



# High irradiance compensated with CO<sub>2</sub> enhances the efficiency of *Haematococcus lacustris* growth

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## ABSTRACT

*Haematococcus lacustris* (*H. lacustris*), a promising source for astaxanthin production, is a light-sensitive microalga that is prone to sluggish growth when subjected to high levels of irradiance. A challenge in *H. lacustris* culture is to find a way to efficiently use illumination to maintain vigorous growth and harvest dense biomass, which is essential for further exploiting the potential for astaxanthin production. Previous studies have shown that in addition to illumination, carbon supply in culture is a key limitation for algae growth. Here, we investigated a combined culture approach involving high light intensity (110 μmol m<sup>-2</sup>s<sup>-1</sup>) and injection of a 1% (v/v) CO<sub>2</sub> air-gas mixture which provided an effective method for *H. lacustris* culture to achieve both a high growth rate and high cell density. The cell number in the group with high light exposure combined with CO<sub>2</sub> enrichment was increased almost four-fold compared with a high light group (110 μmol m<sup>-2</sup>s<sup>-1</sup> without CO<sub>2</sub> injection). Additional experiments suggested a possible mechanism in which elevated CO<sub>2</sub> increases the electron sink capacity, thus alleviating photoinhibition and oxidative damage. The scaled-up photobioreactor demonstrated much better performance, with growth rates improved by 50–350 %, providing further evidence that this new method can improve algal cell production. Overall, our work provides an efficient way for *H. lacustris* culture and manufacture, with potential industrial applications.

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## 1. Introduction

The green algae *Haematococcus lacustris* is a promising resource to produce astaxanthin, which is used as a potent antioxidant in the food industry, healthcare, and clinical treatments [1–3]. Increasing market demand for astaxanthin has led to *H. lacustris* becoming a research hotspot, especially regarding the optimization of culture conditions. Two-stage culture has been widely adopted. During first stage, green cells of *H. lacustris* were cultured with suitable illumination for around one week. It is intended to provide optimal culture condition to reach high biomass production and second stage is involved in stress mode like high irradiance, nitrogen deficiency. When *H. lacustris* cells are exposed to a stress state under external conditions, cellular astaxanthin accumulation is triggered, thus pausing algae growth and causing a transformation into a haematocyst [4,5]. Therefore, obtaining

high-density cells is an essential prerequisite for high astaxanthin production. Several studies of culture-related parameters have been carried out to determine the optimal conditions for *H. lacustris* culture. According to Tjahjono et al., the optimal temperature for *H. lacustris* culture growth is 14–20 °C [6]. Regarding pH, either neutral or alkaline conditions are generally considered to be suitable [7,8]. Significant factors that affect culture growth is light intensity, pH, temperature, and the nutrient composition of the medium [9–13].

The light source plays a crucial part in photosynthesis, as it is the source of energy that drives the reaction. Theoretically, the growth rate of vegetative algae cells increases with increasing light intensity. This trend can be described by an empirical formula [14,15]:

$$\mu = \frac{\mu_{max} \times I_{av}^n}{IK^n + I_{av}^n},$$

Where  $\mu$  is the specific growth rate,  $IK$  is the constant saturation of irradiance,  $\mu_{max}$  is the maximum specific growth rate in culture, and  $n$  is a shape parameter and  $I_{av}$  is the average irradiance on the culture surface.

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However, as *H. lacustris* is a light-sensitive species, growth suppression and morphological changes can be observed under high light intensity, which causes light damage to algae [16,17]. Previous work used relatively low light intensities of around 20–30  $\mu\text{mol m}^{-2}\text{s}^{-1}$  as a culture condition. Traditionally, during the growth stage, the specific growth rate is only 0.15–0.23  $\text{d}^{-1}$  [18,19]. Kaewpintong et al. observed morphological changes in vegetative cells when the light intensity was greater than 50  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , with a concomitant accumulation of astaxanthin. When the light intensity reached 60  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , culture growth was no longer detected [1]. Similarly, Fan et al. found that astaxanthin was greatly accumulated under light intensities greater than 90  $\mu\text{mol m}^{-2}\text{s}^{-1}$  [20]. Under traditional culture conditions, light intensity over a certain limit causes photo-inhibition.

When the light saturation point is exceeded, the transmembrane electrochemical proton concentration increases. Such high irradiance causes stress to vegetative cells, with increased electron transport and elevated ROS (reactive oxygen species) levels [21,22]. High level of ROS suppresses vigorous cell division before high cell density is reached [23]. The cell activates non-photochemical quenching of the excited chlorophyll states (NPQ) as a photo-protective mechanism against photodamage [30,35]. Recent studies reports that NPQ rise at the beginning of the stress condition, but down regulation of NPQ was observed when other photoprotection mechanism was activated. Massive accumulation of astaxanthin was a character of *H. lacustris* under unfavorable condition. Recent study claims that via screening effect, astaxanthin can shield cell from excessive light resulting in down-regulated NPQ [35].

