Two-stage LEDs illuminated plastic bag photobioreactor for production of astaxanthin from *Haematococcus pluvialis* and manipulated egg yolk pigment by astaxanthin

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Abstract

Astaxanthin is one of the most significant secondary metabolites with high price because of its high antioxidant activity. It can be used in commercial applications, including the nutraceutical, pharmaceutical, and aquaculture *Haematococcus pluvialis* contains the richest natural source for astaxanthin, so it has gained a lot of attention for commercial astaxanthin production. It is suggested that *H. pluvialis* can produce astaxanthin through a two-stage process that involves growing biomass under optimal conditions and then subjecting this biomass to stress conditions to cause astaxanthin accumulation. In this study, we used a two-stage LED-illuminated technique to produce high level of astaxanthin in a plastic bag photobioreactor. Plastic bag PBRs have received increased attention in recent years due to low cost and simplicity of controlling the culture. The results demonstrated that the two-stage procedure on plastic bags using shifting red-blue LED had the ability to produce high quality astaxanthin at a low production cost. The highest dry biomass and astaxanthin content were 2.53 g L^{-1} and 5.09%, respectively. The yolk color and egg-laying rate of hens improved when their diets were supplemented with *H. pluvialis* biomass. Hens fed with 0.2%, 0.5%, and 1% biomass rose by 8% (63.81%), 16% (68.57%), and 17% (69.52%), respectively, as compared to controls (59.05%).

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Keywords

Astaxanthin, *Haematococcus pluvialis,* light emitting diodes (LEDs), plastic bag PBRs two-stage process.

1 Introduction

One of the most potent antioxidants among carotenoids, astaxanthin (3,3'-dihydroxyl-,-carotene-4,4'-dione) has numerous uses in the pharmaceutical, nutraceuutical, and food industries. Although astaxanthin has already been discovered in a variety of microorganisms and marine species, including salmon red, flamingo pink, and *Phaffia rhodozyma* yeast. However, the most abundant natural source of astaxanthin, is the microalgae *Haematococcus pluvialis* [1-4]. This species is the only commercialized microalgae in astaxanthin production, as it can accumulate more than 3 – 5% dry weight of metabolite [5,6].

Due to promising health benefits, astaxanthin has a very high market value for use in human and animal nutrition. Although it is currently promoted as a food supplement, it is still mainly used in the feed for poultry and aquaculture[7,8]. With an expected 8% annual growth rate, the market value of astaxanthin is projected to increase from $ 550 million in 2017 to $ 800 million in

2022. The aquaculture and poultry feed segments continued to hold the largest market share [8]. Due to its high antioxidant activity and increasing consumer demand towards natural products, efforts have been made to boost astaxanthin production from natural sources. The freshwater green microalgae *H. pluvialis* can assemble the most from natural sources, and it is already cultivated on an industrial scale to synthesize this carotenoid for human purposes [9]. *H. pluvialis'* market share of astaxanthin is predicted to expand dramatically in the future years.

H. pluvialis has a complicated life cycle with three distinct growth stages: a motile flagellate, a nonmotile palmella, and a resting aplanospore (Figure 1). The cells are flagellate and palmella under flavorable culture conditions. The aplanospore stage occurs under unfavourable conditions, and astaxanthin is synthesized [10]. A two-stage approach that separates the growth and production stages has been widely approved for *H. pluvialis* cultivation to produce astaxanthin. To accomplish huge cell proliferation, the best conditions for growth are offered in the first stage. In the second stage, unfavorable environmental conditions are exploited to stimulate astaxanthin synthesis and accumulation [11, 12].

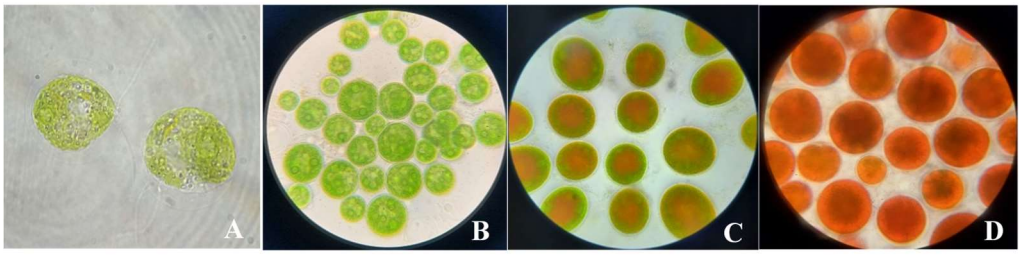

Figure 1 Microscopic images of H. pluvialis during growth and under stress (100X): A: vegetative cell, B: palmella cell, C: beginning of stress, D: aplanospore cell.

