DOI: 10.31276/VJSTE.63(2).81-88



# Effects of red and blue light emitting diodes on biomass and astaxanthin of *Haematococcus pluvialis* in pilot scale angled twin-layer porous substrate photobioreactors

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Received 23 December 2020; accepted 15 March 2021

***Abstract:***

**The production of natural astaxanthin is usually accomplished by suspended cultivation of the microalgae *Haematococcus pluvialis*. In this study, for the purpose of cost reduction, *H. pluvialis* is grown in pilot scale angled twin-layer porous substrate photobioreactors with light energy from red/blue LEDs that can produce red light, blue light, or a combination of blue-red light. The total dry biomass of the microalgae reached a maximum of 40.74 g.m$^{-2}$ under blue-red LEDs. The early initiation of blue-red LED illumination (on day 2) after algae immobilization in the biofilm resulted in the highest accumulation of astaxanthin in the dry biomass, which reached a maximum of 1.3% (w/w) after 10 d of culture.**

***Keywords:*** **angled, astaxanthin, biofilm, *Haematococcus pluvialis*, photobioreactor, porous substrate, twin-layer.**

***Classification number:*** **3.5**

## Introduction

Astaxanthin has been shown to be one of the most powerful antioxidants out of many other carotenoids [1-3]. Astaxanthin can be found in the shells of crustaceans, seafood, the yeast *Phaffia rhodozyma*, and in some bacteria at low concentrations. However, the green alga *Haematococcus pluvialis* is able to synthesize and accumulate astaxanthin in high concentrations (possibly up to 5-6% [w/w] in the dry biomass) and is thus used to produce astaxanthin on an industrial scale [4-7].

Astaxanthin production from *H. pluvialis* is almost exclusively performed with suspended cultivation (open or closed systems). Outdoor open systems can be large shallow ponds, reservoirs, circular ponds or raceways, and the algal suspension is stirred by paddle wheels under sunlight. The amount of astaxanthin can reach 1.5-3.0% of dry biomass. The advantage of this culture system is its simple construction and operation. However, limitations like low light efficiency, loss of culture medium due to evaporation, large space requirements, and high risk of contamination inhibiting the growth of the target alga can occur in these systems [8]. Closed culture systems can limit the risk of contamination, for example, photobioreactors (PBRs) in the form of flat panel, tubular or columnar bioreactors. Environmental conditions (pH, light intensity, temperature, and $CO_2$ concentration) are better controlled and water evaporation and $CO_2$ loss are minimized in closed PBRs. However, some significant disadvantages of closed PBRs are difficulty controlling the cumulative oxygen concentration and costs of setting up and maintaining them in comparison to open ponds [9-11].

The immobilized algal culture model in a twin-

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layer porous substrate photobioreactor (TL-PSBR) has been shown to have several advantages over traditional suspension cultivation systems [12-15]. The TL-PSBR was originally placed vertically and had many advantages such as less water consumption, less restricted gas exchange surface, and easy separation of water from algal biomass when harvested. Additionally, the final cell density (standing crop) can be up to 100 times higher than in suspended cultivation [13, 14].

Recently in Vietnam, studies on culturing *H. pluvialis* in pilot-scale TL-PSBRs have been carried out with high capacity illuminations using a system of 10 high pressure sodium lamps (250 W/lamp) or 8 high-pressure sodium lamps (400 W/lamp). These illuminations stimulate the growth and accumulation of astaxanthin in *H. pluvialis* to achieve high yields [16, 17]. However, these lighting systems consume large amounts of energy that reduce production efficiency and emit considerable heat, which brings difficulty to the control of temperature in the culture environment [17].

Monochromatic illumination from light emitting diodes (LED) has been used in plant culture as well as in microalgae. The LEDs show many advantages such as high efficiency of converting electricity to light (less heat, more energy saving), creating light with a wavelength that matches the absorption spectrum of the pigments in microalgae, and a long service life [18-21]. However, the number of studies on the application of LEDs for the production of astaxanthin from *H. pluvialis* is limited, especially those in immobilized microalgal cultivation such as PSBR systems.

In this study, the effect of the light spectrum from LEDs (red LED, blue LED, and blue-red LED) on biomass growth and astaxanthin accumulation of *H. pluvialis* in angled TL-PSBR was evaluated. From there, the type of LED light most suitable for culturing *H. pluvialis* to produce astaxanthin in TL-PSBRs will be selected for further studies.

## Materials and methods

### *Algal strain and suspended cultivation to prepare microalgae for fixation to biofilm*

The microalga *H. pluvialis* CCAC 0125 (Central Collection of Algal Cultures; https://www.uni-due.de/biology/ccac/) was maintained and cultured in the Biotechnology Laboratory, Nguyen Tat Thanh University, Vietnam. Algae were suspended with BG-11H medium [22] in flasks of 500 ml (for 14 d), 2 l (14 d) and 10 l (20 d) at 23±2°C while illuminated by fluorescent lamps with a light intensity of 30-40 µmol photon $m^{-2}s^{-1}$ and a light/dark cycle of 14 h/10 h.