However, rather than astaxanthin accumulation, we wonder if there is other potential method to down regulate NPQ and mitigate light stress while maintain vigorous cell growth. Study of plants claims that depletion of  $\text{CO}_2$  induces a marked rise in NPQ due to low proton turnover and ATP consumption [21,24,25]. Likewise, the low  $\text{CO}_2$  concentration in algae culture results in slow carbon fixation, leading to NADPH accumulation and photosynthetic electron leakage from the electron transfer chain [36,37]. Cellular oxidation resulting from an excess of photosynthetic electrons is detrimental to cell production [21,22].

By contrast, elevated  $\text{CO}_2$  concentration can lead to NPQ decrease. Increasing the electron sink capacity contributes to accelerate proton drains which reduces unwanted cellular oxidant. After alleviation of photo-inhibition, biomass increases. Thus,  $\text{CO}_2$  supplement in culture can be a feasible method for soothing high light stress and promote cell growth.

This principle has been proven in many  $\text{CO}_2$ -adapted species, including *Chlorella pyrenoidosa* and *Scenedesmus obliquus* [26]. However, its application of *H. lacustris* for green stage cultivation has been relatively rare. Most of the paper published so far on  $\text{CO}_2$  supplement for *Haematococcus* culture has been focused on the second stage for astaxanthin accumulation purpose. Boussiba et al. bubbled air containing 1.5 %  $\text{CO}_2$  with 85  $\mu\text{mol m}^{-2}\text{s}^{-1}$  ( $L_1$ ), or 170  $\mu\text{mol m}^{-2}\text{s}^{-1}$  ( $L_2$ ) meanwhile 1.5 g  $\text{L}^{-1}$   $\text{NaNO}_3$  or 0.15 g  $\text{L}^{-1}$   $\text{NaNO}_3$  was added to the medium. The maximum cell number was around 4 folds higher in the  $L_1$  group than the  $L_2$  group in high light group where massive astaxanthin was observed [38]. Some research adopted gas injection like air bubbling for green stage culture but with moderate light intensity. Chekanov et al. cultured the green cell under light intensity around 40  $\mu\text{mol m}^{-2}\text{s}^{-1}$  to maintain their vigorous growth [39] with  $\text{CO}_2$  compensation. However, the culture of vegetative cell under high light intensity and  $\text{CO}_2$  to reach high cell number has been underexplored. Given the role of high light exposure and  $\text{CO}_2$  concentration in photosynthesis, we hypothesized that the growth rate of light-sensitive *H. lacustris* in vegetative cell culture would be improved by the synergic effects of higher light irradiance and  $\text{CO}_2$  supply.

To test this hypothesis, we set up an experiment in which high irradiance was combined with  $\text{CO}_2$  enrichment. Growth rates and cell productivity were measured, and the method was tested in a scaled-up photobioreactor (PBR) culture of *H. lacustris*. This study aimed to determine the optimal method of *H. lacustris* culture for applications on an industrial scale.

## 2. Material and methods

### 2.1. Strain, cultivation conditions, and experimental design

*H. lacustris* FACHB-712 was obtained from the Culture Collection of Algae at the Institute of Hydrobiology, Chinese Academy of Sciences, and maintained in BBM (Bold Basal Medium) [40] as suggested by the Culture Collection of Algal and Protozoa, Scottish Marine Institute, UK. Precultivation was carried out in a 200-mL flask under the illumination of 20  $\mu\text{mol m}^{-2}\text{s}^{-1}$ .

To investigate the effects of the combination of increased light intensity and carbon enrichment on *H. lacustris* growth, three different irradiances of white light were used: low light intensity (20  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ), medium light intensity (55  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ), and high light intensity (110  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ). Experiments were performed with or without 1% (v/v)  $\text{CO}_2$  supplementation, and each treatment had three replicates. First, bubbling with 1% (v/v) of  $\text{CO}_2$  solved with air, the experiment was performed to provide a sufficient carbon source for the vegetative cells under each illumination level. Then,  $\text{CO}_2$  injection was replaced with a 0.05 (v/v) 1%  $\text{N}_2$  balanced with air supply solved with air while other experimental conditions remained the same. In the initial biomass density (IBD) experiments, IBD was set to  $8.8 \times 10^4$  cells  $\text{mL}^{-1}$  (IBD<sub>2</sub>) or  $1.8 \times 10^5$  cells  $\text{mL}^{-1}$  (IBD<sub>3</sub>). The flasks were incubated in 20 °C under a 12:12=light: dark cycle. Thin liquid level ensured algae in cultivation system obtain analogous light quanta, having little light attenuation effect.

