Influence of light emitting diodes on the growth and astaxanthin production of *Haematococcuspluvialis*

Hai-Linh Tran*.,Kyu-Han Lee& Chang-Hee Hong

LED Agri-bio Fusion Technology Research Center, Chonbuk National University, Iksan Campus, South Korea

E-mail:linhth@jbnu.ac.kr

(Received on Date: **17th March 2015** Date of Acceptance :**20th May 2015**)

ABSTRACT

Microalgae have long been considered promising microorganisms, due to their potential as a source of valuable pharmaceuticals, pigments, carbohydrates, biofuels, and other fine chemicals. High-density microalgalphotobioreactors have been considered for exploiting the biotechnological potential of microalgae. *Haematococcuspluvialis*is considered the richest natural source of astaxanthin for pharmaceutical, nutraceutical, and cosmetic applications. One of key challenges was to provide light sources that are more efficient, to deliver light more efficiently to the microalgal culture. Recent developments in highly efficient light-emitting diodes (LEDs) have made possible the development of LED-based photobioreactors (PBR). We investigated the effects of various LEDs on the growth and astaxanthin production from *Haematococcuspluvialis*. The mixture of red and blue was found to be the most effective light source for growing this microalga. The ratio of mixed red–blue showed the best growth and astaxanthin accumulation from *Haematococcuspluvialis*. The maximum biomass and astaxanthin production with illumination by mixed red–blue LEDs was 1.54 g/L and 20.12 mg/L, respectively. Biomass and astaxanthin were enhanced by a maximum of 4.84 g/L and 124.96 mg/L, with the mixed red–blue illumination at high light intensity 160 $\mu E \cdot m^{-2} \cdot s^{-1}$. Two stage cultures for large scale cultivation using tubular photobioreactor with LED lighting panels was firstly investigated in this study.

Keywords:*Astaxanthin, Haematococcuspluvialis, light emitting diodes, microalga, wavelength*

No. of Figures: *06* ***No. of References:*** *15*

INTRODUCTION

Microalgae have been suggested as good candidates for valuable production because of specific advantages they possess such as higher photosynthetic efficiency, higher biomass production and faster growth when compared with other energy crops (Tran *etal.*, 2010). Microalgae have been considered as great promising microorganism due to its potential as a source of valuable pharmaceuticals, pigments, carbohydrates, biofuels and other fine chemicals. *Haematococcuspluvialis* is green microalga, considered as the best potential source of astaxanthin. Astaxanthin (3,3'-dihydroxy-β, β-carotene-4,4'-dione) is a high value carotenoid pigment with potential applications in various fields, including cosmetics, nutraceuticals, and the food and feed industries (Katsumata *et al.,* 2014). The potent antioxidant properties of astaxanthin have been implicated in various biological activities, demonstrated in both experimental animals and clinical studies. It has both considerable potential and promising applications in human health and nutrition (Guerin *et al.,* 2003). This photosynthetic microorganism requires different culture conditions for vegetative growth and astaxanthin production; astaxanthin accumulates inside *Haematococcus*cell bodies during the transformation of green vegetative cells to red cyst cells under unfavorable environments (Kang and Sim, 2007). Many induction methods, such as nitrogen starvation, exposure to intense light, excess acetate addition, salt stress, and the addition of specific cell division inhibitors, have been developed for transforming green cells to red cysts with high astaxanthin content (Fábregas*et al.,* 1998). Accordingly, the biotechnological exploitation for growth and astaxanthin production potential of microalga requires exploration.

The most relevant environmental factors that affect the growth of microalgae include light, temperature, pH, salinity, nutrients' qualitative and quantitative profiles, and dissolved oxygen, and the levels of heavy metals or synthetic organics (Kumar *et al.,* 2010). Microalgal growth can be affected by reactor operation conditions such as hydraulic residence time, harvesting rates, gas transfer, and mixing equipment, because these factors affect CO_2 availability, shear rates, and light exposure (Kumar *et al.,* 2010). However, although work suggests that nitrogen is a critical factor, it does not work alone, but interacts with other factors such as the light intensity to affect the overall astaxanthin production. The effect of light intensity on astaxanthin production can be largely altered by the algal density in the culture, as the latter affects the light availability for individual cells under a given incident light intensity (Wang *et al.,* 2013). The light regime inside a cultivation system must be considered in the design and scaling-up of photobioreactors, because light energy is a growth limiting substrate. Two properties of light energy are important for astaxanthin accumulation: Light intensity and spectral quality (Kim and Lee, 2001). A high light intensity causes relatively large quantities of astaxanthin to accumulate in *Haematococcus* cells. Spectral quality is defined by the absorption spectrum for chlorophylls and other photosynthetically active pigments