### *Experimental designs*

The experiments were carried out on pilot-scale angled TL-PSBRs [16]. Each chamber was illuminated by a separate set of LEDs. Each LED luminaire was composed of 16 Bridgelux LED chips (Bridgelux, Inc., China) (Fig. 1). The LED chips selected for use were red LEDs, blue LEDs, and blue-red LEDs (1 blue:3 red). The LEDs were installed in a steel frame and automatically controlled to turn on and off by timers. The distance from the LEDs to the biofilms was adjusted to ensure the light intensity on the algal surface was 100-120 µmol photons $m^{-2}s^{-1}$. The light intensity was measured with a photometer Lutron LX-1108 (Taiwan).

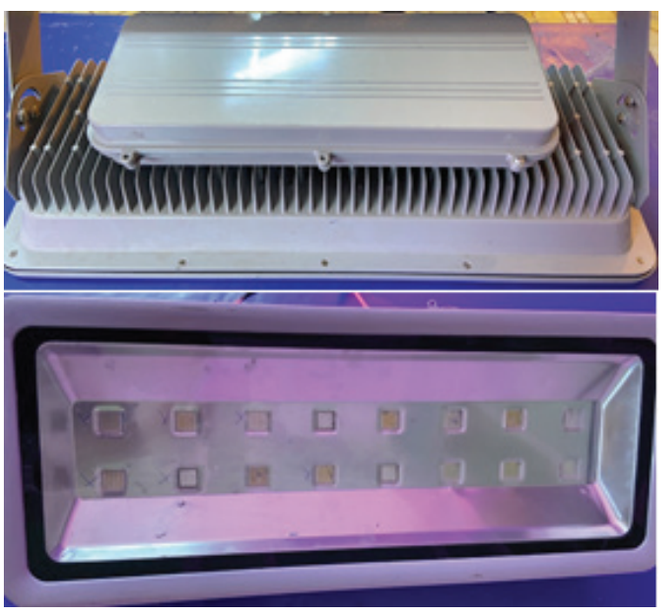

**Fig. 1. The LED luminaires have 16 LED chips used in the experiments.**

*Effects of monochromatic light on growth and astaxanthin accumulation:* experiments were conducted with blue light (wavelength 430-480 nm), red light (wavelength 620-650 nm), simultaneous combinations of red and blue light with a ratio of 3 red:1 blue (Fig. 2). The intensity of the light was 100-120 µmol photon $m^{-2}s^{-1}$.

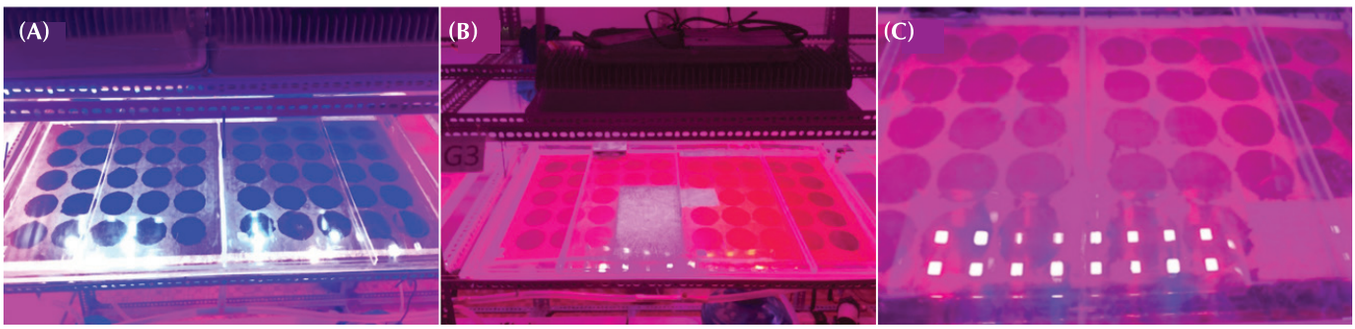


**Fig. 2. The monochromatic LED luminaires used in the experiments: (A) blue light, (B) red light and (C) combinations of red with blue light.**

*Effects of combinations of red LED and blue-red LED illuminations over the culture period:* with the aim of increasing algal biomass in the early days of cultivation (with red light) and stimulating astaxanthin accumulation in the last days of cultivation (with red and blue light), the combination formulas in this experiment were: (1) 10 d with blue and red LEDs, (2) 2 d with red LEDs followed by 8 d with blue and red LEDs, (3) 4 d with red LEDs followed by 6 d with blue and red LEDs, (4) 6 d with red LEDs followed by 4 d with blue and red LEDs, (5) 8 d with red LEDs followed by 2 d with blue and red LEDs, and finally (6) 10 d with red LEDs. The intensity of light was always maintained at 100-120 µmol photon $m^{-2}s^{-1}$.

***Centrifugation to concentrate algae in suspension and immobilization of algae to biofilm in angled TL-PSBRs***

*Centrifugation to collect concentrated algae:* green stage microalgae were concentrated from suspended cultivation (cell density is about $5\times10^5$ cells $ml^{-1}$) by centrifugation at 800 *g* for 5 min with a ROTANTA 460 RC high volume centrifuge (Hettich, Germany). The concentrated algal suspension at the bottom of the centrifuge tube was collected and the dry biomass was determined. To determine the dry biomass, 1 ml of concentrated suspension was added to filter paper (dried and weighed, $m_b$ (g)) and dried at 105°C for *2* h. Dry algae and paper were cooled in the desiccator for 30 min, weighed, and the drying process was repeated until the mass was constant ($m_a$ (g)). The dry biomass of *H. pluvialis* in 1 ml of concentrated algae suspension was calculated as $m_x$ (g/ml)=$m_a$-$m_b$ and repeated 3 times to calculate the mean weight ($m_A$). Total algal biomass obtained was m(g)=$V_A\times m_A$, where $V_A$ is the volume of concentrated algae solution. An initial algal density of 7.5 $g.m^{-2}$ was applied to establish the biofilm.