After the flask experiments, scaled-up cultivation was carried out in a 50L PBR with  $\text{CO}_2$  (1%, v/v) under 110  $\mu\text{mol m}^{-2}\text{s}^{-1}$  light exposure to confirm the potential in industrial manufacturing.

### 2.2. Parameter measurements

Four parameters were monitored every day to evaluate culture growth and condition: cell count, a ratio of normalized variable fluorescence ( $F_v/F_m$ ), cellular ROS level, and astaxanthin content. Cell count was measured manually under a light microscope, using blood cell counter and gravimetric analysis was used to measure dry mass. The overall specific growth rates ( $\mu'_1$ ,  $\text{d}^{-1}$ ) of  $\text{CO}_2$ ,  $\text{N}_2$  injection and IBD experiments were measured using cell count whereas biomass dry weights (W) was calculated to show specific growth rate ( $\mu'_2$ ,  $\text{d}^{-1}$ ) of scale-up bioreactor. The equations are as follow:

$$\mu'_1 = \frac{\ln(C_2/C_1)}{t_2 - t_1}, \mu'_2 = \frac{\ln(W_2/W_1)}{t_2 - t_1},$$

Where  $C_1$  and  $C_2$  represent cell number at times  $t_2$  and  $t_1$ , respectively.  $W_2$  and  $W_1$  are the biomass dry weights ( $\text{g}\cdot\text{L}^{-1}$ ) at times  $t_2$  and  $t_1$ , respectively.

The photosynthetic efficiency ( $F_v/F_m$  value), which represents the maximal photosystem II (PS II) photochemical quantum yield, is a stress indicator. Here,  $F_v/F_m$  was determined using a Phyto-PAM (Walz, Germany) instrument where a saturating light pulse with 5300  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  for 0.8 s was used in the presence of a weak actinic measuring light after 10 min dark adaptation. [43]:

$$F_v/F_m = (F_m - F_0)/F_m$$

where  $F_0$  is the minimal chlorophyll fluorescence intensity in the dark-adapted state,  $F_m$  is maximal chlorophyll fluorescence intensity after applying of saturation light pulse,  $F_v = F_m - F_0$  is the variable chlorophyll fluorescence [22].

ROS levels were estimated following the method of Wang et al. [27] and Zhang et al. [28]. Briefly, the fluorimeter was set to wavelengths of 488 nm excitation and 525 nm emission. The total fluorescence of each sample was divided by the cell count to give the cellular ROS level.

Astaxanthin estimation was carried out with dimethyl sulfoxide following by spectrophotometric analysis according to the method of Choi et al. [29]. Measurements were performed in an Infinite 200 PRO (TECAN, Switzerland) microplate reader in a polypropylene 96-well plate (Corning, USA) with the optical pathlength of 10 mm. Optical density was measured at 492 nm:

$$\text{astaxanthin (mg.L}^{-1}\text{)} = 4.5 \cdot OD_{492} \cdot A \cdot \frac{V_a}{V_b}$$

Where A is the correction factor, the value of which is 1.3569,  $V_a$  is the reagent volume, and  $V_b$  is the sample volume.