(such as astaxanthin and carotenoids) (Kim *et al.,* 2013); supplying a specific wavelength of light will enhance astaxanthin production. Light emitting diodes (LEDs) have recently been recognized as effective light sources and low cost energy sources for microalgae cultivation (Schulze *et al.,* 2014). For these reasons, it is important to develop general knowledge in this field, with an emphasis on the effects of light emitting diodes.

In the present study, the growth and astaxanthin accumulation of *Haematococcuspluvialis* was examined for various light sources. Mixed red–blue light was found to be the most efficient for growth and astaxanthin production in *Haematococcuspluvialis*, and the shifting wavelength was investigated. Herein, *Haematococcuspluvialis* biomass was observed as a potential source of astaxanthin production when cultured using light emitting diodes.

MATERIAL AND METHODS

Microalgal strain and culture systems

*Haematococcuspluvialis*was obtained from Professor Seong-Moon Park's laboratory, Chonbuk National University, South Korea. It was cultivated photoautotrophically in the OHM medium, which consisted of (g/L) KNO_3, 0.41; Na_2HPO_4, 0.03; $MgSO_4{\cdot}7H_2O$, 0.246; $CaCl_2{\cdot}2H_2O$, 0.11; and (mg/L) $C_6H_5FeO_7$, 2.62; $CoCl_2{\cdot}6H_2O$, 0.011; $CuSO_4{\cdot}5H_2O$, 0.012; $MnCl_2{\cdot}4H_2O$, 0.98, Cr_2O_3, 0.075; $Na_2MoO_4{\cdot}2H_2O$, 0.12; SeO_2, 0.005 and in (µg/L) biotin, 25; thiamine, 17.5 and B12, 15 (Fábregas*et al.,* 2000). Flask photobioreactors were made of Duran glass, and air that had been sterilized by filtering through glass fiber was passed into the flasks (Tran *et al.,* 2009). Fluorescent lamps were used as a light source for the seed culture, and the cultivations were maintained at 25 °C.

Biomass estimation

Aliquots of the culture suspension were harvested via centrifugation at 5,000 rpm for 10 minutes, after which the cells were washed twice with distilled water. The dry weight of the algal cells was then measured by pipetting the washed cell suspension onto pre-weighed Nylon filters (Model R04SP0470S, GE OsmonicsLabstore, Minnetonka, MN, USA). After applying a vacuum from a small compressor for 5 minutes, the liquid component of the samples had been substantially removed, leaving the cell pellet. The filter and cell pellet were then placed in the drying oven and dried to constant weight by heating at 80 °C for 24 hours. The filters were then allowed to cool to room temperature in a desiccator and reweighed, after which the dry weight of the algal biomass was determined gravimetrically as the difference between the weight of the filter before and after use; growth was expressed in terms of dry weight (g/L) (Tran *et al.,* 2013).

Astaxanthin measurement

The astaxanthin concentration was analyzed using a spectrophotometer after acetone extraction together with bead-beating using a cell disruptor. The extraction was centrifuged at 13,000 rpm for 10 minutes, determined by the spectrophotometer at OD_{474},and calculated using calibration curves. The astaxanthin standard (Sigma-Aldrich Co. Ltd, U.S.A.) was used for calibration. The level of astaxanthin production (mg/L)

(C_A) was calculated using the following equation:

$$C_A = (OD_{474}-0.0831)/0.1426$$

Morphological change

Morphological changes in the cells were observed via optical microscope (model KB-500, KoreaLabTech Corporation) and were analyzed in photographs taken with a digital camera (Discovery C30 Scientific microscope camera) using the ISCapture version 2.5.1 software, which was supplied by Scienon Technology Co., Ltd.

Light sources and measurement of light intensity

Different light sources were used: Fluorescent lamps (Model FPL-55W, OSRAM Co., Ltd., Korea), blue (λmax 447 nm) and red LEDs (λmax 656 nm) were made by LED Agri-Bio Fusion Technology Research Center, Chonbuk National University, South Korea. The light intensities of the fluorescent lights were adjusted by altering the number of fluorescent lamps and the distance from the photobioreactor. The light intensity of the LED light was controlled using a light controller, thus the light intensities of the LEDs could be adjusted. The light intensities were measured using a quantum sensor (Model SKP 200 38684, Skye Instruments Ltd, United Kingdom).