*Algae immobilization on angled TL-PSBRs:* in order to immobilize the microalgae to create a biofilm, the algal suspension was painted onto the substrate layers with a soft brush. The algae were painted in circles with a radius of 4 cm (each plot had an area of about 50.24 $cm^2$) (Fig. 3) to facilitate sampling at the time of 4, 6, 8, 10 d after immobilization. The volume of concentrated algal suspension needed to immobilize each plot was calculated as:

$$V\ (ml) = \frac{7.5}{m_A} \times 0.005024$$

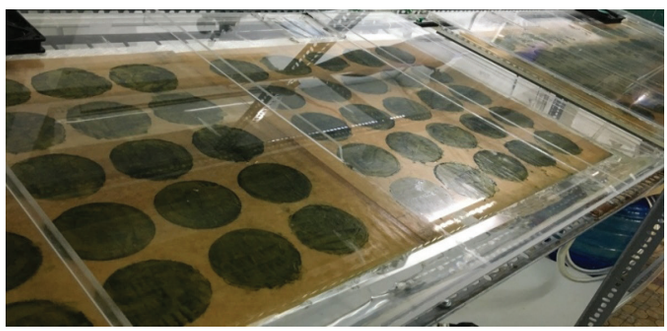

**Fig. 3. Algae were immobilized into circular plots in angled TL-PSBRs.**

***Culture conditions after algal immobilization***

Algal biofilms in TL-PSBRs were cultivated for 10 d. Nutrition for growth of microalgae was provided with 40 l of BG-11H medium from day 1 to day 7, the medium was replaced after 2 or 3 d. After 7 d of culture, the BG-11H medium was replaced with 40 l of N- and P-free medium (stress medium). The culture medium was aerated with 1% $CO_2$ (v/v) added to keep the pH between 6.5-8. Filtered water was added to the culture medium every day to compensate for evaporation, which ensured that the electrical conductivity of the medium was only about 1800-2000 µS/cm. The room temperature during experiments was maintained at 23-26°C.

***The following variables were monitored***

*Environmental factors:* temperature, pH, and electrical conductivity of the BG-11H medium were measured daily during culture in the TL-PSBRs.

*The growth of microalgae:* microalgae growth was monitored by the dry biomass of microalgae obtained per $m^2$ at 4, 6, 8, 10 d after inoculation of algae onto the substrate layer. The fresh algal biomass in a circular area with a radius of 4 cm was scraped with a plastic piece (3×5 cm) and placed in a 10 ml centrifuge tube (pre-weighed, $m_1$). The sample was then dried at 105°C for 2 h. The dry algae and tube were cooled in a desiccator for 30 min, weighed, and the process was repeated the dry weight was constant ($m_2$). The dry weight of microalgae per $m^2$ was calculated as $m(g.m^{-2})=(m_2-m_1)/0.005024$. Three biological samples (n=3) were collected for each treatment at each time for statistical analysis.

*Astaxanthin content in the dry biomass (A%):* to determine the astaxanthin content in *H. pluvialis*, 0.001 g of dry biomass was weighed with a high precision balance and placed in a 2 ml centrifuge tube with cap. Then, 0.5 ml 90% acetone (v/v) was added and shaken vigorously with stainless steel balls (0.5 cm in diameter) for 3 h to allow all cells to rupture (as evaluated by microscopic examination) and release astaxanthin. Then, 1 ml of 90% acetone was added and the sample centrifuged at 6000 *g* for 30 sec. The supernatant was collected and 90% acetone was added to obtain a final volume of 2.0 ml. The procedure was performed in the dark or under weak diffused light to minimize the breakdown of astaxanthin.

The pigment extracts were measured for optical density at a wavelength of 530 nm [22]. The astaxanthin concentration was determined using a standard curve equation constructed with standard astaxanthin (Sigma-Aldrich) dissolved in acetone 90%. (Fig. 4). The standard curve equation is y=0.0577x+0.0131, where y is the OD value and x is the concentration of astaxanthin ($\mu g\ ml^{-1}$). Astaxanthin concentration was determined according to the equation (x) ($\mu g\ ml^{-1}$) = ($OD_{530nm}$ (y)-0.0131)/0.0577.