### 2.3. Statistical analysis

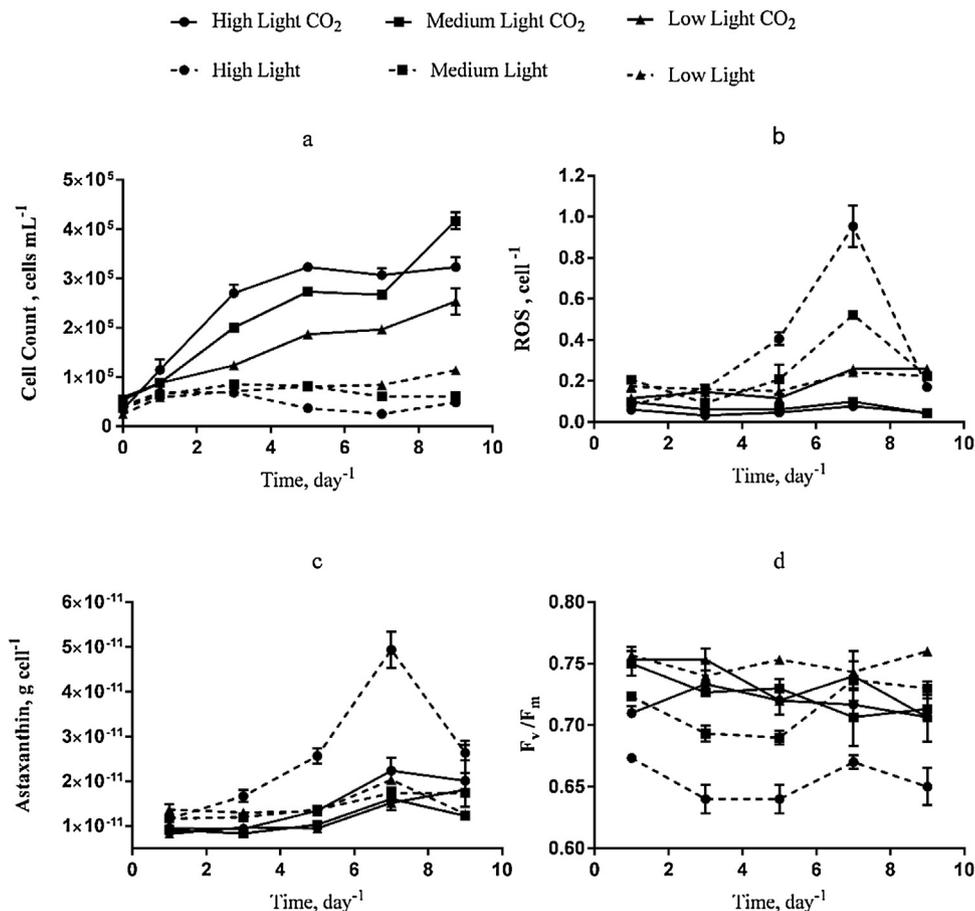
The data were processed by one-way analysis of variance using Origin 8.0. The average value of three replicate samples is expressed by standard error bars in figures. A value of  $P < 0.05$  was considered statistically significant.

## 3. Results and discussion

### 3.1. Synergistic effect of high light intensity and CO<sub>2</sub> injection

We first tested whether increasing CO<sub>2</sub> in *H. lacustris* culture would increase maximum cell number and growth rate. As expected, the CO<sub>2</sub> groups generally showed higher cell counts and growth rates than the non-CO<sub>2</sub> groups. Under differential light intensity, the opposite growth pattern was observed. In the CO<sub>2</sub> groups, culture growth increased with increasing light intensity. The highest growth rate was found in the CO<sub>2</sub> high light intensity group, reaching 0.412 d<sup>-1</sup>. The maximum cell count in the CO<sub>2</sub> groups was  $4.2 \times 10^5$  cells mL<sup>-1</sup> (Fig. 1a) after ten days, approximately 4–6 fold that of the non-CO<sub>2</sub> groups. Conversely, the highest growth rate observed in non-CO<sub>2</sub> group under low light intensity was only 0.257 d<sup>-1</sup>, with a maximum cell count of  $1.2 \times 10^5$  cells mL<sup>-1</sup>, and culture growth under high light intensity was arrested, with cell counts remaining at around  $4.8 \times 10^4$  cells mL<sup>-1</sup>. Those results demonstrated that *H. lacustris* adapts better to high light intensity after CO<sub>2</sub> compensation.

Why did the adaptability of *H. lacustris* to light intensity increase after CO<sub>2</sub> compensation? We hypothesized that CO<sub>2</sub> might alleviate the oxidative damage caused by high irradiance and enhance the photosynthetic activity, which is closely related to cell productivity. Therefore, we further tested cellular ROS levels, anti-oxidant content, and photosynthetic activities in vegetative cells. Consistent with our hypothesis, ROS levels in the non-CO<sub>2</sub> groups



**Fig. 1.** The change of culture growth and condition under different light intensity with or without 1% CO<sub>2</sub> injection. (a) Cell Count, (b) Cellular ROS level, (c) Cellular Astaxanthin Content, (d)  $F_v/F_m$  Value of *H. lacustris* cultured under high light intensity ( $110 \mu\text{mol m}^{-2}\text{s}^{-1}$ ), medium light intensity ( $55 \mu\text{mol m}^{-2}\text{s}^{-1}$ ), low light intensity ( $20 \mu\text{mol m}^{-2}\text{s}^{-1}$ ).

were positively related to light intensity. The highest ROS level, at  $0.96 \text{ cell}^{-1}$ , was observed in the top light group on day 7 of culture (Fig. 1b). The medium-light group showed a similar pattern, with the ROS level peaking at  $0.53 \text{ cell}^{-1}$  (Fig. 1b). By contrast, ROS levels in the high light  $\text{CO}_2$  and medium-light  $\text{CO}_2$  groups were maintained at around  $0.06 \text{ cell}^{-1}$ , about 15 times lower than that of the peak of high light group. Thus, elevating  $\text{CO}_2$  in culture appeared to lead to intermediate cellular ROS levels. Under high irradiance, high NPQ with ROS production occurs, which is detrimental to photosynthetic efficiency and cell productivity [22,35]. These results suggest that  $\text{CO}_2$  injection allows the cellular ROS level to remain stable and low. We assumed that a low ROS level would induce less of a stress response and thus be beneficial in terms of photosynthetic efficiency [41].