Because the cells of *H. pluvialis* are extremely sensitive to environmental conditions, the large-scale production of astaxanthin is still a challenge in Vietnam today. One of the elements that affects both production cost and efficiency is the cultivation system's design. The closed photobioreactor systems will aid in maintaining optimal culture conditions, but they require high equipment costs and complications in operation and cleaning. To solve these drawbacks, the hanging plastic bag photobioreactor (PBR) is regarded as a cost-effective option. This model is used in this study for two-stage LED illumination to boost astaxanthin synthesis. The plastic bag PBR will reduce equipment expenses, maintenance and operating costs, as well as contamination, whereas the two-stage LEDs will stimulate the increase of biomass and astaxanthin. Due to its tiny chip size, extended duration, and ability to narrow illumination to a precise wavelength with minimal power consumption, LEDs are commonly utilized in photobioreactors [13]. Many studies have shown the physiology of *H. pluvialis* was affected by the monochromatic spectrum of LED lights, which red LED seemed to promote biomass growth and the blue LED increased astaxanthin synthesis [14-16]. This research will develop a low-cost and suitable model for large scale astaxanthin-rich biomass production in Vietnam. This process's biomass was also examined by feeding it to hens and analyzing astaxanthin's ability to improve egg yolk pigmentation.

2 Materials and methods

2.1 Strain and culture condition

H. pluvialis (BP1885062*)* was supplied by Professor Hong, Jeonbuk National University, Korea. Microalgae cells were grown in OHM (Optimal Haematococcus Medium) pH 7, which contained 0.41 g KNO_3, 0.03 g Na_2HPO_4, 0.246 g $MgSO_4.7H_2O$, 0.11 g $CaCl_2.2H_2O$, 2.62 mg Fe(III)-citrate H_2O, 0.011 mg $CoCl_2.6H_2O$, 0.012 mg $CuSO_4.5H_2O$, 0.075 mg Cr_2O_3, 0.98 mg $MnCl_2.4H_2O$, 0.12 mg $Na_2MoO_4.2H_2O$, 0.005 mg SeO_2, 25 µg biotin, 17.5 µg thiamine, and 15 µg B12 per 1 L of deionized distilled water. The inoculum was cultivated in 500 mL Erlenmeyer flasks with 200 mL of OHM medium at 20 °C, continuous illumination (45 – 50 µmol m^{-2} s^{-1}) for 5 days, and sterilized-filtered air flow rate of 100 mL min^{-1}. The inoculums were then transferred to plastic bags (30 × 70 cm) containing 5 L fresh OHM medium, which had an initial cell density of 1×10^5 cells mL^{-1}. For growth of biomass, these bags were cultivated under a 12 h light:12 h dark lighting

cycle at 50 µmol m^{-2} s^{-1} red LEDs at 20 ºC. They were continually switched into blue LEDs at 150 µmol $m^{-2}s^{-1}$, 28 ºC to enhance astaxanthin accumulation.

2.2 Growth measurements

Once every 2 days, the cell growth rate and morphology were examined under an optical microscope. Direct counting with a hemocytometer under a microscope was used to evaluate cell density. The dry biomass was determined by centrifuging the culture suspension at 5000 rpm for 10 minutes and freeze drying at −40ºC at 0.1 mBar

2.3 Extraction and deesterification of astaxanthin

According to Sarada et al. (2006) method [17], *H. pluvialis* dried cysts (10 mg) were treated with 4 N HCl (1 mL) at 70ºC for 2 minutes to extract astaxanthin. After centrifuging the treated pellet at 13,000 rpm for 5 minutes, it was washed twice with distilled water and resuspended in 1 mL of acetone 99%. The mixture was ultrasonically extracted in an ice water bath for 20 minutes, then centrifuged at 13,000 rpm for 10 minutes and extracted again until colorless.

Enzymolysis was used to deesterify astaxanthin using 500 µL of total carotenoid extract (0.1 mg mL^{-1}) combined with 500 µL of cholesterol esterase (4 units mL^{-1} in Tris-HCl pH 7.0) as described by Jacobs et al. 1982 [18]. For 0 minutes, the mixture was placed in a thermostatically controlled water bath at 37ºC in darkness, with gentle mixing every 10 minutes. Petroleum ether was used to remove the pigment from the reaction mixture. The mixed petroleum ether extract was dried at room temperature by flushing with nitrogen gas and redissolved in 500 µL of acetone (99%) for HPLC analysis.