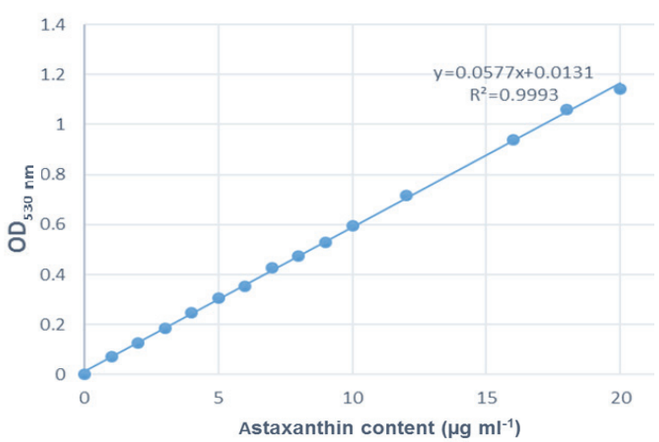


**Fig. 4. Calibration curve for astaxanthin concentration.**

*Ratio of astaxanthin in dry biomass:*

(A%)=[x($\mu g\ ml^{-1}$)x2 ($ml$)x100%]/[0.001($g$)x1,000,000]

Astaxanthin productivity ($mg.m^{-2}d^{-1}$)=[m($mg$)xA%]/10

*Morphological criteria of microalgae under optical microscope:* algal cell morphology was observed with an Olympus CX21 optical microscope (Japan).

***Data analysis***

Statistical analyses were made by using R (version 3.4.2). Tukey's HSD (honestly significant difference) test was use for analysis of difference in experiments. The data were analysed by using Microsoft Excel 365. The presented values were average value (Mean) ±SD (standard deviation) of three replicates (n=3).

## Results

***Effect of monochromatic light on growth and astaxanthin accumulation of H. pluvialis in TL-PSBRs***

*Results of monitoring environmental factors:* monitoring results show that the pH was maintained at 6.6-7.57 throughout the culture period, which was still within the optimal range for the growth of *H. pluvialis*. The temperature of the BG-11H medium was maintained at 21-24°C during experiments (Table 1).

**Table 1. Results of monitoring the pH and temperature of the culture medium.** The same nutrient medium was used for all treatments in an experiment.

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| pH | 6.60 | 6.96 | 6.89 | 7.06 | 7.21 | 7.31 | 7.57 | 7.40 | 7.20 | 7.28 |
| Temperature (°C) | 24.2 | 23.6 | 23.8 | 23.3 | 22.0 | 22.0 | 21.2 | 21.4 | 21.3 | 21.2 |

*Effect of monochromatic light on the growth of H. pluvialis:* the dry biomass for all three treatments increased during the first 6 d (initial dry biomass was 7.5 $g.m^{-2}$) under the same culture conditions and light intensity (range from 100-120 µmol photon $m^{-2}/s$) but different types of monochromatic LED (red, blue or the combination blue and red) (Fig. 5). There was almost no significant change in the dry biomass when illuminated by the blue LED from day 6 to day 10 ($p>0.05$, n=3).

For algae illuminated by the red LED, an increase in dry biomass was observed until day 6, which then decreased after day 8 (Fig. 5). The dry biomass of microalgae illuminated by the combination of blue and red LED, however, increased continuously over the culture period of 10 d and the growth was linear with a rate of 3.06 $g.m^{-2}d^{-1}$.

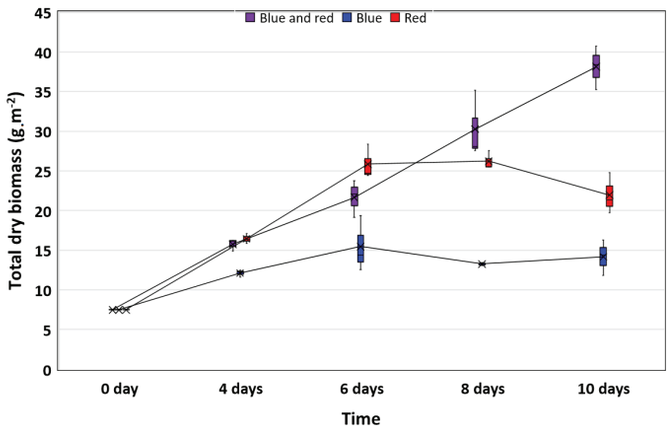


**Fig. 5. Total dry biomass of *H. pluvialis* over time when illuminated with different types of monochromatic light.**

After 10 d of culture, the total dry biomass of microalgae with the red and blue LEDs combination was the highest, in fact, much higher than the blue LED or red LED alone ($p<0.05$, n=3). The dry biomass yield was highest at around 3.06 $g.m^{-2}d^{-1}$ when illuminated with a combination of blue and red LEDs.

*Effect of monochromatic light on astaxanthin accumulation of H. pluvialis:* the percentage of astaxanthin accumulated in the dry biomass of *H. pluvialis* over the culture period is shown in Fig. 6. At day 4, the astaxanthin content in the biomass was highest with LEDs containing blue light (up to 1.7% in the blue and red LED lighting) and then decreased until day 8. This was likely because during the first days of cultivation, the algal cells in the thin biofilm were exposed to relatively high light intensities favouring astaxanthin accumulation. When the biofilm became thicker (i.e., >20 g dry weight/$m^2$, Fig. 7), the percentage of cells in the lower cell layers increased, which are shaded by the upper layers, thus lowering the overall astaxanthin content of the biofilm.