RESULTS AND DISCUSSION

Effects of different kinds of LEDs on the growth and astaxanthin production

In this study, we took steps to determine whether growth and astanxanthin production were affected by wavelength. We used the same experimental setting, except using different light sources such as fluorescent lighting, red, blue, and mixed red–blue LEDs. LEDs were used because it is possible to control their wavelengths precisely. Blue light (wavelength range 400–505 nm, with a peak at 447 nm) and red light (wavelength range 595–700 nm, with a peak at 656 nm) were used. Fluorescent light was used as the control. As shown in Figure 1, the emission spectra of these light sources are very distinctive from one another. The cultures were illuminated at the same total light intensity (40 $\mu E \cdot m^{-2} \cdot s^{-1}$), and the pre-culture of *H. pluvialis* was mixed in OHM medium in a 500 mL Duran flask, with a working volume of 350 mL. Inorganic carbon for microalgae growth was supplied at 1 vvm (aeration volume/medium working volume/minute) using an air pump and air sparger. The schematic diagram of the experimental setup is shown in Figure 2. As seen in Figure 3, the mixed–blue LED setup was found to be the most effective for growth of the microalga. The maximum biomass obtained with mixed red–blue illumination was 1.54 g/L, followed by red, blue and fluorescent light at 1.44, 1.36, and 1.24 g/L, respectively. Compared with monochromatic light, the growth and astaxanthin production of *H. pluvialis* using mixed red–blueLEDs were substantially higher. Ruyters reported that sufficient red and blue light should be provided for the adequate photosynthesis of microalgae and plants (Schulze *et al.*, 2014). In this study, photosynthetic efficiency increased by selectively and simultaneously providing both red and blue light, which provided the necessary wavelength ranges for photosynthesis. Thereby, the biomass and astaxanthin production rates were higher

than for only red light, only blue light, or fluorescent light. These results indicate that mixed LEDs are more favorable and effective for biomass and astaxanthin production in *H. pluvialis*.

Effects of shifting the wavelength and initial light intensity of mixed red–blue LEDs on growth and astaxanthin production

To examine the effect of light intensity and wavelength on growth and astaxanthin production further, *H. pluvialis*cells were cultured under various conditions. One at low light intensity (40 $\mu E \cdot m^{-2} \cdot s^{-1}$), one at high light intensity (160 $\mu E \cdot m^{-2} \cdot s^{-1}$) of mixed red and blue wavelengths, and two experienced shifting wavelengths (red light and fluorescent with low light intensity (40 $\mu E \cdot m^{-2} \cdot s^{-1}$) for eight initial days, then mixed red–blue with high light (160 $\mu E \cdot m^{-2} \cdot s^{-1}$) for six days). Red LED and fluorescent light were used as a control (at 40 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity). The batches were all cultured for 14 days in total. Figure 4 shows that the mixture of red and blue light at a high light intensity (160 $\mu E \cdot m^{-2} \cdot s^{-1}$) was the most effective for growth and astaxanthin production. It displayed the highest biomass (4.84 g/L) and astaxanthin accumulation (124.96 mg/L), followed by the sample of shifting wavelength from fluorescent to mixed red–blue light (high light intensity - 160 $\mu E \cdot m^{-2} \cdot s^{-1}$) with 3.8 g/L and 95.26 mg/L. The next was shifting wavelength from red to mixed red–blue light (high light intensity - 160 $\mu E \cdot m^{-2} \cdot s^{-1}$) (3.4 g/L and 86.26 mg/L), then mixture of red and blue light, red light and fluorescent with low light intensity (40 $\mu E \cdot m^{-2} \cdot s^{-1}$) with (2.46 g/L and 62.86 mg/L), (2.3 g/L and 59.87 mg/L) (1.86 g/L and 40.82 mg/L), respectively. These results suggest that red–blue light, whose wavelength falls on the adsorption spectra of astaxanthin and chlorophylls, has higher efficiency than non-overlapping spectral sources. Furthermore, low light intensities (40 $\mu E \cdot m^{-2} \cdot s^{-1}$) were insufficient to support high-density photoautotrophic cultures or fully induce astaxanthin. However, the efficiency of high light intensities of mixed red–blue (160 $\mu E \cdot m^{-2} \cdot s^{-1}$) have been jumped recently. This clearly suggests that LEDs can be suitable light sources, not only for biomass, but also for astaxanthin accumulation.