2.4 HPLC quantification of astaxanthin

The extract was evaluated using a C18 column (250 mm × 4.5 mm × 5 µm) by High Performance Liquid Chromatography (HPLC) method (Agilent 1100). The mobile phase were acetone (A) and methanol:H_2O (9:1 v:v) (B) at a flow rate of 0.8 mL min^{-1}, volume sample of 20 µL and column temperature of 30 ºC. The following gradient program was used: B was run at 80 to 20% for 25 minutes, 20% for 10 minutes, and 20 to 80% for 5 minutes. At 475 nm, the detection wavelength was measured. As a control, all-trans astaxanthin (Sigma) was employed. The astaxanthin content was calculated using the standard calibration curve (Standard was diluted in acetone 99.9% from 2 ppm to 10 ppm concentration).

2.5 Effect of astaxanthin on egg yolk pigment

H. pluvialis dried biomass was fed directly to hens. *Gallus gallus domesticus* Brisso layers (20 weeks old, n = 105) were randomly assigned to one of five treatment in a single factorial design. Each treatment consisted of three replicates of seven hens. For 5 days, diets were supplemented with five levels (0, 0.1, 0.2, 0.5 and 1%) of *H. pluvialis* dried cyst. The laying hens were given free access to water and each diet during the experiment. Daily egg output was gathered and yolk pigment was examined.

2.6 Statistical analysis: All experiments were simultaneously repeated three times. For all statistical analysis, the SigmaPlot 8.0 tool was used. Results were represent as mean ± standard deviation (S.D.) and $P < 0.05$ is considered the level of significance.

3 Results and discussion

3.1 The morphylogy and growth of *H. pluvialis* at LEDs light shifting

As *H. pluvialis* is extremely reliant on light composition, the use of LED is a great method for increasing astaxanthin pdroduction. Prior research suggested that blue LED at 30ºC boosted the cumulative rate of astaxanthin whereas red LED at 20ºC was the best for cell production [11, 19]. To increase astaxathin production by *H. pluvialis* in a 5 L plastic bag, we adopted a light wavelength shifting strategy (Red - Blue LED light shifting) in the current investigation (Figure 2A). According to Figure 2B, cell density increased for the first 2 days, then transitioned to the growth phase after the 3rd day, and eventually to the stationary phase after 10th day, with no substantial rise in cell number. The maximum cell density was around $8.3 \times 10^5 \pm 0.11$ cells mL^{-1} (an increase of 8.3 times the initial density). Compared to white LED and blue LED, the red LED was found to be better suited for cell proliferation [20]. As red light causes more excitation in chlorophyll electrons, the efficacy of these chlorophyll pigments increases significantly. These electrons produce water hydrolysis, which results in ATP synthesis. As a result, ATP is utilized to synthesize carbohydrates, which aid in the growth of *Haematococcus sp.* Some nuclear genes that produce chloroplastic proteins involved in photosynthesis are expressed in a controlled manner when the phytochrome is activated by red LED light [21, 22].

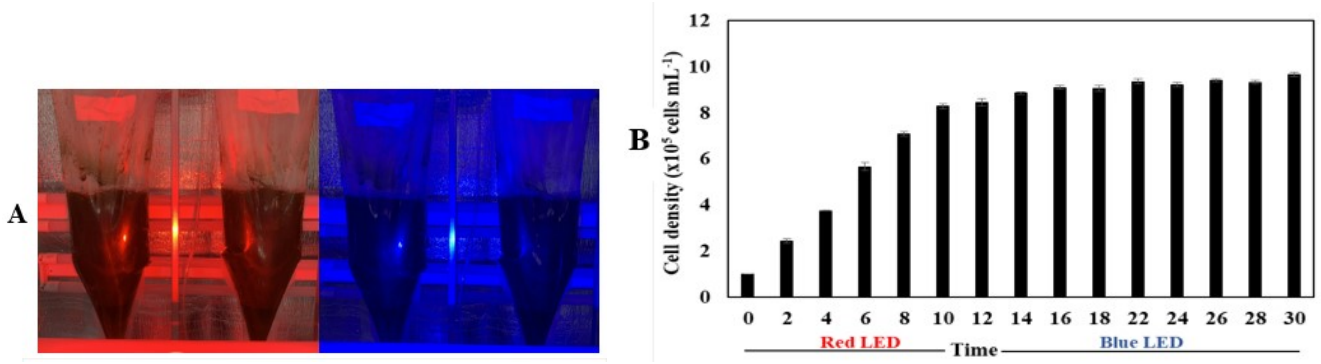

Figure 2 (A) The experimental design of two-stage process on plastic bag PBRs and LEDs. (B) The cell density of *H. pluvialis* at LEDs light shifting.

