EFFECTS OF DIFFERENT WAVELENGTHS AND PHOTOPERIODS ON THE ACCUMULATION OF PHYCOCYANIN BY *Synechocystis* SP. CACIAM 05

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ABSTRACT

The study aimed to optimize the growth and synthesis of phycocyanin in *Synechocystis* sp. CACIAM 05 through the influence of light and periodicity (light:dark) in cultivation. Red LED light and continuous photoperiod enhanced biomass production. On the other hand, for the production of phycocyanin by *Synechocystis* sp. CACIAM 05, blue LED light proved to be the most suitable wavelength under partial light exposure.

Keywords: Cyanobacteria. Pigment accumulation. Optimization. Light emitting diodes. Photoperiod.

1 INTRODUCTION

Phycocyanin (PC) is a type of phycobiliprotein that acts as a light-harvesting pigment.[1] Phycobiliproteins are biotechnologically significant products produced by high-value sources such as cyanobacteria.[2] In a cultivation system, the light source is essential for the development of photosynthetic microorganisms, where light must be provided at appropriate duration and wavelength.[3] Thus, light-emitting diodes (LEDs) can be used as an effective artificial source for cultivating cyanobacteria, serving as an optimal parameter for inducing this metabolite.[4]
Therefore, with the aim of promoting the synthesis of phycobiliprotein by *Synechocystis* sp. CACIAM 05 for biotechnological applications, the study focused on optimization and evaluating the production of this biocompound by manipulating different wavelengths of LEDs (blue, red and white), along with varying photoperiods (partial and continuous).

2 MATERIAL & METHODS

The cyanobacterium *Synechocystis* sp. CACIAM 05 was obtained from the Amazonian Collection of Cyanobacteria and Microalgae (CACIAM), located at the Laboratory of Biomolecular Technology (LTB) within the Institute of Biological Sciences (ICB) at the Federal University of Pará (UFPA). In the cultivation of CACIAM 05, different light-emitting diodes (LEDs) were used: white (400-700 nm), red (630-675 nm) and blue (450-475 nm), along with different photoperiods: partial$_{(p)}$ (13:11 h (light:dark)) and continuous$_{(i)}$ (24 h (light)). The experiment with white light served as the control and was conducted only with a partial photoperiod. The light intensity was fixed at µmol m^{-2} s^{-1}. Cultures were performed in triplicate over a 20-day period at a temperature of 23 ± 2 °C, using *erlenmeyers* flasks containing 300 mL of BG-11 medium with an initial biomass concentration of 0,002 g/L. At the end of the experiment, the total biomass production yield (g/L) was measured. The obtained biomass was centrifuged at 4000 x g for 10 minutes, and productivity was determined gravimetrically using a precision balance and calculated using Equation 1.[5]

$$P(mg/L/dia) = \frac{C_t - C_0}{t - t_0} \quad (1)$$

The extraction of PC was performed in the dark with modifications, involving the addition of 10 mL of phosphate buffer to 60 mg of lyophilized biomass from the cyanobacterium.[6,7] Subsequently, to facilitate the extraction process, maceration and sonication methods were applied to the samples.[8] The amount of PC was calculated according to Equation 2. Meanwhile, the purity (EP) of the phycocyanin extract was spectrophotometrically monitored by the A_{615}/A_{280} ratio (Equation 3). The extraction yield was defined based on Equation 4 .[9]

$$C_{PC(mg/mL)} = \frac{A_{615} - 0.474 * A_{652}}{5.34} \quad (2)$$

$$EP = \frac{A_{615}}{A_{280}} \quad (3)$$

$$Yield = \frac{PC * V}{DB} \quad (4)$$

3 RESULTS & DISCUSSION

The highest biomass concentration ($p \leq 0.05$) was observed in the experiment with red LEDs under continuous light (Table 1). This result was approximately 2 times greater compared to the control experiment, which used white LED light and a partial photoperiod. Biomass production using $blue_{(i)}$ and $red_{(p)}$ LEDs did not show significant growth-promoting effects ($p > 0.05$) in the cyanobacterium *Synechocystis* sp. CACIAM 05 when compared to the control group (Table 1), resulting in biomass production of 0.48 g/L and 0.42 g/L, respectively. Therefore, only the experiments with $Red_{(i)}$ and $blue_{(p)}$ LEDs yielded statistically significant results in terms of biomass concentration, biomass productivity, and specific growth rate ($p \leq 0.05$).