The growth and astaxanthin accumulation of *H. pluvialis*in large-scale cultivation using a tubular photobioreactor

Both tubular and flat panel photobioreactors have been scaled up to large-scale, yet neither has been demonstrated as clearly superior to the other (AciénFernández*et al.,* 2013). Although the production of *H. pluvialis* in outdoor using tubular PBR has been done (Olaizola and Huntley, 2003), no data mentioned to the production of *H. pluvialis* in indoor using light emitting diodes as light sources. The horizontal tubular photobioreactor (PBR) used in this experiment was made by total 68 ea of glass tube which connected by the glass connector (each tube with long 68 cm and diameter 2.5 cm), the working volume in tubular was 25L and the working volume in the container was 25L, and its total working volume for this type PBR is 50L. The culture solution from container was connected to the tubular PBR by a centrifugal pump; the speed

was controlled by a controller. Air was supplied at the bottom of the container. In this kind of photobioreactor, light dilution is obtained by applying LEDs lighting panels (included red, blue and mixed of red: blue) to the tubular PBR. It was experimentally characterized for developing scalable industrial photobioreactors with LED illumination. The schematic diagrams of the tubular photobioreactor are described in Figure 5. A two-stage cultivating process of *H. pluvialis*cells for large scale production of astaxanthin comprising: (stage 1) growing the cells under controlled environmental conditions for optimal growth so called "green stage", this step using fluorescent lamp as light sources with intensity (40 $\mu E \cdot m^{-2} \cdot s^{-1}$); (stage 2) *H. pluvialis*cells from stage 1 were moved to culture in tubular PBR under the illumination of mixed red–blue LED (160 $\mu E \cdot m^{-2} \cdot s^{-1}$) and the red, blue light (100 $\mu E \cdot m^{-2} \cdot s^{-1}$), this step for the induction and astaxanthin accumulation so called "red stage". The stage 1 was cultured for 8 days; the stage 2 was cultured for next 6 days. The batches were all cultured for 14 days in total. As seen in Figure 6, the biomass and astaxanthin production under the illumination of mixed red–blue light (1.66 g/L and 36.78 mg/L, respectively) was higher than for blue light (1.5 g/L and 32.06 mg/L) and red light (1.36 g/L and 27.89 mg/L), respectively. It was confirmed that mixed of red and blue LEDs can improve the quality and quantity of microalgal biomass and their integration into microalgal production systems (photobioreactors) should be considered.

ACKNOWLEDGEMENTS

This work was supported by the Industrial Technology Research Infrastructure Program (N0000004) funded by the Ministry of Knowledge Economy (MKE, Korea).

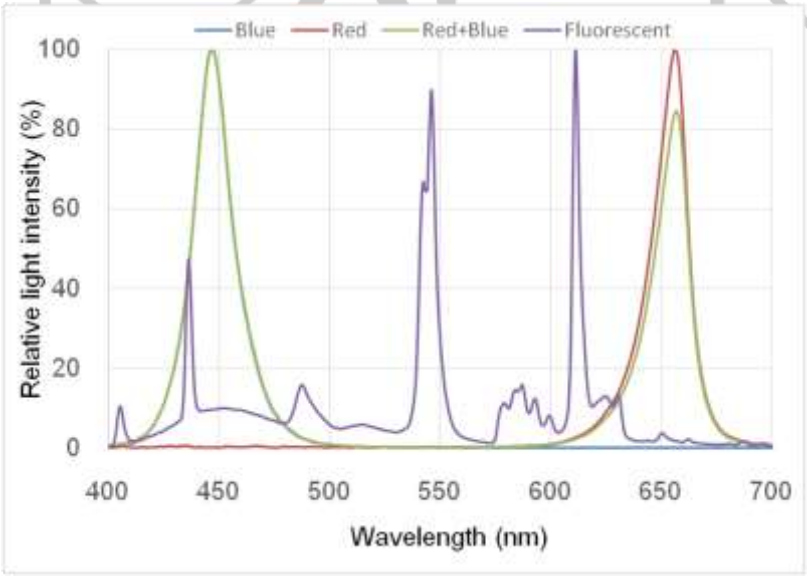

Fig 1: *Emission spectra of blue, red, mixed red–blue LEDs, and fluorescent light.*