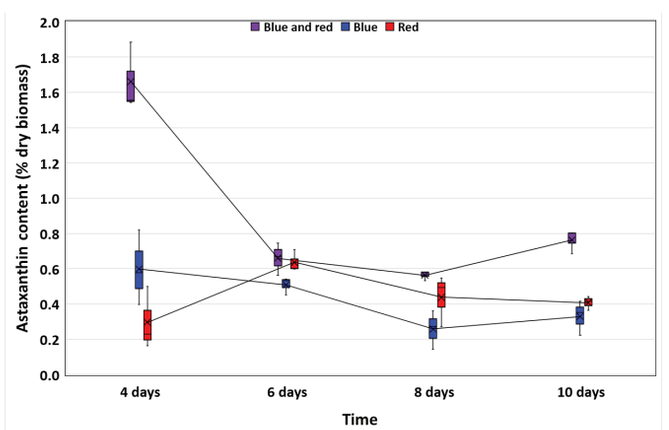


**Fig. 6. Percentage of astaxanthin in dry biomass of *H. pluvialis* over time when illuminated with different types of monochromatic light.**

Under red LED illumination, astaxanthin accumulation was highest at day 6 (Fig. 6) and then gradually decreased. After 10 days, the percentage of astaxanthin in the dry biomass was highest in the blue and red LED combination treatment (average 0.76%, Fig. 6), which is significantly higher than that of using only red or blue LEDs ($p<0.05$, n=3).

Therefore, the total dry biomass and the percentage of astaxanthin in the dry biomass after 10 days of culture were highest in the treatment with the combination of blue and red LEDs, thus the astaxanthin amount ($mg.m^{-2}$) was also highest in this treatment (Table 2) and the difference was statistically significant compared with the other two treatments ($p<0.05$, n=3).

**Table 2. Astaxanthin standing crop per square meter ($mg.m^{-2}$) after 10 days when illuminated with different types of monochromatic light.**

| Blue and red LEDs | Blue LED | Red LED |
|---|---|---|
| 291.99±44.73[a] | 46.99±18.37[b] | 89.75±15.85[c] |

Note: [a, b, c] letters in the same row indicate statistically significant difference ($p<0.05$).

*Effect of monochromatic light on biofilm surface and microscopic morphology of H. pluvialis:* the macroscopic appearance of the algal biofilm surface over the culture period at different LED exposures is shown in Fig. 7. In general, the thickness of the biofilms increased over time in

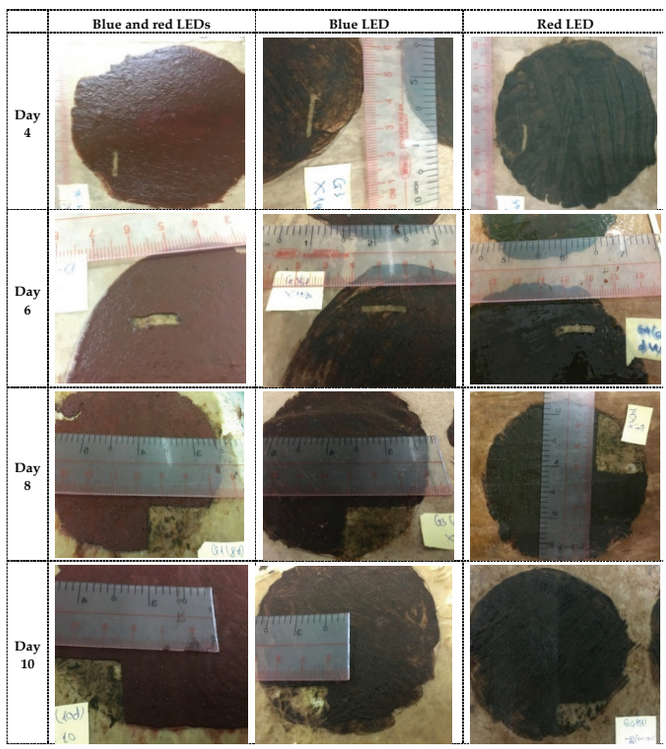


**Fig. 7. Biofilm surface of *H. pluvialis* when illuminated with different types of monochromatic light.**

accordance with the determinations of the total dry biomass, of which the thickest was that of the blue and red LED treatment.

The biofilm surface colour was also markedly different between the 3 treatments. After 10 d, the algal surface under red LED was still green in accordance with the very low level astaxanthin content while the biofilm surface from the combined blue and red LED illumination had a red colour.

***Effects of the combinations of red LED and blue-red LED illuminations over the culture period on H. pluvialis in TL-PSBRs***

Previous studies have shown that red light stimulates the growth of *H. pluvialis* while blue light stimulates astaxanthin accumulation [23-25]. Therefore, this experiment investigated the effect of combining red LED illumination in the early days and blue-red LED illumination in the following days to investigate the ability to stimulate both algal growth and accumulation of astaxanthin.