To analyze cell anti-stress responses, astaxanthin content was investigated. According to Kobayashi et al., astaxanthin showed strong antioxidant effect in cyst against oxidative stress [11]. Fan et al claimed that astaxanthin accumulation was triggered as a result of photoprotection process [42] which were in consistent of our results. High light group show higher light protective activities while higher astaxanthin accumulation was observed. It is difficult to achieve high biomass concentrations once the cell shifts to astaxanthin accumulation and haematocysts formation has occurred. Thus, maintaining low levels of astaxanthin during the cultivation process is crucial for culture growth. Consistently, the  $\text{CO}_2$  groups, which had higher cell counts and lower ROS levels, showed relatively low levels of astaxanthin, with an average of  $1.5 \times 10^{-11} \text{ g cell}^{-1}$ , whereas in the non- $\text{CO}_2$  groups, astaxanthin levels increased with ROS levels. The maximum astaxanthin content of the high light group peaked at  $4.9 \times 10^{-11} \text{ g cell}^{-1}$ , almost double the content in  $\text{CO}_2$  high light group. For the medium and

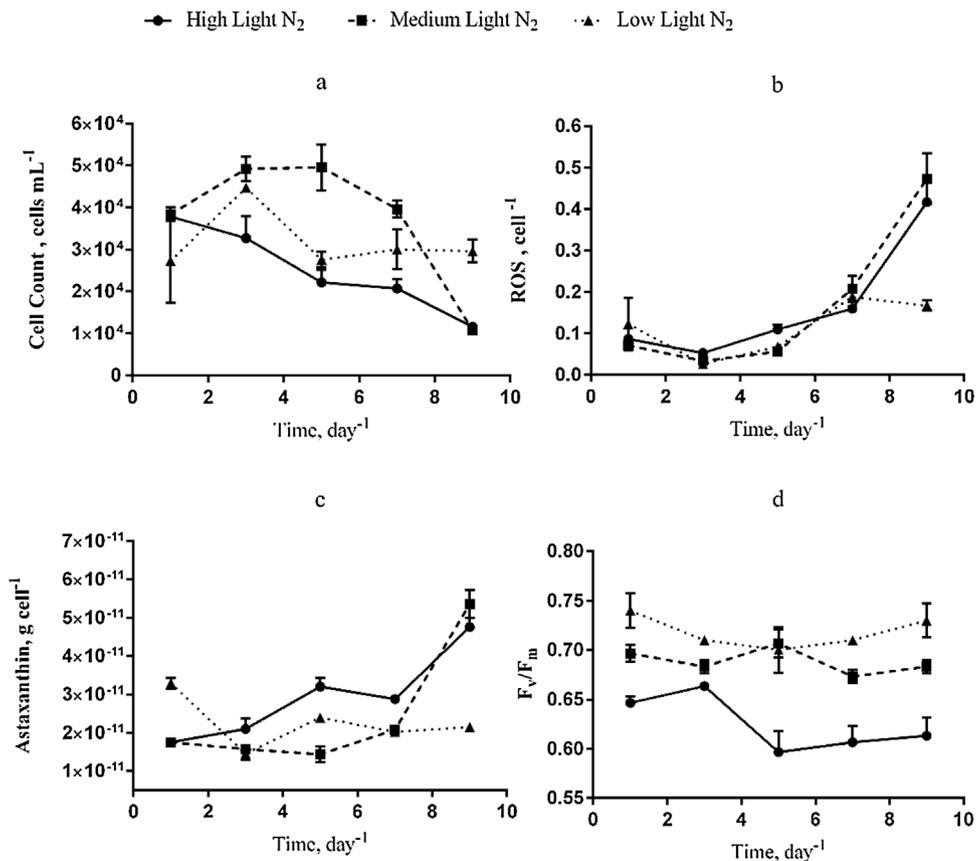
low light intensity treatments, groups with  $\text{CO}_2$  also showed lower astaxanthin levels (Fig. 1c).