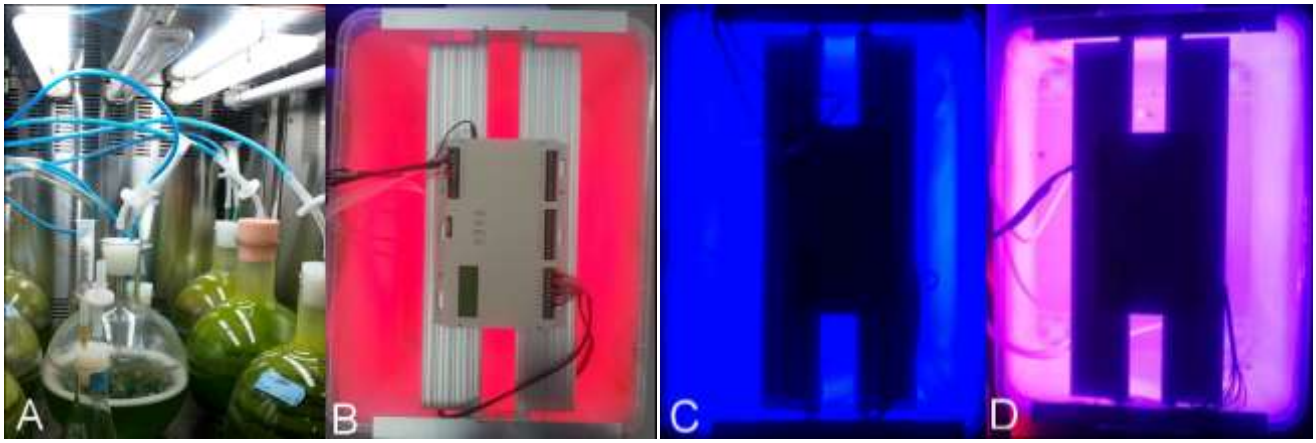

Fig 2: *Schematic diagram of the experimental setup for different light illuminations (A: Fluorescent light, B: Red LED, C: Blue LED, D: A mixture of red and blue LEDs).*

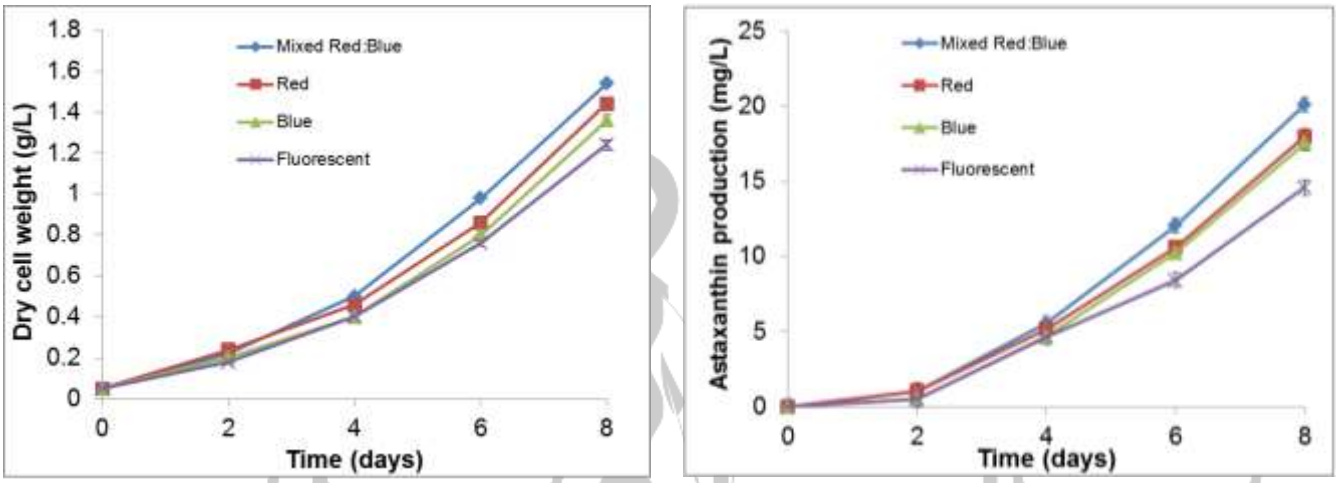

Fig 3: *Biomass concentration and astaxanthin production of H. pluvialis under various light sources*