Table 1 Effects of light conditions and photoperiods on biomass production (X, g L^{-1}), biomass productivity (P, mg L^{-1} d^{-1}) and specific growth rate (μ, d^{-1}).

Experiment	LED	Photoperiod	X (g/L)	P (mg/L/day)	μ, d^{-1}
1	Blue	Partial (13:11 h)	$0,31 \pm 0,04^{c}$	$15,23 \pm 2,13^{c}$	$0,24 \pm 0,008^{c}$
2	Blue	Integral (24 h)	$0,48 \pm 0,015^{b}$	$23,70 \pm 0,79^{b}$	$0,27 \pm 0,001^{b}$
3	Red	Partial (13:11 h)	$0,42 \pm 0,02^{b}$	$20,75 \pm 1,03^{b}$	$0,27 \pm 0,002^{b}$
4	Red	Integral (24 h)	$0,82 \pm 0,06^{a}$	$40,90 \pm 3,04^{a}$	$0,30 \pm 0,003^{a}$
5	White (control)	Partial (13:11 h)	$0,39 \pm 0.03^{b}$	$19,23 \pm 1,76^{b}$	$0,26 \pm 0,004^{b}$

The provided data represents the mean ± standard deviation (n=3). Treatments that share the same letter in the same column indicate that there is no significant difference between the values ($p>0.05$)

The growth of cyanobacteria under blue light is notably slower, resulting in lower biomass production. This occurs due to the lower efficiency with which cyanobacteria utilize blue light for photosynthesis compared to other photosynthetic organisms.[10] Alternatively, red LEDs cover the absorption spectrum of phycobiliproteins (550 nm to 620 nm) within the range of 620 to 645 nm, leading to greater energy utilization and consequently higher biomass production by the cells.[11] Therefore, an increase in biomass production was also observed under red light for *Spirulina* sp. LEB 18, *Haematococcus* sp., and *H. pluvialis*.[12,13,14]
The content and yield of phycocyanin (PC) in *Synechocystis* sp. CACIAM 05 after exposure to different spectral light treatments and periodicity are summarized in Figure 1. The highest amount ($p < 0.05$) of PC, 0.49 mg mL^{-1}, was obtained using blue LED light (partial photoperiod), resulting in a yield of 0.083 mg g^{-1}. Meanwhile, the PC content under exposure to other light treatments—white (control), blue (continuous), red (partial), and red (continuous)—did not show significant differences among them, with average values of 0.15 mg mL^{-1}, 0.32 mg mL^{-1}, 0.19 mg mL^{-1}, and 0.11 mg mL^{-1}, yielding 0.025 mg g^{-1}, 0.054 mg g^{-1}, 0.032 mg g^{-1}, and 0.019 mg g^{-1}, respectively. Similarly, blue light also photo-stimulated phycocyanin accumulation in *A. platensis*, *Porphyridium purpureum*, and *P. mucicola*.[15,16,17]

