Evaluation of Air Flow Rate on the Growth of *Haematococcus pluvialis*

Bahar Aslanbay, Zeliha Demirel and Esra Imamoglu

***Abstract*—** Microalgae *Haematococcus pluvialis* is reported as a large producer of carotenoid. The main goal of this study was to investigate and compare the effects of air flow rates under controlled cultivation conditions to enhance the growth rate of *Haematococcus pluvialis*. The cells were cultivated at three different air flow rates (1 vvm, 2 vvm and 3 vvm) in the sterile bottles for 9 days. The experiments were performed under the same growth conditions. The maximum specific growth rate of 0.246 day^{-1}, which corresponded to the doubling time of 2.82 day, was obtained at the flow rate of 2 vvm under the light intensity of 65 µE $m^{-2}s^{-1}$ in BG11 medium for 9 days of *H. pluvialis* cultivation period.

***Keywords*—**air flow rate, carotenoid, growth, microalgae.

I. Introduction

The unicellular fresh water microalga, *Haematococcus pluvialis* Flotow (Volvocales, Chlorophyceae) is green-colored, biflagellate, and motile in its vegetative stage [1], [2]. This freshwater organism has an atypical life cycle in that it is able to go from a vegetative cell state rich in chlorophylls and proteins to an encysted state under stress conditions. At this encysted state, *H. pluvialis* is surrounded by a thick cell wall and can produce high amounts of secondary metabolites including carotenoids, especially astaxanthin [3] which has attracted considerable attention in recent years [4].

Carotenoids have benefits to the prevention of chronic diseases such as heart disease, cancer, and age degradation (to prevent premature aging). They are potential to be developed for food and pharmaceutical industry as a food coloring agent and production of drugs [5], [6]. The carotenoid fraction of green vegetative *Haematococcus* cells consists of mostly lutein (75-80%) and β-carotene (10-20%). Whereas in red cysts, predominate carotenoid is astaxanthin [7]. Furthermore, the vegetative form is equally worthy of attention due to the amount of proteins (27%) and carbohydrates (40%) [2].

Many researches have been conducted to investigate the controlled factors in the cultivation of *H. pluvialis*. The aim of this study was to investigate and compare the effects of air flow rates under controlled cultivation conditions to enhance the growth rate of *H. pluvialis*.

Bahar Aslanbay is with the Department of Bioengineering, University of Ege, 35100, Izmir, Turkey (e-mail: baharaslanbay@gmail.com).

Zeliha Demirel is with the Department of Bioengineering, University of Ege, 35100, Izmir, Turkey (e-mail: zelihademirel@gmail.com).

Esra Imamoglu is with the Department of Bioengineering, University of Ege, 35100, Izmir, Turkey (corresponding author's phone: +90232388495; fax:+902323884955 ; e-mail: esraimamoglu@yahoo.com).

II. Materials And Methods

A. Algal strain and inoculum preparation

Haematococcus pluvialis Flotow EGE MACC-32 was obtained from the Culture Collection of Microalgae at the University of Ege, Izmir, Turkey. Stock culture of *H. pluvialis* was grown photoautotrophically in BG11 medium [2] at 22 ± 2°C (65 µE $m^{-2}s^{-1}$) in 2-L sterile bottle for 14 days. For the preparation of the inoculum, the cells from the stock culture were collected and concentrated by centrifugation (1160 *g*, 5 min) and the supernatant was removed. The collected cells were incubated aseptically in 250 mL flasks containing 100 mL of BG11 medium under the light intensity of 22 µE $m^{-2}s^{-1}$ at 20±2 °C for four days. Air was supplied to the culture at a flow rate of 1 vvm. A 4-day-old culture cells were used as inoculum at 10% volume for all experiments.

B. Growth conditions

The experiments were performed in 1-L sterile bottles. 4-day old culture (100 ml, approximately 1 x 10^5 cells ml^{-1}) was inoculated into 900 ml sterilized fresh media in 1000 ml sterile bottles. The bottles were incubated for 9 days at 22±2 °C under the light intensity of 65 µE $m^{-2}s^{-1}$ at various airflow rates. Air was supplied to the culture by air pump continuously and three different air flow rates (1, 2 and 3 vvm) were adjusted by flow meter (RST electronic Ltd. Sti, LZM-6T, Turkey). Illumination was provided by LED downlight lamps (Cata 10 W CT-5254) from two sides of the bottles. Light intensity was measured by a quantum meter (Lambda L1-185) on the surface of the PBR.