Together, these results demonstrated that  $\text{CO}_2$  injection alleviated oxidant damage in vegetative cells. Therefore, we predicted that photosynthetic activity would be improved in  $\text{CO}_2$  groups. To test this prediction, we used a Phyto-PAM instrument to measure  $F_v/F_m$  (Fig. 1d). This value represents the maximal PS II photochemical quantum yield, reflecting the potential efficiency of the electron transport rate in PS II reaction centres. Generally, unfavourable conditions triggered the rapid closure of reaction centres in *H. lacustris* cells, as indicated by a decrease in  $F_v/F_m$  [30]. In the  $\text{CO}_2$  groups, low ROS levels, and thus less oxidant damage to the photosynthetic apparatus, may have contributed to  $F_v/F_m$  being sustained at a high level of around 0.72 under various light intensities.

Conversely,  $F_v/F_m$  decreased with increasing light intensity. The  $F_v/F_m$  in the medium-light group was approximately 6% lower than that of a small light group, while the average value in the high light group was 12 % lower. High  $F_v/F_m$  values reflect high levels of photosynthetic activity and efficient light energy utilization, indicating a more robust cell condition in the high light group with  $\text{CO}_2$  injection.

### 3.2. $\text{N}_2$ injection or IBD increase as a potential substitute for $\text{CO}_2$ injection

To further investigate why the treatment combining high light intensity and  $\text{CO}_2$  enrichment led to lower cellular ROS levels and higher specific growth rates, two questions were posed. First, did gas injection reduce the accumulation of dissolved oxygen, leading to a decrease in cellular ROS levels and reducing oxidant damage in



**Fig. 2.** The effect of  $\text{N}_2$  injection on growth and condition of *H. lacustris* under different light intensity (a) Cell Count, (b) Cellular ROS level, (c) Cellular Astaxanthin Content, (d)  $F_v/F_m$  Value.

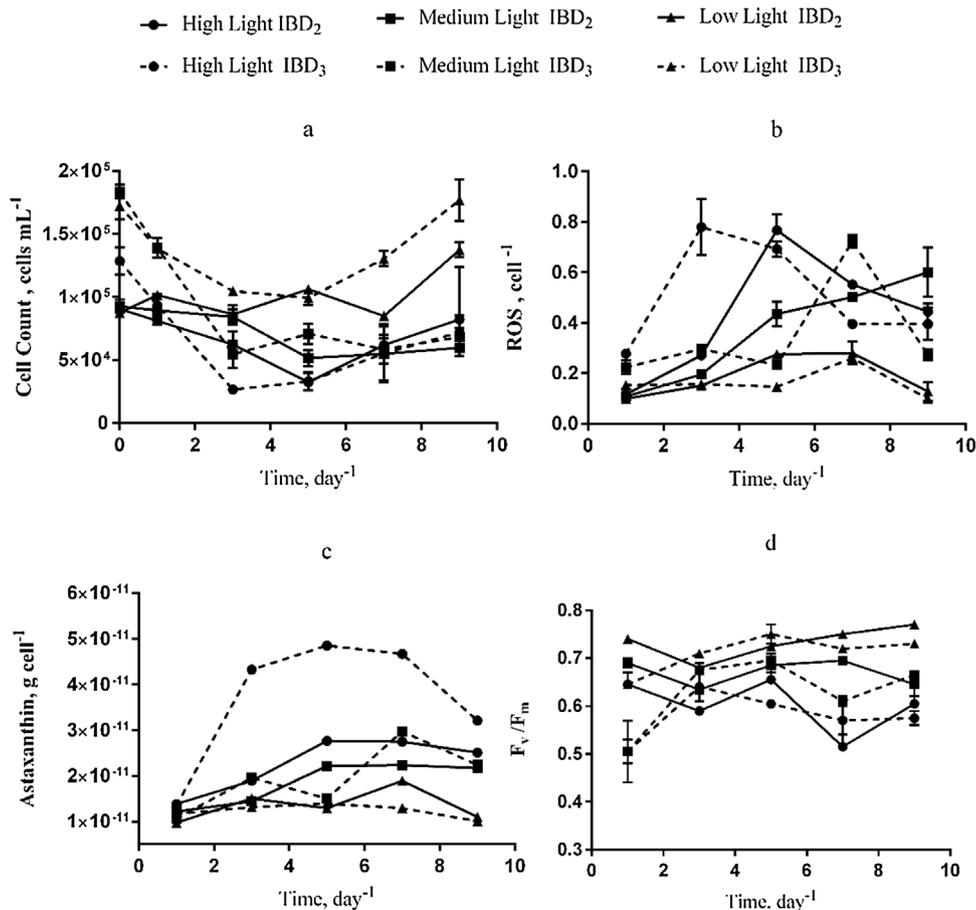
the CO<sub>2</sub> injection groups under different light intensities? Second, did light attenuation caused by self-shading under high biomass conditions result in less photooxidative loss, facilitating carbon assimilation and culture growth?

