EVALUATION OF LIGHT MODULATION ON BIOMASS PRODUCTION AND β-CAROTENE ACCUMULATION IN *Synechocystis* SP. CACIAM 05

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ABSTRACT

The study aimed to cultivate the cyanobacterium *Synechocystis* sp. CACIAM 05 under the manipulation of different light-emitting diodes (blue, red, and white LEDs) and different exposure times (partial and full), in terms of biomass production and pigment accumulation. The maximum concentrations of biomass (0.82 g/L), chlorophylls (5.64 µg/mL), total carotenoids (2.04 µg/mL), and β-carotene (1.27 mg/L) were obtained when red LEDs and a full photoperiod (24 h) were applied to the cultivation, demonstrating the potential of LEDs in the biostimulation of high-value bioproducts.

Keywords: Antioxidant. Bioprocesses. Carotenoids. Cyanobacteria. Pigments.

1 INTRODUCTION

Cyanobacteria are photosynthetic microorganisms that can be employed for various biotechnological purposes and exhibit extensive biological activity.[1] Light sources, both natural and artificial, are utilized for the cultivation of these microorganisms. However, variations in natural light availability can directly inhibit the growth of cyanobacteria.[2] Thus, light-emitting diodes (LEDs) can be utilized as an effective artificial source for the cultivation of cyanobacteria, being ideal for both growth and the induction of specific metabolites.[3]

Therefore, the modulation of light quality can be effectively used to optimize photosynthetic microorganisms and the production of value-added bioproducts. In this context, the present study aimed to cultivate the cyanobacterium *Synechocystis* sp. CACIAM 05 under the manipulation of different light-emitting diodes (blue, red, and white LEDs) and different exposure times (partial and continuous) to assess their impacts on growth. Additionally, the study also investigated the effects of optimizing these parameters on biomass production and the accumulation of bioactive pigments (chlorophylls, total carotenoids, and β-carotene).

2 MATERIAL & METHODS

The cyanobacterium *Synechocystis* sp. CACIAM 05 originates from the Amazonian Collection of Cyanobacteria and Microalgae (CACIAM), housed in the Laboratory of Biomolecular Technology (LTB) within the Institute of Biological Sciences (ICB) at the Federal University of Pará (UFPA). The experiments were conducted in triplicates over a period of 20 days at a temperature of 23 ± 2°C, using 500 mL erlenmeyer flasks containing 300 mL of BG-11 medium with an initial biomass concentration of 0.002 g/L. The light intensity was constant and fixed at 100 µmol m^{-2} s^{-1}. Subsequently, the cultures were treated with different light-emitting diodes: white (400-700 nm), red (630-675 nm), and blue (450-475 nm), and with different photoperiods, $partial_{(p)}$ (13 h light and 11 h dark) and $integral_{(i)}$ (24 h light and 0 h dark), aiming to evaluate their influences on the production of bioactive pigments in the cultivation of CACIAM 05. The treatment with white light was considered as control and conducted only with a partial photoperiod. The content of photosynthetic pigments, chlorophylls, and total carotenoids was measured every 2 days using a UV-Visible spectrophotometer, and concentrations were calculated according to Equations 1, 2, and 3, respectively.[4]

$$C_a(\mu g/mL) = 12{,}21A_{663} - 2.81A_{646} \quad (1)$$

$$C_b\ (\mu g/mL) = 20{,}13A_{646} - 5.03A_{663} \quad (2)$$

$$C_{car}\ (\mu g/mL) = \frac{1000A_{470} - 3.27C_a - 104C_b}{229} \quad (3)$$

The productivity per dry biomass was determined gravimetrically. The dry weight (DW) was measured using a precision balance and calculated based on the difference between the final and initial weights, and productivity was calculated using equation 4.[5]

$$P(mg/L/day) = \frac{C_t - C_0}{t - t_0} \quad (4)$$

The quantification of β-carotene was conducted on the 20th day of cultivation and measured using a UV-Visible spectrophotometer at wavelengths of 412 nm, 431 nm, 460 nm, and 480 nm, and concentration were calculated according to equations 5.[6]

$$\beta - carotene\ \left(\frac{\mu g}{mL}\right) = -0.430A_{412} + 0.251A_{431} - 4.376A_{460} + 13.216A_{480} \quad (5)$$

