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Impact of light and dark (L/D) period on the biosynthesis of astaxanthin in green alga *Haematococcus pluvialis*

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ABSTRACT

The aim of the present study was to investigate the impact of light and dark (L/D) period on biosynthesis of astaxanthin in green alga *Haematococcus pluvialis*. The astaxanthin was extracted from *H. pluvialis* with dimethyl sulfoxide, and the astaxanthin content was determined by the ultraviolet spectrophotometer. *H. pluvialis* was incubated under 10:14 h (L/D) period exposed to sunlight at 32 ± 2°C temperature, the astaxanthin biosynthesis was compared with the culture which was incubated in controlled air-conditioned culture room of 16:8 h (L/D) period at 25 ± 2°C. Highest astaxanthin content was found as 0.045 µg/mL in *H. pluvialis* culture which was exposed to sunlight under 10:14 h (L/D) on the 9th day of study. Under 16:8 h (L/D) period at 25 ± 2°C, the highest astaxanthin content was found as 0.04 µg/mL on the 17th day of study. From our present investigation, it is apparent that the 10:14 h (L/D) period is more effective in promoting astaxanthin content of green alga *H. pluvialis*.

1. INTRODUCTION

Photosynthetic cells are important for the production of organic matters. Microalgae have umpteen sources of important pharmaceuticals pigments and biochemicals [1]. Astaxanthin (3, 3'-dihydroxy-β, β'-carotene-4, 4' dione) a red carotenoid pigment. *Haematococcus pluvialis* is considered as the best natural source of astaxanthin. The antioxidant properties of astaxanthin are 500 and 38 times better than β-carotene and Vitamin E, respectively. This makes it capable of protecting against inflammation, ultraviolet radiation photooxidation, aging and age-related macular degeneration, cancer, and used in cosmetic, food, and feed industries, in addition to maintaining normal liver and heart functions [2]. However, cultivation of *H. pluvialis* is very difficult on a large scale due to its slow growth and risk of contamination in open cultures. The life cycle of unicellular green microalga *H. pluvialis* has two stages depending on its environmental conditions, green motile and red non-motile form. Under favorable conditions, the cells are green capable to swim with the help of two flagella. Under unfavorable conditions, the green vegetative cells cease to be motile and enter a resting stage. The resting stage is marked by red color due to the accumulation of astaxanthin [3-5]. Nutrient limitation or supplement [6], high light intensity [7,8], cell concentration, light path, mixing rate, and the geometry of the cultivation vessel [7] are the factors which influence on the accumulation of astaxanthin in *H. pluvialis*. The effect of light is undoubtedly the most important factor in the astaxanthin accumulation [9]. In the present investigation, the efforts were made to study the impact of L/D period on astaxanthin biosynthesis in green alga *H. pluvialis*.

2. MATERIALS AND METHODS

2.1. Procurement and Maintenance of *H. pluvialis* Culture

The *H. pluvialis* used in the present investigation was procured from Culture Collection of Algae at the University of Texas, Austin, USA.

The culture of *H. pluvialis* was maintained in both liquid and solid Bold's basal medium (BBM) [10]. The axenic cultures were incubated in controlled air-conditioned culture room maintained at 25 ± 2°C under 16:8 h (L/D) of light intensity of 35 µmol/m/s.

2.2. Design of Experiment for the Culture of *H. pluvialis*

The culture was incubated under sunlight for 10:14 h (L/D), outside the controlled air-conditioned culture room. The intensity of light was measured at the surface of flask with lux meter. The average light and temperature were found to be 50 µmol/m/s and 30 ± 2°C, respectively. Another set of flasks of *H. pluvialis* culture were incubated in controlled air-conditioned culture room, under the light-dark period of 16:8 h (L/D) at 25 ± 2°C. For the preparation of the inoculum, the

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cells from the stock culture were centrifuged at 2800× *g* for 5 min, the supernatant was discarded and the pellet was washed with the sterilized double distilled water thrice. The pellet was homogenized in 1 ml BBM and transferred aseptically in a 250 ml conical flask containing 100 ml of fresh BBM and incubated under continuous illumination 35 µmol/m/s, at 25 ± 2°C for 4 days. A 4-day-old culture was used as an inoculum for the experiment. The experiment was performed in 250 ml conical flasks. 4-day-old culture approximately 1×10^6 cells/mL was inoculated into 100 ml sterilized fresh medium in 250 ml flasks and incubated separately in the controlled air-conditioned culture room, under the 16:8 h (L/D) at 25 ± 2°C and under the sunlight of 10:14 h (L/D) period. Cultures were shaken thrice a day with rotary flask shaker.

2.3. Extraction of Astaxanthin

The harvested biomass of *H. pluvialis* was first treated with a solution of 5% KOH in 30% methanol to destroy the *Chl.* The supernatant was discarded and remaining pellet was treated with dimethyl sulfoxide for the extraction of astaxanthin [9]. The absorbance of the combined extracts was determined at 492 nm, and the amount of astaxanthin was calculated [10].