*Effects of the combinations of red LED and blue-red LED illuminations over the culture period on growth:* under the same culture conditions and light intensity (100-120 µmol photon $m^{-2}/s$), the total biomass of microalgae generally increased with culture time for all 6 treatments (initial dry biomass was 7.5 $g.m^{-2}$) (Fig. 8). The total dry biomass and biomass productivity were highest in the treatment with the the greatest number of days of blue-red LED (0 day of red +10 days of blue-red). After 10 days of culture, the total dry biomass in this treatment (0 day of red +10 days of blue-red) was the highest at 38.1 $g.m^{-2}$, which is much higher than the other treatments and the difference was statistically significant ($p<0.05$, n=3). The lowest total dry biomass

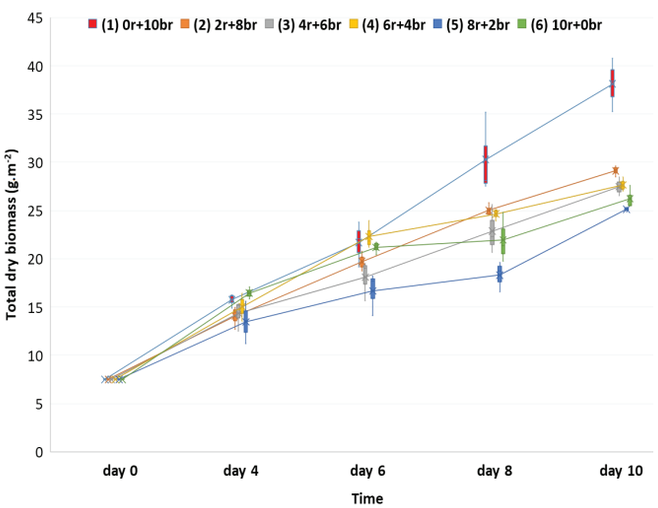


**Fig. 8. Total dry biomass of *H. pluvialis* over time when illuminated by LEDs.** r: red LED, br: blue-red LED.

occurred in treatment (5) (8 days with red LEDs +2 days with blue and red LEDs) and treatment (6) (10 days with red LEDs +0 day with blue and red LEDs), which was 25.1 and 26.28 $g.m^{-2}$, respectively. Thus, the combination of blue and red lighting from the beginning still showed the highest dry biomass increase after 10 d of culture.

*Effects of the combinations of red LED and blue-red LED illuminations over the culture period on the astaxanthin accumulation of H. pluvialis:* the percentage of astaxanthin accumulated in the dry algal biomass over the culture time is shown in Fig. 9. The percentage of astaxanthin in the dry biomass reached its highest value of 1.2% after 10 d in treatment (2:2 days red LEDs followed by 8-d blue-red LEDs) and the difference was statistically significant compared with treatments (1), (4), (5) and (6) ($p<0.05$, n=3).

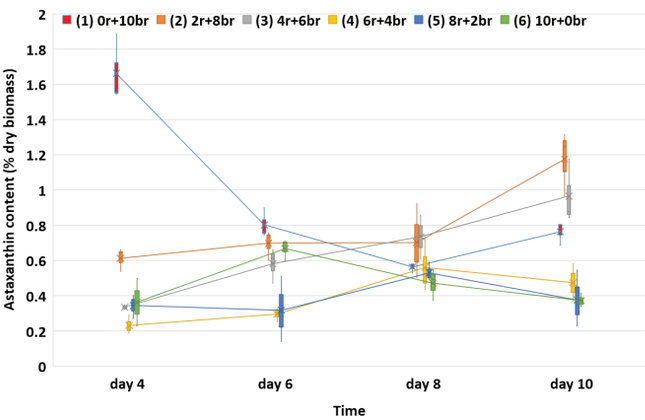


**Fig. 9. Content of astaxanthin in the dry biomass of *H. pluvialis* over time when illuminated by LEDs.** r: red LED, br: blue-red LED.

These results occurred because in treatments with more blue-red light, the microalgal layers in the biofilm were stimulated to accumulate more astaxanthin upon transition to the red phase. Conversely, the more days of red LED illumination, the greater the number of cells in the green phase in the biofilm layers, which results in a low percentage of astaxanthin in the dry biomass. On day 10, the astaxanthin contents in the dry biomass of the six treatments were distributed into two distinct groups: one with a higher astaxanthin content (1, 2, and 3) and one with a significantly lower percentage of astaxanthin in the biomass (4, 5, and 6) (Fig. 9). This suggests that a minimum of 6-d exposure to blue LEDs is required to attain higher astaxanthin values.

The results of determining the ratio of astaxanthin in dry biomass also showed that the percentage of astaxanthin when fully illuminated with blue-red LEDs reached the highest value at day 4 but then decreased gradually. In the early days of blue-red LED illumination, cells had

mainly accumulated astaxanthin and changed into the red phase but the dry biomass of microalgae did not increase as significantly. Thus, the content of astaxanthin in dry biomass would be high.

Table 3 summarizes the amount of astaxanthin ($mg.m^{-2}$) obtained in 6 treatments with combination LED illumination. The results show that treatments (1), (2), and (3) gave the highest astaxanthin productivity and the difference between these treatments was not statistically significant ($p>0.05$, $n=3$), but was much higher than the rest of the treatments ($p<0.05$, $n=3$).

**Table 3. Astaxanthin standing crop per square meter ($mg.m^{-2}$) after 10 d.**

| (1) 0 r+10 br | (2) 2 r+8 br | (3) 4 r+6 br | (4) 6 r+4 br | (5) 8 r+2 br | (6) 10 r+0 br |
|---|---|---|---|---|---|
| 292.0±44.7[a] | 342.9±60.3[a] | 264.1±42.1[a] | 130.8±27.5[b] | 94.6±40.1[c] | 97.5±10.1[c] |

Note: [a, b, c] letters in the same row indicate statistically significant difference. $p<0.05$, r: red LED, br: blue-red LED.