3 RESULTS & DISCUSSION

Cyanobacterial pigments, such as chlorophylls and carotenoids, are essential pigments for the development of photosynthetic microorganisms, playing important roles in light absorption and conversion into biochemical energy. [7,8] It can be observed that both the quality of light provided and the duration of light exposure had a direct impact on the growth of the cyanobacterium *Synechocystis* sp. CACIAM 05 (Figure 1A). In the different light regimes tested - white light (control), $red_{(p)}$, $red_{(i)}$, $blue_{(p)}$, and $blue_{(i)}$ - exposure to red LED light for 24 hours a day showed significantly promotive effects ($p < 0.05$) on the growth of CACIAM 05 from the 2nd day, with a 66.86% increase in cell density on the 20th day. During the same period, the concentrations of chlorophyll-a for the cultures with $blue_{(p)}$ LED light, $blue_{(i)}$, $red_{(p)}$, and control were 1.54 µg/mL, 3.30 µg/mL, 3.44 µg/mL, and 3.38 µg/mL, respectively. Exposure to blue light under partial photoperiod demonstrated significant inhibitory effects ($p < 0.05$) on the growth of CACIAM 05, resulting in a 54.44% reduction observed on the 20th day. However, no significant difference was detected between treatments with $blue_{(i)}$ light, $red_{(p)}$, and the control ($p > 0.05$) (Figure 1A).

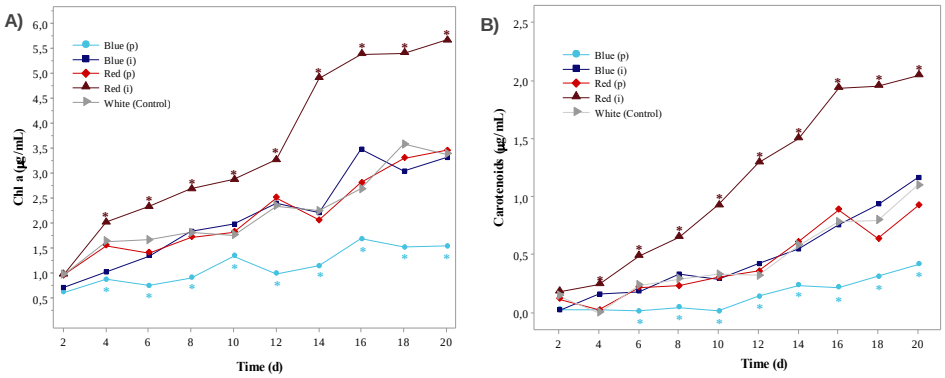

Figure 1 Effects of light modulation on chlorophyll (A) and carotenoid (B) contents in cyanobacterium CACIAM 05. Light exposure time - (p): partial photoperiod and (i): integral photoperiod. Data are represented in biological triplicates (*n=3*). *: Statistically significant difference ($p < 0.05$) compared to the control.

Similarly, when comparing the effects of red, blue, and white LEDs and photoperiods on the production of total carotenoids, $red_{(i)}$ LED light significantly ($p < 0.05$) increased the carotenoid content in *Synechocystis* sp. CACIAM 05 from the 2nd day, with an 85.45% increase on the 20th day (Figure 1B), corresponding to 2.04 µg/mL. In comparison, when subjected to the same wavelength (red), but under a partial photoperiod (13L:11D), the maximum total carotenoid content was 0.92 µg/mL. Additionally, it was observed that shorter exposure time to blue LED light also demonstrated significant effects ($p < 0.05$) on carotenoid accumulation (1.16 µg/mL). However, the influence of $blue_{(i)}$ LED light and $red_{(p)}$ LED light on carotenoid accumulation was also not statistically significant ($p > 0.05$). Red light plays a crucial role in elevating electrons in the light-harvesting complexes composed of chlorophyll-a or b. In cyanobacteria, accessory pigments in the phycobilisome absorb light in the range of 560-660 nm, where chlorophyll absorption is highly limited, transferring energy to the photosystems. Thus, the red wavelength maintains the linear flow of electrons, balancing energy between the two photosystems. [9,10] Therefore, the lowest concentration of chlorophyll a in *Arthrospira maxima* was obtained through cultivation with blue LED light, while red LED light directly influenced the production of this pigment.[11] A similar trend was observed in the cultivation of *Nostoc sphaeroides*.[12] Meanwhile, red light significantly increased the content of chlorophyll a in *Microcystis aeruginosa* cells.[8]

The highest biomass concentration and productivity ($p < 0.05$) were observed in the experiment with red LEDs under a continuous light photoperiod (0.82 g/L and 40.90 mg/L/day, respectively). This result was approximately 2 times higher compared to the control experiment, which used white LED light and a partial photoperiod (0.39 g/L and 19.23 mg/L/day). Biomass production and productivity using $blue_{(i)}$ LEDs and $red_{(p)}$ did not show significant promotive effects ($p > 0.05$) on the growth of cyanobacterium CACIAM 05, reaching a biomass concentration of 0.48 g/L and 0.42 g/L, respectively, and productivity of 23.70 mg/L/day and 20.75 mg/L/day, respectively. However, the culture exposed to $blue_{(p)}$ LED light showed statistically significant differences ($p < 0.05$), but biomass production (0.42 g/L) and productivity (20.75 mg/L/day) were lower compared to all other treatments.

