green microalga *Botryococcus braunii* (NIES-836) for biofuel production. *Environ. Prog. Sustain. Energ.* 2015, 34, 269–275.
- [13] Qin, J. G., Li, Y., Optimization of the growth environment of *Botryococcus braunii* strain CHN 357. *J. Freshwater Ecol.* 2006, 21, 169–176.
- [14] Sakamoto, K., Baba, M., Suzuki, I., Watanabe, M. M., et al., Optimization of light for growth, photosynthesis, and hydrocarbon production by the colonial microalga *Botryococcus braunii* BOT-22. *Bioresour. Technol.* 2012, 110, 474–479.
- [15] Wang, S. K., Stiles, A. R., Guo, C., Liu, C. Z., Microalgae cultivation in photobioreactors: an overview of light characteristics. *Eng. Life Sci.* 2014, 14, 550–559.

- [16] Schnurr, P. J., Espie, G. S., Allen, D. G., The effect of light direction and suspended cell concentrations on algal biofilm growth rates. *Appl. Microbiol. Biotechnol.* 2014, 98, 8553–8562.
- [17] Tuantet, K., Temmink, H., Zeeman, G., Janssen, M., et al., Nutrient removal and microalgal biomass production on urine in a short light-path photobioreactor. *Water Res.* 2014, 55, 162–174.
- [18] Jain, A., Voulis, N., Jung, E. E., Doud, D. F. R., et al., Optimal intensity and biomass density for biofuel production in a thin-light-path photobioreactor. *Environ. Sci. Technol.* 2015, 49, 6327–6334.
- [19] Largeau, C., Caradevall, E., Berkalo, C., Dhamliencourt, P., Sites of accumulation and composition of hydrocarbons in *Botryococcus braunii*. *Phytochemistry* 1980, 19, 1043–1051.
- [20] Wang, S. K., Wang, F., Stiles, A. R., Guo, C., et al., *Botryococcus braunii* cells: Ultrasound-intensified outdoor cultivation integrated with *in situ* magnetic separation. *Bioresour. Technol.* 2014, 167, 376–382.
- [21] Martínez, L., Morán, A., García, A. I., Effect of light on *Synechocystis* sp. and modelling of its growth rate as a response to average irradiance. *J. Appl. Phycol.* 2012, 24, 125–134.
- [22] Carvalho, A. P., Silva, S. O., Baptista, J. M., Malcata, F. X., Light requirements in microalgal photobioreactors: an overview of biophotonic aspects, *Appl. Microbiol. Biotechnol.* 2011, 89, 1275–1288.
- [23] Lee, C., Kim, M., Sanjay, K., Kwag, J., et al., Biomass production potential of *Chlorella vulgaris* under different CO₂ concentrations and light intensities. *J. Anim. Sci. Technol.* 2011, 53, 261–268.
- [24] Atta, M., Idris, A., Bukhari, A., Wahidin, S., Intensity of blue LED light: a potential stimulus for biomass and lipid content in fresh water microalgae *Chlorella vulgaris*. *Bioresour. Technol.* 2013, 148, 373–378.
- [25] Yan, C., Zheng, Z., Performance of photoperiod and light intensity on biogas upgrade and biogas effluent nutrient reduction by the microalgae *Chlorella* sp. *Bioresour. Technol.* 2013, 139, 292–299.
- [26] Xu, L., Wang, F., Chen, C., Liu, C. Z., Improved algal oil production from *Botryococcus braunii* by feeding nitrate and phosphate in an airlift bioreactor. *Eng. Life Sci.* 2012, 12, 171–177.
- [27] Niehaus, T. D., Okada, S., Devarenne, T. P., Watt, D. S., et al., Identification of unique mechanisms for triterpene biosynthesis in *Botryococcus braunii*. *Proc. Natl. Acad. Sci. U.S.A.* 2011, 108, 12260–12265.
- [28] Okada, S., Murakami, M., Yamaguchi, K., Hydrocarbon production by the Yayoi, a new strain of the green microalga *Botryococcus braunii*. *Appl. Biochem. Biotech.* 1997, 67, 79–86.
- [29] Mandotra, S. K., Kumar, P., Suseela, M. R., Nayaka, S., et al., Evaluation of fatty acid profile and biodiesel properties of microalga *Scenedesmus abundans* under the influence of phosphorus, pH and light intensities. *Bioresour. Technol.* 2016, 201, 222–229.
- [30] Sun, Y., Liao, Q., Huang, Y., Xia, A., et al., Application of growth-phase based light-feeding strategies to simultaneously enhance *Chlorella vulgaris* growth and lipid accumulation. *Bioresour. Technol.* 2018, 256, 421–430.
- [31] Xiao, Y., Zhang, J., Cui, J., Yao, X., et al., *Nannochloropsis oceanica* IMET1 under high light intensity and nitrogen replete conditions. *Algal Res.* 2015, 11, 55–62.
- [32] Krzemińska, I., Piasecka, A., Nosalewicz, A., Simionato, D., et al., Alterations of the lipid content and fatty acid profile of *Chlorella protothecoides* under different light intensities. *Bioresour. Technol.* 2015, 196, 72–77.
- [33] Klyachko-Gurvich, G., Tsoglin, L. N., Doucha, J., Kopetskii, J., et al., Desaturation of fatty acids as an adaptive response to shifts in light intensity. *Physiol. Plant.* 1999, 7, 240–249.
- [34] Ma, R., Thomas-Hall, S. R., Chua, E. T., Eltanahy, E., et al., LED power efficiency of biomass, fatty acid, and carotenoid production in *Nannochloropsis* microalgae. *Bioresour. Technol.* 2018, 252, 118–126.
- [35] Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., et al., Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J.* 2008, 5, 621–639.
- [36] Sukenik, A., Livne, A., Variations in lipid and fatty acid content in relation to acetyl CoA carboxylase in the marine *Prymnesiophyte Isochrysis galbana*. *Plant Cell Physiol.* 1991, 32, 371–378.