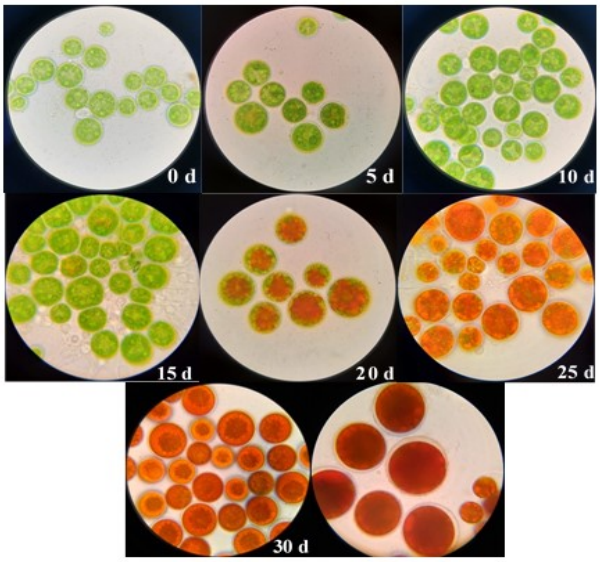

Figure 3 The daily morphylogical changes of *H. pluvialis* cells at LEDs light shifting.

Under the light microscope, changes in cell morphology were noted, and the results were given in Figure 3. On the first 5 days of cultivation, the cells were a mixture of motile and nonmotile cells, but by the end of 10 days, almost all of the cells were green nonmotile cells. From the 10th to the 15th day, the cells grew larger and there was minimal orange-red pigmentation in the mid-region, thus the plastic bags turned blue LED. Red pigmentation gradually occupied the entire cell volume after 20 days of induction by blue LED. The redness of the cells deepened as the stress continued, resulting in the formation of a big red cyst at the end of 30 days.

3.2 The biomass and astaxanthin content at LEDs light shifting

The astaxanthin content (column) in the blue LED stage increased significantly from day 22 to day 30; whereas the dried biomass (Figure 4) remained unchanged. Dry biomass and astaxanthin content were 2.53 0.13 g L^{-1} and 5.09 ± 0.11%, respectively, after 30 days of cultivation. Blue light has been demonstrated to promote the accumulation of astaxanthin; when exposed to intense of blue light for an extended period of time, the blue light receptor gene phot was upregulated, leading to coordinated upregulation of astaxanthin biosynthetic pathway genes, culminating in high astaxanthin content [23].

According to previous studies, LED light produced at short wavelengths (380 – 470 nm) could cause *H. pluvialis* to undergo morphological change and boost astaxanthin accumulation by up to 5-6% per dry cell [20]. Red and blue photons could increase the content of astaxanthin from *H. pluvialis*. In order to increase astaxanthin accumulation and improve the light regime inside a photobioreactor, LEDs could be an ideal light source for internal illumination. The maximal astaxanthin levels under blue or red light supplements were likewise about 20% higher than those of control under white light [14]. Blue light had been shown a higher energy level than red light, only a few photons of blue light were needed to obtain the same energy intensity utilizing red light [21]. Wong et al. 2016 [12] studied the effect of strong light intensity of 170 mol $m^{-2}s^{-1}$ on astaxanthin production in *H. pluvialis* when exposed to varied wavelengths (white plasma, blue and red LEDs light) and photoperiods (24:0 and 12:12 light:dark (L:D) cycle). The astaxanthin levels under white, red, and blue LEDs were 3.5%, 2.6%, and 4.9%, respectively, with a photoperiod (L:D cycle) of 12:12. While astaxanthin content increased (4.7%, 2.8%, and 5.5%, respectively) under a 24:0 L:D cycle.

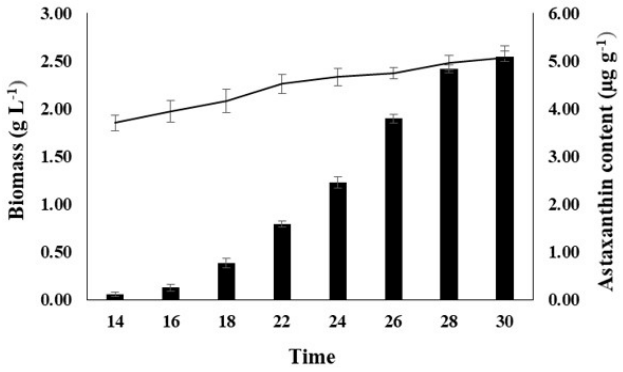

Figure 4 The biomass and astaxanthin content at blue LED stage.