The growth of the cyanobacterium *Synechocystis* sp. CACIAM 05 under blue light was notably slower, resulting in lower biomass production. This occurs because *Synechocystis* sp. utilizes blue light less efficiently for photosynthesis, directly influencing growth, as blue light creates an imbalance between photosystems I and II.[10] Given that the predominant photosynthetic pigments in cyanobacteria absorb energy in wavelength ranges of approximately 430 to 680 nm (chlorophyll-a) and 550 to 620 nm (phycobiliproteins), red LEDs cover the absorption spectrum of these pigments in the range of 620 to 645 nm. This results in a greater utilization of energy and, consequently, in higher biomass production by the cells.[13] Therefore, under red light, an increase in biomass production by *Spirulina plantensis* was also detected.[14] *Haematococcus*.[15] *Nostoc sphaeroides.*[12] *Arthrospira platenses*.[16] *Spirulina* sp. LEB 18.[13] *H. pluvialis.*[17] *Picochlorum atomus.*[18]

The production of β-carotene can be affected by various factors, including light quality. In *Dunaliella salina*, it was observed that β-carotene production was significantly higher when exposed to red LED light compared to white and blue light.[19] On the other hand, the maximization of β-carotene in *Phormidium* sp. was more induced by white light.[20] Meanwhile, in *Chlorella ellipsoidea*, blue light increased the β-carotene content.[21] In this study, treatments with $red_{(i)}$ LED light, $red_{(p)}$, $blue_{(i)}$, $blue_{(p)}$, and white light (control) showed different effects on β-carotene biosynthesis (Figure 2). The treatment with red LED light and integral

photoperiod significantly increased ($p < 0.05$) the β-carotene content, reaching a maximum content of 0.22% in *Synechocystis* sp. CACIAM 05. On the other hand, under the same light pattern but with a partial photoperiod, there was a reduction in the β-carotene content, reaching 0.12%. This trend was also observed in treatments with blue light (integral) (0.14%) and blue light (partial) (0.05%) (Figure 2).

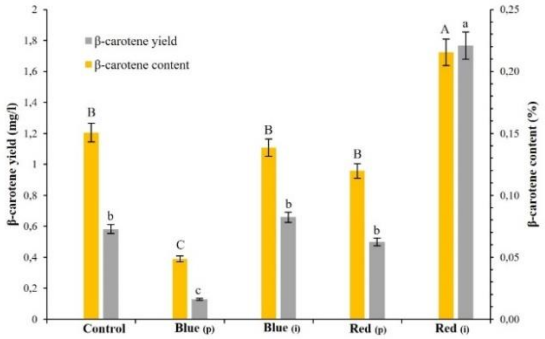

Figure 2 Effects of light modulation on β-carotene accumulation and content. The provided data are mean ± standard deviation (*n=3*). Treatments sharing the same lowercase letter indicate no significant difference between values ($p > 0.05$). Treatments sharing the same uppercase letter indicate no significant difference ($p > 0.05$).

Based on the β-carotene content data and total carotenoid production, the maximum β-carotene yield in CACIAM 05 was 1.27 mg/L ($p < 0.05$) in cells cultured under integral photoperiod and red LED light. However, cultures exposed to the same light but under a partial photoperiod had a negative impact on β-carotene accumulation, experiencing a reduction of approximately 14% compared to the control. Cultures under $red_{(i)}$ light were approximately 3.6 times more efficient in β-carotene production than those grown under $red_{(p)}$ light (Figure 2). Additionally, no significant differences ($p > 0.05$) were observed in β-carotene production under $red_{(p)}$ LED light (0.50 mg/L) and $blue_{(i)}$ LED light (0.66 mg/L), unlike the culture subjected to $blue_{(p)}$ light treatment (0.13 mg/L) ($p < 0.05$). However, blue LED light under a partial regime demonstrated the most pronounced inhibitory effect, resulting in a 77.58% decrease in β-carotene accumulation. Thus, it can be inferred that cultures subjected to red LED light under integral photoperiod over the 20-day cultivation period had a direct impact on growth, consequently resulting in higher biomass production and β-carotene accumulation.

4 CONCLUSION

This study demonstrated that light manipulation, considering its quality and duration, effectively stimulated biomass production and the target bioproduct. The results indicated that the use of red LEDs under continuous photoperiod resulted in higher efficacy in promoting growth kinetics and β-carotene synthesis. In contrast, red and blue LEDs under partial and continuous photoperiods, respectively, did not show significant stimulation, resulting in relatively lower β-carotene concentrations due to decreased biomass at harvest. It is relevant to note that various strategies have been widely studied to increase biomass and carotenoid production in cyanobacteria, including light intensity, light quality and exposure time. The synergy between these approaches can further enhance yields. In summary, these findings provide valuable insights for determining the optimal conditions of light quality and exposure, aiming to enhance biomass and β-carotene productivity in cyanobacteria. These compounds can be explored in various biotechnological applications, including the treatment of diabetic wounds.

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