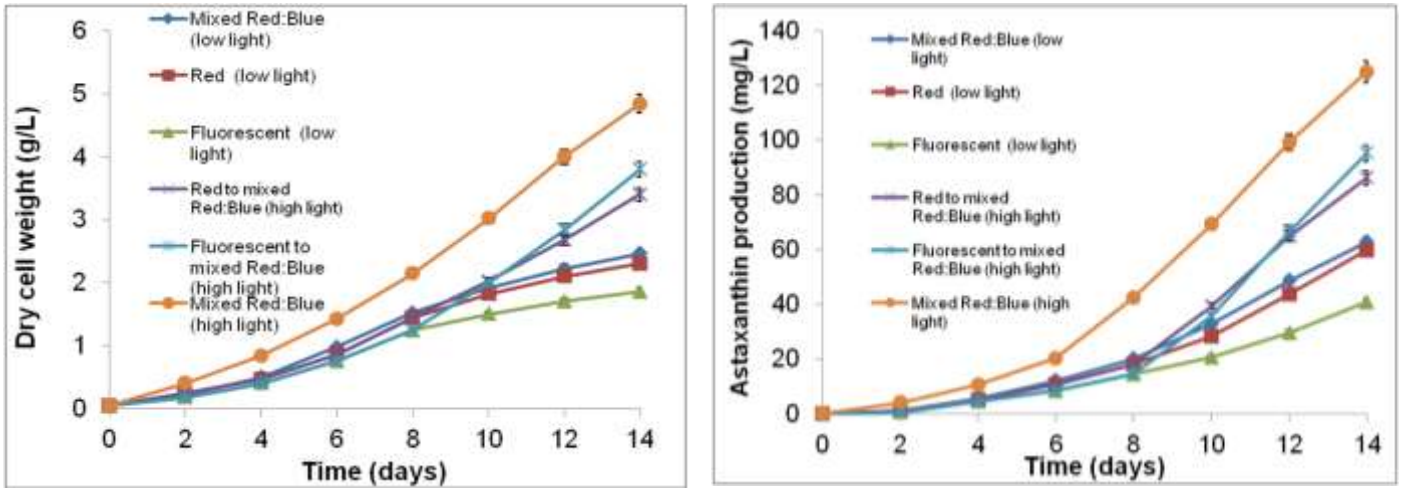

Fig 4: *Effect of shifting wavelength and light intensity of LEDs on biomass concentration and astaxanthin production of H. pluvialis.*

Fig 5: *Schematic diagrams of 50L tubular photobioreactor (A: tubular PBR with LEDs lighting panels, B: controller for a centrifugal pump, C: the container)*