The chromatograms of extracted samples before and after deesterification were indicated in Figure 5. Following cholesterol esterase's deesterification of many astaxanthin esters, only one prominent peak of free astaxanthin was seen (Figure 5B). In *H. pluvialis*, astaxanthin occurred mostly as mono- and diesters of different fatty acids and contributed for up to 95% of total secondary carotenoids [22]. These pigments were found outside of the chloroplast in lipid globules. Furthermore, astaxanthin accumulation was linked to fatty acid production. Oleic acid and triacylglycerol (TAG) interacted during the process to form a significant portion of cytoplasmic lipid droplets [23]. The intracellular depot for astaxanthin accumulation was formed by this class of neutral lipids. For the esterification of fatty acids, before being deposited inside the hydrophobic environment of lipid droplets, astaxanthin's polar hydroxyl groups were esterified using fatty acids. The culture stress caused microalgae cells to accumulate secondary carotenoid (astaxanthin) in their cytoplasm. It clarified how light and nutritional stress induced lipid synthesis and accumulation in *H. pluvialis* [24].

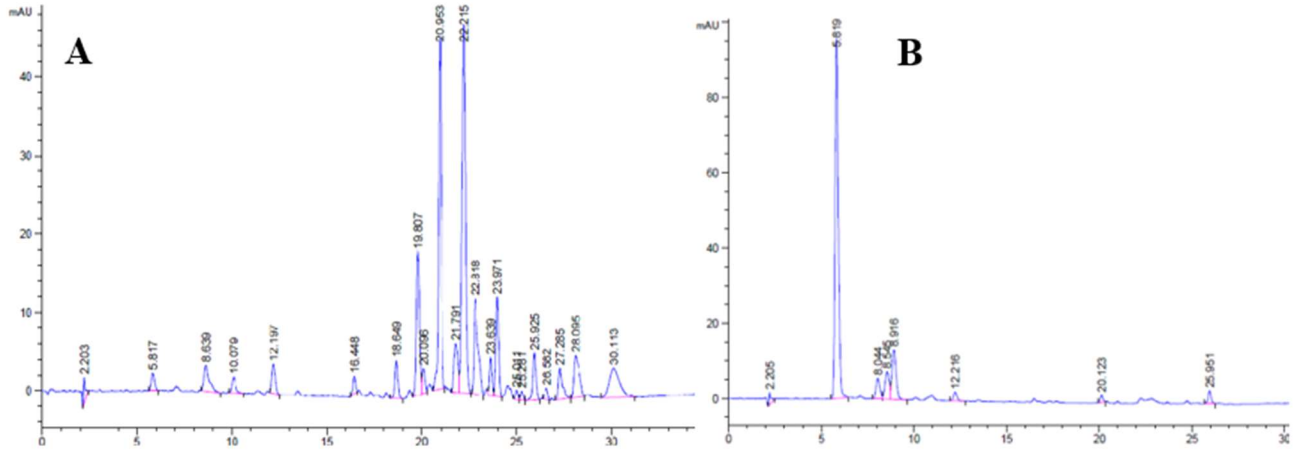

Figure 5 The HPLC chromatograms of astaxanthin content after 30 days cultivation before and after deesterification.

3.3 Effect of astaxanthin on egg yolk pigment

The dried H. pluvialis biomass was fed to hens to investigate the effect of astaxanthin on improving egg yolk colour. Eggs were gathered, and the color of the yolks pigment was examined. The results in Figure 6 showed that the yolk color was improved by adding H. pluvialis biomass to the combination, and the change in yolk color depended on the amount of astaxanthin added to the diet. After 15 days of feeding, the color of the yolk was dramatically elevated in diets supplemented with 0.5 and 1% astaxanthin-rich biomass. In comparison to hens fed without biomass, the egg-laying rate of hens given algae-based biomass was also higher. The egg-laying rate of hens fed 0.2%, 0.5%, and 1% increased by 8% (63.81%), 16% (68.57%), and 17% (69.52%), respectively, as compared to control (59.05%).

Consumers' preferences for yolk color vary greatly around the world. Deeper colors, on the other hand, command large premiums in most marketplaces. Instead of using artificial coloring agents, the bakery and food processing industries prefer deeper colored yolks. The color of the yolk is an essential indicator of egg quality. To compensate for the lack of a natural dietary source of the color, astaxanthin is routinely added to meals [25]. These farm animals need astaxanthin for pigmentation and for healthy growth and survival [26]. According to studies, astaxanthin made hens' skin, feet, and beaks more yellow in color. Carotenoids were moved from the intestinal mucosa to

the blood via lymphatic arteries, then from the blood to the liver, and finally from the liver to surrounding tissues by very low density lipoprotein (VLDL) [27].