To answer the first question, N<sub>2</sub> was injected into the medium instead of CO<sub>2</sub> to blow out dissolved oxygen without adding carbon sources. As shown in Fig. 2b, the ROS levels of the three N<sub>2</sub> groups remained at 0.09 cell<sup>-1</sup>, and there was no significant increase in the astaxanthin content of any group, which would seem to favour culture growth (Fig. 2c). However, the cell counts of the three groups were maintained at around 4 × 10<sup>4</sup> cells mL<sup>-1</sup> (Fig. 2a), indicating no visible culture growth. Both the ROS levels and astaxanthin content of the high and medium light intensity N<sub>2</sub> groups were elevated after seven days cultivation, peaking at around 0.47 cell<sup>-1</sup> and 5.4 × 10<sup>-11</sup> g cell<sup>-1</sup> (Fig. 2b, c), respectively, on day 9. Similarly, compared with the other two groups, in which F<sub>v</sub>/F<sub>m</sub> remained at approximately 0.7, the F<sub>v</sub>/F<sub>m</sub> of the high light N<sub>2</sub> group decreased to 0.61 during the culture period (Fig. 2d). N<sub>2</sub> injection may have caused a decrease in dissolved oxygen levels in the culture medium, contributing to low astaxanthin and ROS levels initially. However, dissolved CO<sub>2</sub> declined in the medium at the same time, leading to inadequate carbon sources for culture growth and thus reduced cell productivity. Photodamage accumulated under high light, indicating that the elevated cellular ROS and astaxanthin levels became detrimental to culture growth in the long term. Based on these results, we concluded that CO<sub>2</sub> injection treatment cannot be substituted by N<sub>2</sub> injection, and oxygen blow-out was not the reason for the observed decrease in ROS levels and photosynthetic protection.

Cultures with different IBD were used to address the second question cultivation. Fig. 3a. depicts the growth curves of *H. lacustris* cultures with two different IBDs of around 1 × 10<sup>5</sup> cells mL<sup>-1</sup> (IBD<sub>2</sub>) and 9 × 10<sup>4</sup> cells mL<sup>-1</sup> (IBD<sub>3</sub>). When the IBD was increased further, culture growth under high light intensity decreased to around 6.0 × 10<sup>4</sup> mL<sup>-1</sup>. Groups under 20 μmol m<sup>-2</sup> s<sup>-1</sup> treatment had stable F<sub>v</sub>/F<sub>m</sub> values of around 0.70–0.75, whereas the F<sub>v</sub>/F<sub>m</sub> of the high light groups declined to 0.61 by the end of the cultivation process (Fig. 3d).

Further analysis of the ROS and astaxanthin data showed that increasing IBD alleviated the severe photoinhibition caused by high light intensity to some extent. The astaxanthin content of the high light intensity IBD<sub>3</sub> group on day 5 was almost double that of the high light intensity IBD<sub>2</sub> group (Fig. 3c). The first ROS level peak at 0.78 cell<sup>-1</sup> (Fig. 3b) occurred on day 3 in the IBD<sub>3</sub> high light intensity group, followed by a peak for the IBD<sub>2</sub> high light intensity group at 0.77 cell<sup>-1</sup> on day 5, and for the IBD<sub>3</sub> medium light group at 0.72 cell<sup>-1</sup> on day 7. IBD elevation without carbon enrichment led to light attenuation but had little impact on cell productivity. Overall, our results demonstrated that neither high IBD nor addition of N<sub>2</sub> could fully explain the rapid growth of *H. lacustris*.

Therefore, CO<sub>2</sub> restriction during the culture phase is a possible explanation for growth suspension under high light intensity. The limitation of CO<sub>2</sub> supply in the atmosphere limits the carbon assimilation process, causing ATP and NADPH accumulation, whereas a sufficient CO<sub>2</sub> supply should promote dark reactions by timely transferring ATP and NADPH from light reactions [24,25] and facilitates photon uptake, resulting in a relatively low NPQ level, which manifests as efficient photochemistry and eventually



**Fig. 3.** The effect of different IBD on growth and condition of *H. lacustris* under different light intensity (a) Cell Count, (b) Cellular ROS level, (c) Cellular Astaxanthin Content, (d) F<sub>v</sub>/F<sub>m</sub> Value.