## Discussion

*H. pluvialis* and other green algae do not use all wavelengths of visible light spectrum (wavelengths between 400 and 700 nm) for photosynthesis, but only absorb mainly blue, purple, and red light. Fluorescent lamps contain 60% of the light that is suitable for photosynthesis. When using a combination of red and blue light, this ratio was up to 84%, increasing the amount of red light, the amount of light received by the photosynthetic chain was increased by 91% [26]. Red light provides a higher number of photons than white light for enhanced algal growth [26]. The use of blue light stimulated more algae proliferation and astaxanthin accumulation [24-27].

The two types of monochromatic LED light used in this study were red light (wavelength about 620-650 nm) and blue light (wavelength about 430-480 nm). In two-step suspension cultivations, previous studies using monochromatic light from LEDs have shown an energy efficiency in stimulating growth and accumulation of astaxanthin in the green microalga *H. pluvialis*. In particular, the red LED light played a major role in stimulating growth to increase the dry biomass but did not stimulate astaxanthin accumulation, so the astaxanthin productivity was usually quite low and similar to the results of this study. Meanwhile, blue LED light stimulated the microalgae to accumulate astaxanthin but was not effective in increasing the total dry biomass [21, 24, 25]. Consequently, the combination of these two monochromatic light sources had the effect of both stimulating biomass growth and the accumulation of astaxanthin in suspension cultivations [18-20, 27].

The results of this study are consistent with previous research, although immobilized microalgal cultivation displays many differences compared to suspended cultivation. This study showed that LEDs have great potential for the cultivation of *H. pluvialis* in a TL-PSBR both by increasing biomass and stimulating astaxanthin accumulation. The simultaneous use of blue and red LEDs produced the highest dry biomass productivity that reached 4.74 $g.m^{-2}d^{-1}$ under the experimental conditions used in this study and a culture period of 10 d. Previous research on immobilized *H. pluvialis* cultivation showed dry biomass productivities between 3.7-11.25 $g.m^{-2}d^{-1}$ after 7 to 15 d using fluorescence lamps [28, 29] or high power and high pressure sodium lamps as the light source [17, 30]. Recently, the use of monochromatic LEDs to cultivate immobilized algae in biofilms has been applied on *Chlorella* [31]. Our initial research using LED light to grow *H. pluvialis* in pilot scale angled TL-PSBRs resulted in a relatively low astaxanthin yield. For the application of LEDs in angled TL-PSBRs, further studies need to be performed to increase productivity of astaxanthin accumulation by variation of environmental parameters and the use of other strains or mutants [7, 32, 33].

## Conclusions

The combination of blue and red LEDs with an intensity of 100-120 µmol photons $m^{-2}s^{-1}$ in immobilized *H. pluvialis* cultivation resulted in the highest dry biomass and astaxanthin accumulation compared to using only blue or red LEDs.

## COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

## REFERENCES

[1] Y.M. Naguib [2000], "Antioxidant activities of astaxanthin and related carotenoids", *J. Agric. Food Chem.*, **48(4)**, pp.1150-1154.

[2] M. Guerin, M.E. Huntley, M. Olaizola (2003), "Haematococcus astaxanthin: applications for human health and nutrition", *Trends Biotechnol.*, **21(5)**, pp.210-216.

[3] I. Faraone, et al. (2020), "Astaxanthin anticancer effects are mediated through multiple molecular mechanisms: a systematic review", *Pharmacol. Res.*, **155**, DOI: 10.1016/j.phrs.2020.104689.

[4] G. Panis, J. Rosales Carreon (2016), "Commercial astaxanthin production derived by green alga *Haematococcus pluvialis*: a microalgae process model and a techno-economic assessment all through production line", *Algal Research*, **18**, pp.175-190.

[5] M.M. Shah, et al. (2016), "Astaxanthin-producing green

microalga *Haematococcus pluvialis*: from single cell to high value commercial products", *Front. Plant. Sci.*, **7**, DOI: 10.3389/fpls.2016.00531.

[6] K.S. Khoo, et al. (2019), "Recent advances in biorefinery of astaxanthin from *Haematococcus pluvialis*", *Bioresource Technol.*, **288**, DOI: 10.1016/j.biortech .2019.121606.

[7] X. Li, et al. (2020), "Biotechnological production of astaxanthin from the microalga *Haematococcus pluvialis*", *Biotechnol. Adv.*, **43**, DOI: 10.1016/j.biotechadv. 2020.107602.

[8] M. Olaizola, M. Huntley (2003), "Recent advances in commercial production of astaxanthin from microalgae", *Recent Advances in Marine Biotechnology*, Science Publishers, **9**, pp.143-164.

[9] L. Brennan, P. Owende (2010), "Biofuels from microalgae - a review of technologies for production, processing, and extractions of biofuels and co-products", *Renewable and Sustainable Energy Reviews*, **14(2)**, pp.557-577.

[10] O. Giuseppe, S. Piero, M. Antonio (2014), "Advances in photobioreactors for intensive microalgal production: configurations, operating strategies and applications", *J. Chem. Technol. Biotechnol.*, **89(2)**, pp.178-195.