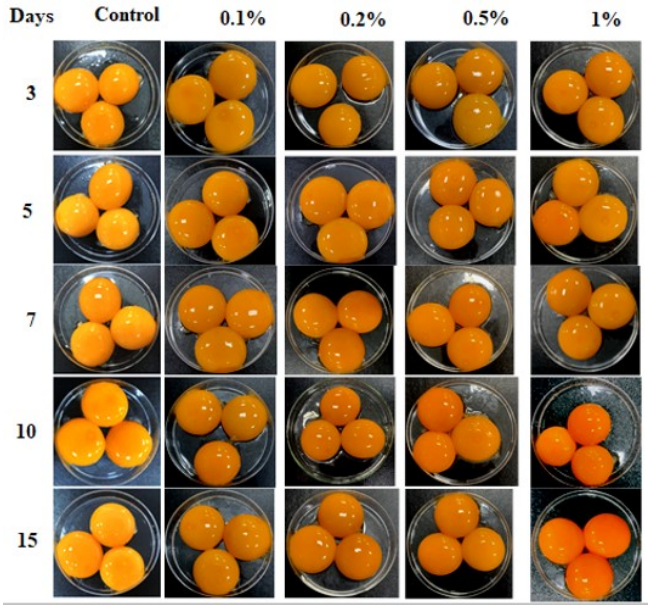

Figure 6 Effect of astaxanthin on yolk color.

The algal meal diet boosted fertility, accelerated weight gain, greatly raised breast muscle weights, and improved feed utilization in broilers [28]. There had also been reports of astaxanthin, a natural pigment, having similar effects on the yolk color in the layer [29, 30]. However, the influence of astaxanthin on yolk color depends on a variety of parameters, including genetics, astaxanthin concentration in diets, the timing of feedings, experimental circumstances, and so on, resulting in inconsistent results among research. Amount of astaxanthin added to the diet not only determines the color of yolk but also the price of product so we choose the range of biomass levels from 0.1 to 1%.

4 Conclusion

This study shown that *H. pluvialis* grown in plastic bags has a tremendous potential for producing astaxanthin at high levels. After 30 days of cultivation under shifting red-blue LED illumination, the maximum dry biomass and astaxanthin content were 2.53 ± 0.13 g L^{-1} and 5.09 ± 0.11%, respectively. Additionally, our preliminary studies also demonstrated that astaxanthin-rich biomass could boost egg-laying rate and yolk color. The use of plastic bags with LED lighting is a promising low-cost method for the manufacture of high-quality astaxanthin, and *H. pluvialis* biomass has the potential to be used in poultry feed to enhance yolk pigment.

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References

1. Hata, N., Ogbonna, J. C., Hasegawa, Y.,Taroda, H. & Tanaka, H. (2001). Production of astaxanthin by *Haematococcus pluvialis* in a sequential heterotrophic-photoautotrophicculture. *Journal of Applied Phycology*, 13, 395-402. DOI: 10.1023/A:1011921329568
2. Ambati, R. R., Phang, S. M., Ravi, S., Aswathanarayana, R.G. (2014). Astaxanthin: sources, extraction, stability, biological activities and its commercial applications-a review. *Marine Drugs*, 12, 128-152. DOI: 10.3390/md12010128
3. Ganzaya, P., Hitomi, T., Toru, O., Masatoshi, K., Motoni, K. & Shinobu, F. (2014). Effect of dietary astaxanthin rich yeast, *Phaffia rhodozyma*, on meat quality of broiler chickens. *Animal Science Journal*, 85, 895-903. DOI: 10.1111/asj.12221
4. Lee, J. Y., Hong, M., Chang, W. S., Sim, S. J. (2015). Enhanced carbon dioxide fixation of *Haematococcus pluvialis* using sequential operating system in tubular photobioreactors. *Process Biochemistry*, 50, 1091-1096.
5. Olaizola, M. (2000). Commercial production of astaxanthin from *Haematococcus pluvialis* using 25,000-liter outdoor photobioreactors. *Journal of Applied Phycology*, 12, 499-506. DOI: 10.1023/A:1008159127672