C. Analytical procedures

Samples were taken at indicated times, and the following growth parameters were measured immediately; the cell concentration was determined by counting triplicate samples in a Neubauer hemocytometer. The cellular turbidity (optical density) was measured at 680 nm in UV/VIS spectrophotometer (GE Healthcare Ultrospec 1100 pro, UK). Dry weight was determined by filtering a 5-ml culture sample through pre-weighed GF/C filter (Whatman, UK) and drying the cell mass at 105 °C for two hours. Algal pigments were extracted with dimethyl sulfoxide (DMSO) as reported by Wellburn et al. [8].

The specific growth rate (µ) of the cells was calculated from the initial logarithmic phase of growth for at least 48 h, as $\mu = (\ln X_2 - \ln X_1)/dt$, where X_2 is the final cell concentration, X_1 is the initial cell concentration and dt is the time required for the increase in concentration from X_1 to X_2.

Doubling time (DT) was also calculated as DT = ln 2/μ. The data were analyzed using one-way analysis of variance (ANOVA). Results were reported as mean values with standard deviations (n=3) unless otherwise indicated.

III. Results And Discussion

The cells were cultivated at three different air flow rates (1 vvm, 2 vvm and 3 vvm) in the sterile bottles for 9 days. The experiments were performed under the same growth conditions. As shown in Figure 1, the cell concentration significantly increased after 5 days of cultivation period in different flow rates. During the cultivation, the growth increased 12 times in terms of the initial cell concentration at the flow rate of 2 vvm under the light intensity of 65 μE $m^{-2}s^{-1}$ for *H. pluvialis*. Additionally, it was recorded that the cell concentration was 42% higher at the flow rate of 3 vvm compared to the flow rate of 1 vvm at the end of the cultivation period. It is also worth noting that the air flow rate of 2 vvm attracted attention, especially concerning the yield and productivity. As reported by Damiani et al. [9], the maximum cell concentration of $11x10^5$ cells ml^{-1} was obtained in Bold's Basal Medium under the light intensity of 90 μE m^{-2} s^{-1} with a 12:12 h light:dark cycle photoperiod with the continuous bubbling of air (500–700 cm^3 min^{-1}) containing 0.30 cm^3 min^{-1} of CO_2 for the growth of *H. pluvialis* after 12 days of period.

Noteworthy that chlorophyll plays an essential role for capturing CO_2 and solar energy to generate the metabolic flux for the microalgal growth [10]. As seen in Figure 2a, the similar chlorophyll-a contents of 4.33±0.03 g L^{-1} and 4.22±0.02 g L^{-1} were obtained on the 7th day at the flow rates of 2 vvm and 3 vvm, respectively. The maximum chlorophyll-a content, 7.06±0.29 g L^{-1}, was obtained at the flow rate of 2 vvm, and the low chlorophyll-a content was found at the flow rate of 1 vvm. On the other hand, the chlorophyll-b content reached a peak value of 5.69±0.23 g L^{-1} on day 9 while the minimum chlorophyll-b content was obtained at the flow rate of 1 vvm only with the value of 3.78±0.09 g L^{-1} (Figure 2 b).

As shown in Figure 3, total carotenoid contents were close to each other in all experiments during the first 5 days; however the largest changes occurred after the day of 5. The maximum carotenoid content of 2.49±0.03 g L^{-1} was found at the flow rate of 2 vvm under the light intensity of 65 μE $m^{-2}s^{-1}$ for *H. pluvialis*, which indicated that cells could adjust well to the growth conditions. The carotenoid content decreased by 27% at the air flow rate of 1 vvm in comparison with the flow rate of 2 vvm.

The maximum specific growth rate of 0.246 day^{-1}, which corresponded to the doubling time of 2.82 day, was obtained at the flow rate of 2 vvm (Table 1). As expected, increasing the air flow rate from 2 vvm to 3 vmm reduced to the growth rate of *H. pluvialis*. As reported by Imamoglu et al. [11], the maximum specific growth rate of 0.271 day^{-1} was found in flat plate PBR for the semi-continuous cultivation of *H. pluvialis*.