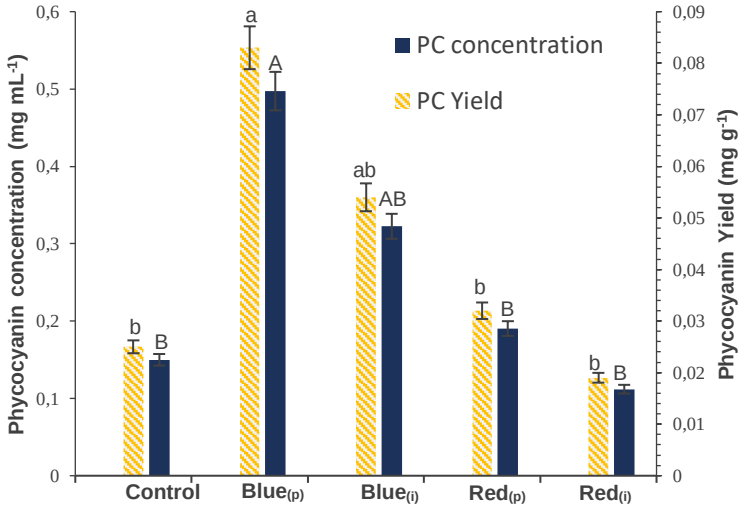

Figure 1 Effects of light conditions on phycocyanin concentration (mg mL^{-1}) and yield (mg g^{-1}). Treatments sharing the same lowercase letter indicate no significant difference between values ($p > 0.05$). Treatments sharing the same uppercase letter also indicate no significant difference ($p > 0.05$).

The comparison between full and partial photoperiod treatments with blue LED illumination showed no statistically significant difference ($p>0.05$) in phycocyanin concentration and yield (Figure 1). However, the PC concentration under partial photoperiod (13:11 h) was slightly higher than that observed under continuous photoperiod (24 h). This suggests that cyanobacterium CACIAM 05 maintains comparable growth efficiency even with a shorter light exposure time. Therefore, to achieve higher pigment concentration and improve cost-effectiveness, partial light exposure can be utilized. Partial illumination also favored PC accumulation in *Spirulina* and *P. amphigranulata* USMAC18.[18,19] Thus, partial photoperiod significantly reduces electricity consumption, as light sources operate for shorter periods, extending the lifespan of LED bulbs and reducing related costs.
Similarly, the treatment with blue LED light and partial photoperiod achieved the highest purity, corresponding to 12 (Figure 2). Meanwhile, treatments with $Red_{(p)}$, $Red_{(i)}$, $Blue_{(i)}$, and the control LEDs reached concentrations of 3.6, 2.0, 6.3, and 1.7, respectively. These experimental results align with previous studies. Lee *et al*. (2016) observed that although blue light led to slower growth, it exhibited higher phycocyanin production rates in terms of both content and purity. A purity of 0.7 is considered food-grade, 3.9 is reactive-grade, and samples above 4.0 are considered analytical-grade.[20]

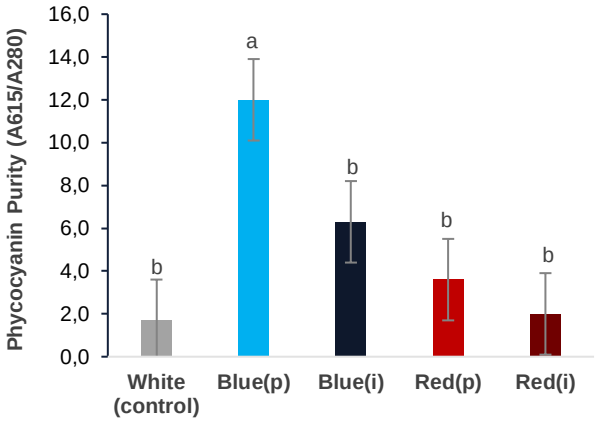

Figure 2 Effects of light-emitting diodes (LEDs) and different photoperiods on phycocyanin purification. Treatments sharing the same lowercase letter indicate no significant difference between values ($p > 0.05$). Treatments sharing the same uppercase letter also indicate no significant difference ($p > 0.05$).

Various studies indicate that blue light is most effective for phycocyanin (PC) production in cyanobacteria due to its specific wavelength. Blue light is not easily absorbed by these organisms, resulting in the production of large amounts of phycocyanin to capture light energy. Additionally, under rapid growth conditions, these microorganisms require significant nitrogen, and they can use phycocyanin as an alternative nitrogen source for biomass production. The use of blue light can slow down cyanobacterial growth, allowing for nitrogen accumulation in the form of phycocyanin.[21]

4 CONCLUSION

This study revealed that stress induced by different LEDs and photoperiods effectively stimulated biomass and phycocyanin production. The results showed that the treatment with red LED and partial photoperiod achieved the maximum biomass concentration of 0.82. Alternatively, blue LED under partial photoperiod resulted in the highest PC concentration of 0.49, yield of 0.083, and purity of 12. In comparison, other treatments exhibited lower concentrations of the target pigment. It's worth noting that various strategies are widely used to enhance phycocyanin production and reduce associated costs. Therefore, partial photoperiod can be employed to achieve higher phycocyanin concentration in *Synechocystis* sp. CACIAM 05 and improve cost-effectiveness. These results provide valuable insights for the high-purity phycocyanin production through cultivation of a cyanobacterium collected in the Amazon region.