6. Hussein, G., Sankawa, U., Goto, H., Matsumoto, K., Watanabe, H. (2006). Astaxanthin, a carotenoid with potential in human health and nutrition. *Journal of Natural Products*, 69, 443-449. DOI: 10.1021/np050354+
7. Shah, M. M. R., Liang, Y., Cheng, J. J. & Daroch, M. (2016). Astaxanthin-producing green microalga *Haematococcus pluvialis*: from single cell to high value. *Frontiers Plant Science*, 7, 531. DOI:10.3389/fpls.2016.00531.
8. Li, X., Wang, X., Duan, C., Yi, S., Gao, Z., Xiao, C., Wang, G., Li, J. (2020). Biotechnological production of astaxanthin from the microalga *Haematococcus pluvialis*. *Biotechnology Advances*, 43, 10762. DOI:org/10.1016/j.biotechadv.2020.107602.
9. Villaro, S., Ciardi, M., Espana, A. M., Zurano, A. S, Fernandez, G. A & Lafarga, T. (2021). Microalgae derived astaxanthin: Research and consumer trends and industrial use as food. *Food,* 10, 2303. DOI:org/10.3390/foods10102303.
10. Niizawa, I., Espinaco, B. Y., Leonardi, J. R., Heinrich, J. M., Sihufe, G. A. 2018. Enhancement of astaxanthin production from *Haematococcus pluvialis* under autotrophic growth conditions by a sequential stress strategy. *Preparative Biochemistry & Biotechnology*, 48(6), 528-534. DOI:10.1080/10826068.2018.1466159.
11. Xi, T., Kim, D. G., Roh, S. W., Choi, J. S., Choi, Y. E. (2016). Enhancement of astaxanthin production using *Haematococcus pluvialis* with novel LED wavelength shift strategy. *Applied Microbiology and Biotechnology,* 100, 6231-6238. DOI: 10.1007/s00253-016-7301-6
12. Wong, Y. K., Ho, Y. H., Ho, K. C., Lai, Y. T., Tsang, P. M., Chow, K. P., Yau, Y. H., Choi, M. C., Ho, R. S. C. (2016). Effects of light intensity, illumination cycles on microalgae *Haematococcus pluvialis* for production of astaxanthin. *Journal of Aquaculture & Marine Biology*, 2(2), 1-6. DOI: 10.15436/2381-0750.16.1083
13. Zhao, Y. J., Hui, Z., Chao, X., Nie, E., Li, H. J., He, J., Zheng, Z. (2011). Efficiency of two-stage combinations of subsurface vertical down-flow and up-flow constructed wetland systems for treating variation in influent C/N ratios of domestic wastewater. *Journal of Ecological Engineering*, 37, 1546-1554. DOI: 10.1016/j.ecoleng.2011.06.005
14. Kim, Z. H., Lee, H. S., & Lee C. G. (2009). Red and blue photons can enhance the production of astaxanthin from *Haematococcus pluvialis*. *Algae,* 24(2), 121-127. DOI: 10.4490/ALGAE.2009.24.2.121
15. Ma, R., Thomas-Hall, S. R., Chua, E. T., Alsenani, F., Lu, Y., Schenk, P. M. (2018). Gene expression profiling of astaxanthin and fatty acid pathway in *Haematococcus pluvialis* in response to diferent LED lighting conditions. *Bioresource Technology*, 250, 591-602. DOI: 10.1016/j.biortech.2017.11.094
16. Lee, C., Ahn, J. W., Kim, J. Y., Choi, Y. E. (2018). Comparative transciptome analysis of *Haematococcus pluvialis* on astaxanthin biosynthesis in response to irradiation with red or blue LED wavelength. *World Journal of Microbiology and Biotechnology*, 34(7), 96. DOI:10.1007/s11274-018-2459-y. DOI: 10.1007/s11274-018-2459-y
17. Sarada, R.,Vidhyavathi, R., Usha, D., & Ravishankar A. G. (2006). An efficient method for extraction of astaxanthin from green alga *Haematococcus pluvialis*. *Journal Agriculture Food Chemistry*, 54(20), 7585-7588. DOI: 10.1021/jf060737t
18. Jacobs, P. B., LeBoeuf, R. D., McCommas, S. A. & Tauber, J. D. (1982). The cleavega of carotenoid esters by cholesterol esterase. *Comparative Biochemistry and Physiology*, 72B, 157-160. DOI: 10.1016/0305-0491(82)90027-X
19. Pereira, S., & Otero, A. (2020). *Haematococcus pluvialis* bioprocess optimization: Effect of light quality, temperature and irradiance on growth, pigment content and photosynthetic respone. *Algal Research*, 51:102027. DOI:org/10.1016/j.algal.2020.102027.
20. Katsuda, T., Lababpour, A., Shimahara, K., Katoh, S. (2004). Astaxanthin production by *Haematococcus pluvialis* under illumination with LEDs. *Enzyme and Microbial Technology*, 35(1), 81-86. DOI: 10.1016/j.enzmictec.2004.03.016
21. Juneja, A., Ceballos, R. M., & Murthy, G. S. (2013). Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: a review. *Energies,* 6(9), 4607-4638. DOI: 10.3390/en6094607
22. Lee, Y. K., Zhang, D. H. (1999). Production of astaxanthin by Haematococcus. In: Cohen Z, ed. *Chemicals from Microalgae*, 175-195.
23. Zhekisheva, M., Boussiba, S., Khozin-Goldberg, I., et al. (2002). Accumulation of oleic acid in *Haematococcus pluvialis* (Chlorophyceae) under nitrogen starvation or high light is correlated with that of astaxanthin esters. *Journal of Phycology*, 38(2), 325-331. DOI: 10.1046/j.1529-8817.2002.01107.x
24. Zhekisheva, M., Zarka, A., Khozin-Goldberg, I., et al. (2005). Inhibition of astaxanthin synthesis under high irradiance does not abolishtriacylglycerol accumulation in the green alga *Haematococcus pluviali* (Chlorophyceae). *Journal of Phycology*, 41, 819-826. DOI: 10.1111/j.0022-3646.2005.05015.x
25. Torrissen, O. J., Hardy, R. W., & Shearer, K. D. (1989). Pigmentation of salmonids -carotenoid deposition and metabolism. CRC Crit. Rev. *Aquaculture Science*, 1, 209-225.