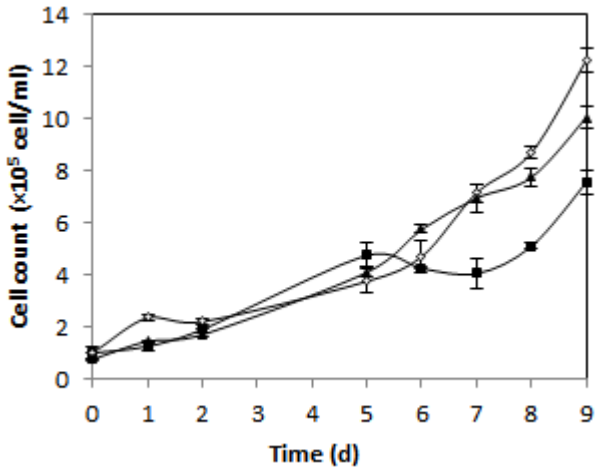

Fig. 1 Cell count profiles of *H. pluvialis*: (■) 1 vvm, (◊) 2 vvm, (▲) 3 vvm.

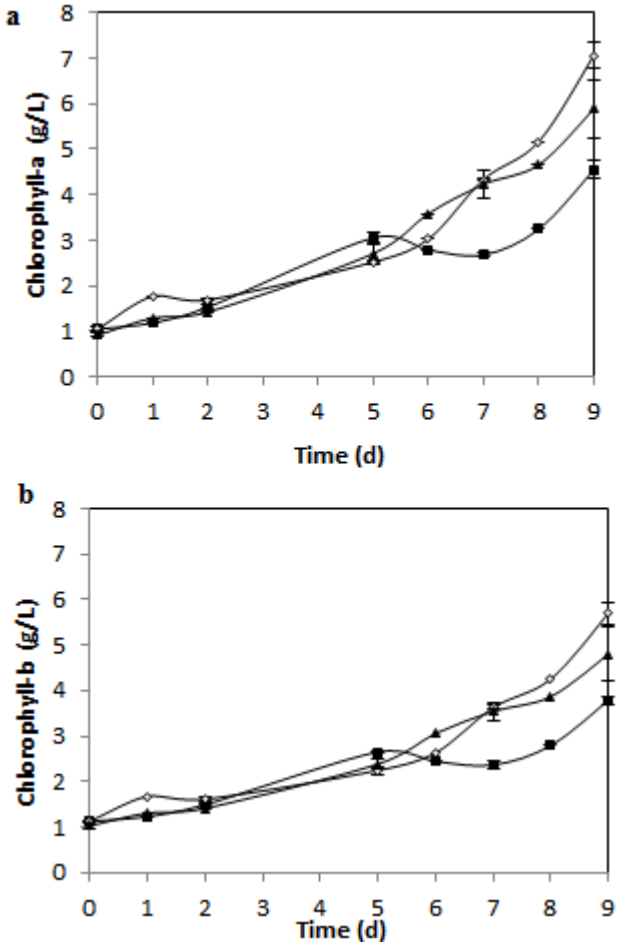

Fig. 2 Chlorophyll profiles of *H. pluvialis*: (a) Chlorophyll-a content, (b) Chlorophyll-b content. (■) 1 vvm, (◊) 2 vvm, (▲) 3 vvm.

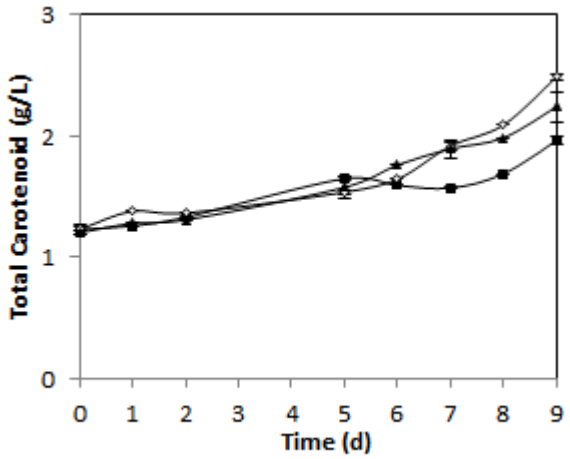

Fig. 3 Total Carotenoid contents of *H. pluvialis*: (■) 1 vvm, (◊) 2 vvm, (▲) 3 vvm.