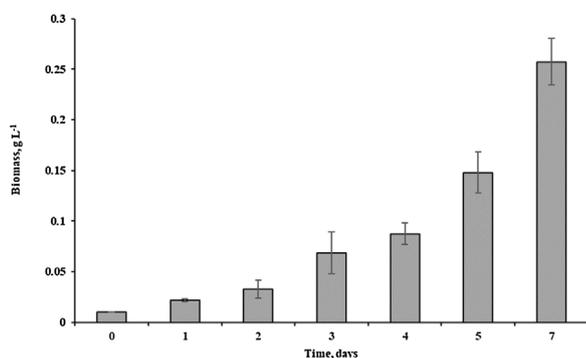


Fig. 4. The growth curve of *H. lacustris* in the photobioreactor.

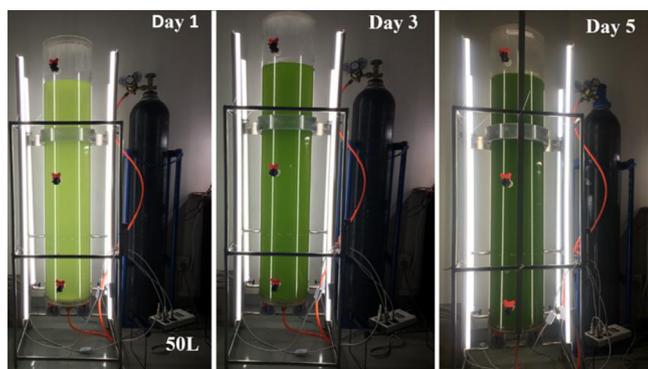


Fig. 5. Pictures demonstrating the growth of *H. lacustris* in a photobioreactor.

contributes to an increase in biomass [21,30,31]. The maintenance of cellular ROS and astaxanthin at relatively low levels is a prerequisite for sustaining high photosynthetic rates, which in turn contributes to an increased cell count.

### 3.3. Scaled-up experiment

Based on the suitable culture conditions determined at flask level, a scaled-up analysis in 50 L PBR was conducted to test the validity of these conditions on a larger scale (Fig. 5).

During the experiments, the *H. lacustris* vegetative cells showed significant growth, with the dry mass rising from 0.01 g L<sup>-1</sup> to 0.26 g L<sup>-1</sup> within seven days and the specific growth rate reaching 0.46 d<sup>-1</sup> (Fig. 4). Traditionally, a linear rather than a log growth trend has been observed in bioreactor tests, with the final dry mass reaching only around 0.06 g L<sup>-1</sup> and a specific growth rate of 0.21 d<sup>-1</sup>. Clearly, the combination of high light intensity and CO<sub>2</sub> injection in our PBR operation is an efficient way to achieve high-density culture, indicating that this first-stage culture method is feasible for use in industrial-scale applications. Many studies have aimed to improve the growth of *H. lacustris*. Vega-Estrada et al. cultivated these algae in a split-cylinder internal-loop airlift bioreactor, achieving an overall specific growth rate of about 0.13 d<sup>-1</sup> [32].

Similarly, Wang et al. demonstrated a specific growth rate of around 0.18 d<sup>-1</sup> with the optimal combination of initial biomass and nitrogen concentration [33]. Lee et al. designed a sequential operating system in tubular PBRs to enhance the carbon fixation of *H. lacustris*, with a specific growth rate of approximately 0.1 d<sup>-1</sup> [34]. Kaewpintong et al. implemented high-density cultivation with the optimal airlift bioreactor design and optimal conditions; the specific growth rate reached 0.31 d<sup>-1</sup> [1]. Compared with these examples from the literature, our PBR showed much better

performance, with improvements of about 50–350 % in terms of growth rate.

## 4. Conclusions

This study demonstrates the superiority of a combination of constant CO<sub>2</sub> supply and high light intensity during *H. lacustris* cultivation. Elevating CO<sub>2</sub> supply during vegetative cell culture can alleviate the photodamage caused by high light intensity, resulting in low ROS levels and high photosynthetic activity. The combination of high irradiance and CO<sub>2</sub> injection was tested by scaled-up experiment, enabling the optimal growth of *H. lacustris*. Thus, this culture method is a potential tool for boosting *H. lacustris* cultivation in manufacturing to produce valuable microalgal bioproducts.

## Author statement

Kebi Wu and Kezhen Ying designed experiments with the assistance of Jin Zhou and Zhonghua Cai; Kebi Wu, Kezhen Ying, Lu Liu carried out experiments; Kebi Wu and Lu Liu analyzed experimental results and data. Kebi Wu wrote the manuscript. Jin Zhou and Zhonghua Cai assisted in polishing and revision.

## Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.btre.2020.e00444>.

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