26. Yang, Y. X., Kim, Y. J., Jin, Z., Lohakare, J. D., Kim, C. H., Ohh, S. H., Lee, S. H., Choi, J. Y., & Chae, B. J. (2006). Effects of dietary supplementation of astaxanthin on production performance, egg quality in layers and meat quality in finishing pigs. Asian-Australas. *Journal of Animal Science*, 19(7), 1019-1025. DOI: 10.5713/ajas.2006.1019
27. Tyssandier, V., Choubert, G., Grolier, P., & Borel, P. (2002). Carotenoids, mostly the xanthophylls, exchange between plasma lipoprotein. *International Journal for Vitamin and Nutrien Research*, 72(5), 300-308. DOI: 10.1024/0300-9831.72.5.300
28. Inborr, J. (1998). Haematococcus, the Poultry Pigmentor. *Feed Mix*, 6, 31-34.
29. Rosemond, A. D., Mulholland, P. J., Brawley, S. (2000). Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. *Canadian Journal of Fisheries and Aquatic Sciences*, 57(1), 66-75. DOI: 10.1139/cjfas-57-1-66
30. Elwinger, K., Lignell, A., & Wilhelmson, M. (1997). Astaxanthin rich algal meal (*Haematococcus pluvialis*) as carotenoid source in feed for laying hens. *Proceedings of the VII European Symposium on the Quality of Eggs and Egg Products. Poznan, Poland*, 52-59.

Sản xuất astaxanthin từ *Haematococcus pluvialis* trên mô hình túi treo bằng đèn LEDs hai giai đoạn và ứng dụng astaxanthin trong việc cải thiện sắc tố lòng đỏ trứng

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Tóm tắt Astaxanthin được biết đến là một trong những chất chuyển hóa thứ cấp có giá trị cao nhờ hoạt tính chống oxy hóa mạnh. Astaxanthin được ứng dụng trong nhiều lĩnh vực như thực phẩm chức năng, dược phẩm, nuôi trồng thủy sản và chất tạo màu trong thực phẩm. *Haematococcus pluvialis* được biết đến là nguồn cung cấp astaxanthin tự nhiên dồi dào nhất nên đã nhận được sự quan tâm đáng kể trong sản xuất astaxanthin thương mại. Các nghiên cứu trước đây đã đề xuất rằng việc sản xuất astaxanthin bởi *H. pluvialis* có thể đạt được trong quy trình nuôi cấy hai giai đoạn, tạo ra sinh khối trong điều kiện tăng trưởng tối ưu và cho sinh khối này tiếp xúc với các điều kiện stress để tạo ra sự tích tụ astaxanthin. Mặc dù phương pháp này có vẻ khá đơn giản nhưng việc sản xuất ở quy mô lớn với chi phí thấp vẫn là một thách thức. Nghiên cứu này tiến hành sản xuất astaxanthin trên mô hình túi treo kết hợp chiếu sáng đèn LED hai giai đoạn. Việc sử dụng túi treo sẽ giúp tiết kiệm chi phí sản xuất và dễ kiểm soát các điều kiện nuôi cấy. Kết quả cho thấy, quy trình nuôi cấy hai giai đoạn trên túi treo bằng đèn LED hai giai đoạn đỏ - xanh có tiềm năng sản xuất astaxanthin hàm lượng cao với chi phí thấp. Hàm lượng sinh khối khô và astaxanthin tối đa lần lượt là 2,53 g/L và 5,09%. Khi khẩu phần ăn của gà mái được bổ sung sinh khối *H. pluvialis*, màu sắc lòng đỏ và tỉ lệ đẻ trứng được cải thiện. Cụ thể, gà mái được cho ăn sinh khối 0,2%, 0,5% và 1% tăng lần lượt 8% (63,81%), 16% (68,57%) và 17% (69,52%); so với 59,05% của nghiệm thức đối chứng.

Từ khóa Astaxanthin**,** *Haematococcus pluvialis*, LEDs, túi treo, nuôi cấy hai giai đoạn.