TABLE I
RESULTS OF OBTAINING PARAMETERS OF *H. PLUVIALIS*

	1 vvm	2 vvm	3 vvm
Cellular turbidity (OD)	0.450±0.005	0.652±0.003	0.557±0.002
Dry weight ($g\ L^{-1}$)	0.552±0.05	0.697±0.08	0.629±0.06
Biomass productivity ($g\ L^{-1}\ d^{-1}$)	0.061±0.005	0.077±0.008	0.070±0.006
Specific growth rate (μ, d^{-1})	0.197	0.246	0.230
Doubling time (DT, d)	3.516	2.821	3.014

IV. CONCLUSION

These results have demonstrated that maximal growth rate of *H. pluvialis*, 0.246 d^{-1}, was obtained at the flow rate of 2 vvm under continuous illumination (65 µE $m^{-2}s^{-1}$) in BG11 medium for 9 days of cultivation period. The primary barriers of microalgal processes are the energy consumption and the cost of investment, especially in R&D activities for microalgal cultivation. These expenses may be reduced by the use of optimum process conditions (such as temperature, light, flow rate and etc.) providing to reach the high volumetric productivities and high biomass concentrations.

ACKNOWLEDGMENT

This study was a part of Cost action ES1408 and the authors would like to thank the Scientific and Technological Research Council of Turkey (TUBITAK) with the project number of 115M014 for the financial support.

REFERENCES

[1] L. Fan, A. Vonshak and S. Boussiba, "Effect of temperature and irradiance on growth of *Haeamatococcus pluvialis*," *J. Phycol.* vol. 33, pp. 829–833, 1994.

[2] E. Imamoglu, F.V. Sukan and M.C. Dalay, "Effect of Different Culture Media and Light Intensities on Growth of *Haematococcus pluvialis*," *Int. J. Nat. Eng. Sci*., vol. 1, no. 3, pp. 5-9, 2007.

[3] M.M. Mendes-Pinto, M.F.J. Raposo J. Bowen, A.J. Young and R. Morais, "Evaluation of different cell disruption processes on encysted cells of *Haematococcus pluvialis*: effects on astaxanthin recovery and implications for bio-availability," *J. Appl. Phycol*., vol. 13, pp. 19–24, 2001.

[4] F. Ba, A.V. Ursu, C. Laroche and G. Djelveh, "*Haematococcus pluvialis* soluble proteins: Extraction, characterization, concentration /fractionation and emulsifying properties," *Bioresour. Technol*., vol. 200, pp. 147-152, 2016.

[5] N.K. Wusqy and F.F. Karwur, "Astaksantin dari bakteri laut: biosintesis, manfaat, dan potensi produksi massal," *Squalen*., vol. 5, no. 1, pp. 33-38, 2010.

[6] E.A. Suyono, Aminin, L. Pradani, U. Mu'avatun, R.N. Habiba, Ramdaniyah and E.F. Rohma, "Combination of blue, red, white, and ultraviolet lights for increasing carotenoids and biomass of Microalga *Haematococcus pluvialis*," *Procedia Environ. Sci*., vol. 28, pp. 399 – 405, 2015.

[7] B. Renstrom, G. Borch, O. Skulberg and S. Liaaen-Jensen, "Optical purity of (3S,3'S)-astaxanthin from *Haematococcus pluvialis*," *Phytochem*., vol. 20, no. 11, pp. 2561-2564, 1981.

[8] A.R. Wellburn,"The Spectral Determination of Chlorophylls a and b as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution," *J. Plant Physiol*., vol. 144, pp. 307-313, 1994.

[9] M.C. Damiani, C.A. Popovich, D. Constenla and P.I. Leonardi, "Lipid analysis in Haematococcus pluvialis to assess its potential use as a biodiesel feedstock," *Bioresour. Technol*., vol. 101, pp. 3801–3807, 2010.

[10] E. Imamoglu, Z. Demirel and M.C. Dalay, "Process optimization and modeling for the cultivation of *Nannochloropsis* sp. *and Tetraselmis striata* via response surface methodology," *J. Phycol*., vol. 51, pp. 442-453, 2015.

[11] E. Imamoglu, M.C. Dalay and F.V. Sukan, 2010, "Semi-continuous Cultivation of *Haematococcus pluvialis* for Commercial Production," *Appl. Biochem. Biotechnol*., vol. 160, no.3, pp. 764-772, 2010.