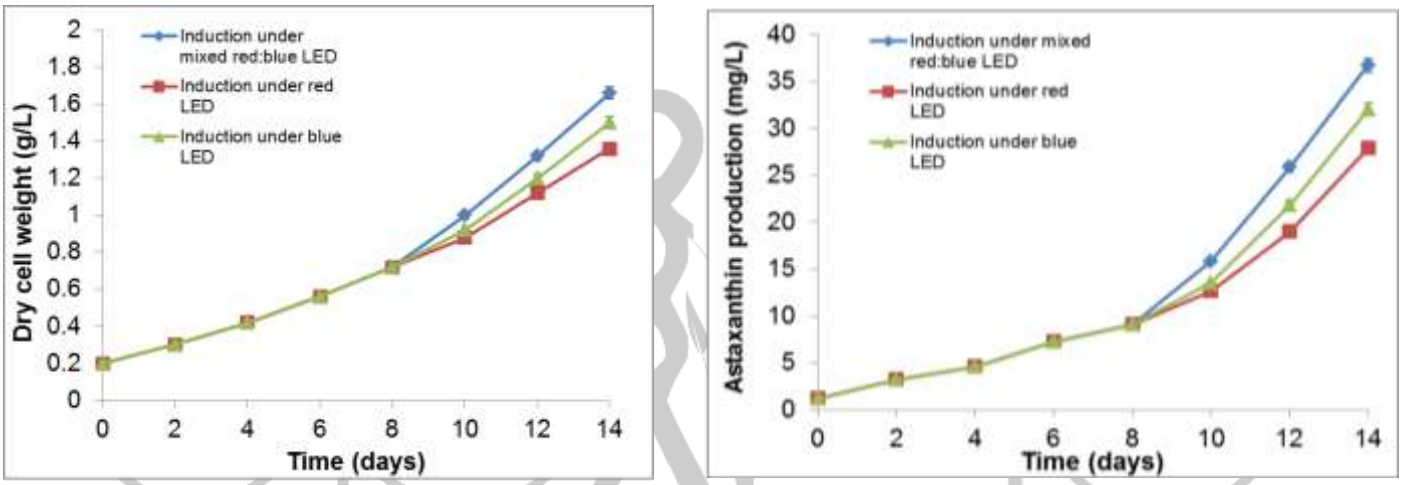

Fig 6: *The growth and astaxanthin accumulation of H. pluvialis in large-scale cultivation using a tubular photobioreactor.*

REFERENCES

Acién Fernández FG, Fernández Sevilla JM, and Molina Grima E. Photobioreactors for the production of microalgae. *Reviews in Environmental Science and Bio/Technology*. 12: 131-151, 2013.

Fábregas J, Domínguez A, Álvarez DG, Lamela T, and Otero A. Induction of astaxanthin accumulation by nitrogen and magnesium deficiencies in *Haematococcus pluvialis*. *Biotechnol Lett.* 20: 623-626, 1998.

Fábregas J, Domínguez A, Regueiro M, Maseda A, and Otero A. Optimization of culture medium for the continuous cultivation of the microalga *Haematococcus pluvialis*. *Appl Microbiol Biotechnol.* 53: 530-535, 2000.

Guerin M, Huntley ME, and Olaizola M.*Haematococcus* astaxanthin: applications for human health and nutrition. *Trends Biotechnol.* 21: 210-216, 2003.

Kang CD and Sim SJ. Selective Extraction of Free Astaxanthin from *Haematococcus* Culture Using a Tandem Organic Solvent System. *Biotechnol Progr.* 23: 866-871, 2007.

Katsumata T, Ishibashi T, and Kyle D. A sub-chronic toxicity evaluation of a natural astaxanthin-rich carotenoid extract of *Paracoccus carotinifaciens* in rats. *Toxicology Reports.* 1: 582-588, 2014.

Kim N-J and Lee C-G. A theoretical consideration on oxygen production rate inmicroalgal cultures. *Biotechnol. Bioprocess Eng.* 6: 352-358, 2001.

Kim T-H, Lee Y, Han S-H, and Hwang S-J. The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using*Scenedesmus* sp. for wastewater treatment. *Bioresource Technol.* 130: 75-80, 2013.

Kumar A, Ergas S, Yuan X, Sahu A, Zhang Q, Dewulf J. Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions. *Trends Biotechnol.* 28: 371-380, 2010.

Olaizola, M., Huntley, M.E. Recent advances in commercial production of astaxanthin from microalgae. In: Fingerman, M.,Nagabhushanam, R. (Eds.), Biomaterials and Bioprocessing. Science Publishers, 2003.

Tran H-L, Hong S-J, and Lee C-G. Evaluation of extraction methods for recovery of fatty acids from *Botryococcus braunii* LB 572 and *Synechocystis* sp. PCC 6803. *Biotechnol. Bioprocess Eng.* 14: 187-192, 2009.

Tran H-L, Kwon J-S, Kim ZH, Oh Y, and Lee C-G. Statistical optimization of culture media for growth and lipid production of *Botryococcus braunii* LB572. *Biotechnol. Bioprocess Eng.* 15: 277-284, 2010.

Tran H-L, Ryu Y-J, Seong D, Lim S-M, and Lee C-G. An effective acid catalyst for biodiesel production from impure raw

feedstocks. *Biotechnol. Bioprocess Eng.* 18: 242-247, 2013.

Schulze PSC, Barreira LA, Pereira HGC, Perales JA, and Varela JCS. Light emitting diodes (LEDs) applied to microalgal production. *Trends Biotechnol.* 32: 422-430, 2014.

Wang J, Sommerfeld MR, Lu C, and Hu, Q. Combined effect of initial biomass density and nitrogen concentration on growth and astaxanthin production of *Haematococcus pluvialis* (Chlorophyta) in outdoor cultivation. *Algae.* 28: 193-202, 2013.