REFERENCES

[1]LEE, SH., LEE, JE., KIM, Y., LEE, SY. Appl Biochem Biotechnol. 178. 382–395.
[2]KHAZI, M. I., DEMIREL, Z., DALAY, M. C. J. of Applied phycology. 30. 1513–1523.
[3]KWAN, P. P., BANERJEE, S., SHARIFF, M., MD. YUSOFF, F. Aquac. Res. 52. 4. 1337–1347.
[4]LIMA, S., SCHULZE, P. S. C., SCHULER, L. M., RAUTENBERGER, R., SÁNCHEZ-MORALES, D., SANTOS, T. F. J. of Biotec. 325. 15–24.
[5]FIORE, M. F., MOON, D. H., TSAI, S. M., LEE, H., TREVORS, J. T. J. Microb. M. 39 (2). 159–169.
[6]BENNETT, A., BOGORAD, L. J. Cell Biol. 58 (2). 419–35.
[7]SHAHID, A., USMAN, M., ATTA, Z., MUSHARRAF, S. G., MALIK, S. ELKAMEL. A., SHAHID, M. Bioresour. Technol. 333. 125194.
[8]JIANG, L., YU, S., CHEN, H., PEI, H. B. Bioresour. Technol. 378. 129009.
[9]SILVEIRA, S. T., BURKERT, J. F. M., COSTA, J. A. V., BURKERT, C. A. V., KALIL, S. J. Bioresour. Technol. 98. 8. 1629–1634.
[10]LUIMSTRA, V. M., SCHUURMANS, J., VERSCHOOR, A., HELLINGWERF, K., HUISMAN, J., MATTHIJS, H. Phot. Res. 138 (2). 177–189.
[11]MARKOU, G. App. Bioch. and Biotec. 172 (5). 2758–2768.
[12]PRATES, D. D. F., DUARTE, J. H., VENDRUSCOLO, R. G., WAGNER, R. BALLUS, C. A. Bioresour. Technol. 306. 123184.
[13]CHEIRSILP, B., WANTIP, K., CHAI-ISSARAPAP, N., MANEECHOTE, W., PEKKOH, J. Env. Tech. & Innov. 28. 102620.
[14]XI, T., KIM, D., ROH, S., CHOI, J., CHOI, Y. Ap. Microb. and Biotec. 100 (14). 6231-6238.
[15]JUNG, C. H. G., WALDECK, P., SYKORA, S., BRAUNE, S., PETRICK, I., KUPPER, J.-H., JUNG, F. Life. 12. 895.
[16]COWARD, T., FUENTES-GRUNEWALD, C., SILKINA, A., OATLEY- RADCLIFFE, D. L., LLEWELLYN, G. Bioresour. Technol. 221. 607–615.
[17]KHATOON, H., LEONG, L. K., RAHMAN, N. A., MIAN, S., BEGUM, H., BANERJEE, S., ENDUT, A. Bioresour. Technol. 249. 652–658.
[18]PRATES, D. F., RADMANN, E. M., DUARTE, J. H. MORAIS, M. G., COSTA, J. A. V. Bioresour. Technol. 256. 38–43.
[19]KHAN, Z., MAZNAH, W. O. W., MERICAN, M. S. M. F., CONVERY, P., NAJIMUDIN, N., ALIAS, S. A. Polar Science. 20. 3–8.
[20]WU, HL., WANG, GH., XIANG, WZ., LI, T., HE, H. Int. J. Food Prop. 19. 2349–2362.
[21]HSIEH-LO, M., CASTILLO, G., OCHOA-BECERRA, M. A., MOJICA, L. Algal Research. 42. 101600.

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