[11] F.G. Acién, et al. (2017), "1 - Photobioreactors for the production of microalgae, in microalgae-based biofuels and bioproducts", *Microalgae-Based Biofuels and Bioproducts*, Woodhead Publishing, pp.1-44.

[12] R.M. Benstein, et al. (2014), "Immobilized growth of the peridinin-producing marine dinoflagellate Symbiodinium in a simple biofilm photobioreactor", *Mar. Biotechnol.*, **16(6)**, pp.621-628.

[13] T. Li, B. Podola, M. Melkonian (2016), "Investigating dynamic processes in a porous substrate biofilm photobioreactor - a modeling approach", *Algal Research*, **13**, pp.30-40.

[14] T. Naumann, et al. (2013), "Growing microalgae as aquaculture feeds on twin-layers: a novel solid-state photobioreactor", *Journal of Applied Phycology*, **25(5)**, pp.1413-1420.

[15] E.C.M. Nowack, B. Podola, M. Melkonian (2005), "The 96-well twin-layer system: a novel approach in the cultivation of microalgae", *Protist*, **156(2)**, pp.239-251.

[16] H.D. Tran, et al. (2019), "Cultivation of *Haematococcus pluvialis* for astaxanthin production on angled bench-scale and large-scale biofilm-based photobioreactors", *Vietnam Journal of Science, Technology and Engineering*, **61(3)**, pp.61-70.

[17] T.T. Do, et al. (2019), "Biomass and Astaxanthin productivities of *Haematococcus pluvialis* in an angled twin-layer porous substrate photobioreactor: effect of inoculum density and storage time", *Biology (Basel)*, **8(3)**, DOI: 10.3390/biology8030068.

[18] T. Xi, et al. (2016), "Enhancement of astaxanthin production using *Haematococcus pluvialis* with novel LED wavelength shift strategy", *Appl. Microbiol. Biotechnol.*, **100(14)**, pp.6231-6238.

[19] S. Pereira, A. Otero (2020), "*Haematococcus pluvialis* bioprocess optimization: effect of light quality, temperature and irradiance on growth, pigment content and photosynthetic response", *Algal Research*, **51**, DOI: 10.1016/j.algal.2020. 102027.

[20] S. Cui, et al. (2020), "Design of automatic illumination culture system for *Haematococcus pluvialis* based on LED", *Proceedings of 2019 Chinese Intelligent Automation Conference*, pp.393-400.

[21] R. Ma, et al. (2018), "Blue light enhances astaxanthin biosynthesis metabolism and extraction efficiency in *Haematococcus pluvialis* by inducing haematocyst germination", *Algal Research*, **35**, pp.215-222.

[22] A.C. Kiperstok (2016), *Optimizing Immobilized Cultivation of Haematococcus Pluvialis for Astaxanthin Production*, PhD thesis, University of Cologne, 128pp.

[23] Y. Li, et al. (2012), "Accurate quantification of astaxanthin from *Haematococcus crude* extract spectrophotometrically", *Chinese Journal of Oceanology and Limnology*, **30(4)**, pp.627-637.

[24] Y.-H. Ho, et al. (2018), "Maximization of astaxanthin production from green microalga *Haematococcus pluvialis* using internally-illuminated photobioreactor", *Advances in Bioscience and Bioengineering*, **6**, pp.10-22.

[25] K.T. Pham, et al. (2018), "Influence of inoculum size, $CO_2$ concentration and LEDs on the growth of green microalgae *Haematococcus pluvialis* flotow", *Vietnam Journal of Science and Technology*, **60**, pp.59-65.

[26] J.H. Jou, et al. (2015), "Plant growth absorption spectrum mimicking light sources", *Materials (Basel)*, **8(8)**, pp.5265-5275.

[27] K.-H. Lee, C.-H. Hong (2015), "Effects of LED irradiation on the growth and Astaxanthin production of *Haematococcus lacustris*", *Biosciences Biotechnology Research Asia*, **12**, pp.1167-1173.

[28] W. Zhang, et al. (2014), "Attached cultivation of *Haematococcus pluvialis* for astaxanthin production", *Bioresource Technol.*, **158**, pp.329-335.

[29] M. Wan, et al. (2014), "The effective photoinduction of *Haematococcus pluvial*is for accumulating astaxanthin with attached cultivation", *Bioresource Technol.*, **163**, pp.26-32.

[30] A.C. Kiperstok, et al. (2017), "Biofilm cultivation of *Haematococcus pluvialis* enables a highly productive one-phase process for astaxanthin production using high light intensities", *Algal Research*, **21**, pp.213-222.

[31] H. Yuan, et al. (2020), "Light-emitting diode power conversion capability and $CO_2$ fixation rate of microalgae biofilm cultured under different light spectra", *Energies*, **13**, DOI: 10.3390/en13071536.

[32] P.I. Gómez, et al. (2016), "Intraspecific variability among Chilean strains of the astaxanthin-producing microalga *Haematococcus pluvialis* (Chlorophyta): an opportunity for its genetic improvement by simple selection", *Journal of Applied Phycology*, **28(4)**, pp.2115-2122.

[33] N. Wang, et al. (2016), "Enhancement of astaxanthin production from *Haematococcus pluvialis* mutants by three-stage mutagenesis breeding", *J. Biotechnol.*, **236**, pp.71-77.