3. RESULTS AND DISCUSSION

Under 16:8 h (L/D) period at 25 ± 2°C, the highest astaxanthin biosynthesis was found 0.04 µg/mL on 17th day of study while as the highest astaxanthin biosynthesis under 10:14 h (L/D) period was reported as 0.045 µg/mL on 10th day of study as seen in Figure 1.

H. pluvialis is one of the best sources of astaxanthin (3, 3'dihydroxy-β, β'-carotene-4, 4'-dione), a ketocarotenoid pigment. Light, temperature, pH, turbidity, nutrients, and aeration, cell concentration, light path, mixing rate, and the geometry of the cultivation vessel [7] are the factors responsible for the growth of photosynthetic organisms. Among them, the light is the most crucial factor for the growth and the accumulation of pigments. The impact of light is one of the most important factors of the astaxanthin accumulation in *H. pluvialis*. A suitable light source with adequate light intensity is required to accumulate a high level of astaxanthin. The quality of light, such as wavelength and/or emission spectra of light, also affects the performance of algae cultivations [3] as well as astaxanthin production. In the present study, L/D period was investigated. *H. pluvialis* culture was incubated under 16:8 h (L/D) and sunlight of 10:14 h (L/D). Under 16:8 h (L/D) period at 25 ± 2°C, the highest astaxanthin biosynthesis was found 0.04 µg/mL on 17th day of study while as the highest astaxanthin biosynthesis under 10:14 h (L/D) period was reported as 0.045 µg/mL on 10th day of study as seen in Figure 1. Numerous literature on impact of light quantity and light quality were available, but light and dark period data are very limited, particularly in *H. pluvialis*. From our present investigation, it is apparent that the 10:14 h (L/D) period is more effective in promoting biosynthesis of astaxanthin content in green alga *H. pluvialis*. The role of secondary carotenoids such as carotene and astaxanthin is to protect algae against photooxidative damage under high irradiances [11,12]. This could be a reason for the earlier formation of astaxanthin in *H. pluvialis*.

4. CONCLUSION

From our present investigation, it is apparent that the 10:14 h (L/D) period is more effective in promoting the biosynthesis of astaxanthin content in green alga *H. pluvialis*. Astaxanthin from *Haematococcus* will expand not only the consumer space but also medical institution worldwide; therefore, optimal biosynthesis method of astaxanthin is important for the human welfare.

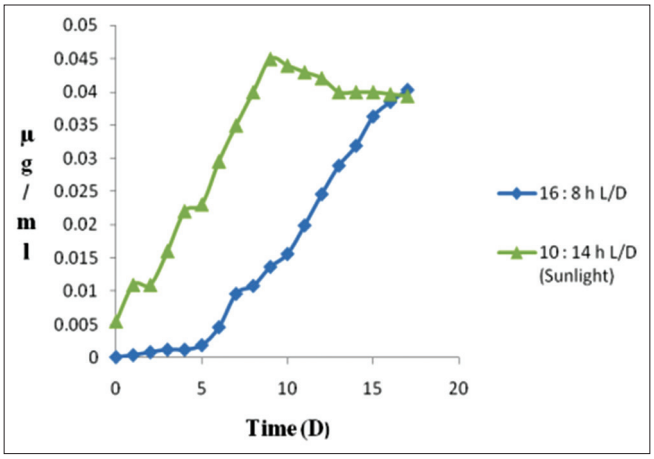

Figure 1: Impact of L/D period on the biosynthesis of astaxanthin in green alga *Haematococcus pluvialis*

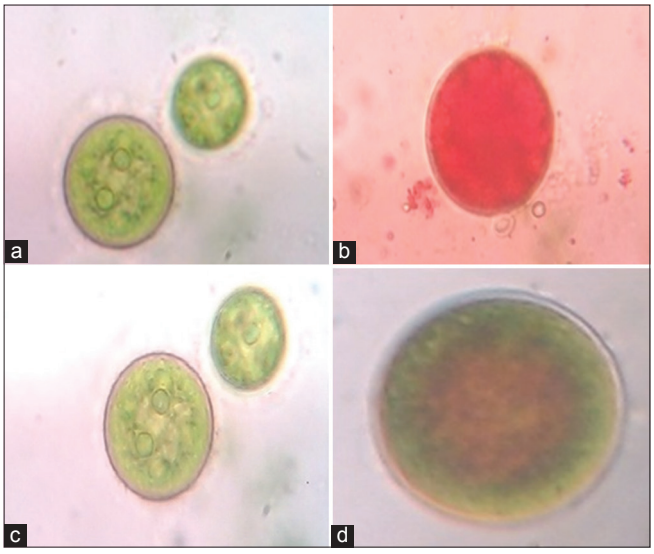

Plate 1: Microscopic view (×100) of *Haematococcus pluvialis*. A and B: 7th day and 16th day of study in 14:10 h (L/D) period. C and D; 7th day and 16th day of study in 16:8 h (L/D) period, respectively

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