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Research Article

Evaluation of different scale-up strategies for *Haematococcus pluvialis* cultivation in airlift photobioreactors

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ABSTRACT

Large scale algal biomass production can be very challenging due to the potential issues of sustainability, environmental ethics, and economic concerns. A strategic approach to the transition from the laboratory to the industrial scale allows the prediction of process characteristics, design and analysis of large scale systems, and reduction of extra costs. In this study, a scale-up procedure that considered different approaches was carried out by selecting the *Haematococcus pluvialis* as a model organism. Three scale-up parameters (constant mixing time (t_m), volumetric power consumption rate (P/V), and oxygen mass transfer coefficient (k_La)) were tested for biomass production in a 2-L airlift photobioreactor and they were compared with those obtained from a 1-L aerated cultivation bottle. Among three strategies, the maximum cell concentration, 4.60±0.20×10^5 cells/mL, was obtained in a constant volumetric power consumption rate experiment. Also, total carotenoid amount showed similar changes with the cell concentration and reached the maximum concentration of 2.02±0.11 mg/L under constant P/V experiment. However, the cultivation bottle presented the highest biomass amount of 0.62 g/L and specific growth rate of 0.38 day^{-1} of all of the photobioreactors. This result might be attributed to the low aeration rates or improper configuration of the system, which created a non-homogenous culture medium and led to ineffective mass transfer.

Keywords: *Haematococcus pluvialis*, Scale-up, Airlift photobioreactor, Biomass production, Carotenoid

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INTRODUCTION

The mass cultivation of microalgae has recently aroused interest because of several advantages, including the rich content of algal biomass, potential beneficial effects on the environment and their great capacity to create renewable energy. Large scale cultivation systems for microalgae production are highly preferable due to the increased growth rate, higher biomass productivity, and requirement of small areas. In addition, it has great potential in a clean and renewable environment by capturing atmospheric CO_2 and treating wastewater with the recovery of nutrients and pollutants (Bendetti et al., 2018). Industrial microalgae cultivation can be done in open ponds and in controlled, closed systems, which are called photobioreactors (PBR). The major advantages of open ponds include low cost, high production capacity, and ease of building and operating. Despite these advances, there are some drawbacks, such as the risk of contamination, low mass and heat transfer efficiency, ineffective mixing, water loss, and poor process control (Wang et al., 2013). Therefore, numerous closed PBRs, such as flat plate, airlift, bubble column, membrane, etc., have been designed and used instead of open ponds. An airlift PBR is a common closed cultivation system that has a cylindrical transparent bubble column and a draft tube located through the center of the PBR (Aslanbay Guler et al.,

2020). This draft tube creates two regions, the downcomer and riser, and gas is sparged through the riser by moving the liquid in the riser zone upwards. The movement of the liquid with the driving force of gas bubbles creates a recirculation between the riser and downcomer regions and provides an effective flow pattern. In this way, airlift PBR has the characteristic advantage of creating a circular mixing pattern and thus, good heat and mass transfer (Ding et al., 2021). Furthermore, airlift PBRs provide proper operating conditions for many microalgae species by causing less shear stress than other types of PBRs (Azhand et al., 2020).

Haematococcus pluvialis is a freshwater green microalga commonly characterized by its high astaxanthin content (up to 5% of dry weight) (Deniz, 2020). Astaxanthin is a red-colored pigment that has strong antioxidant properties owing to its molecular structure. Its biosynthesis from *H. pluvialis* involves two major steps, which are green biomass production and red astaxanthin induction under specific stress conditions (Ranjbar et al., 2008). In order to reach high metabolite content, the concentration of green cells should be maximized during the first step of cultivation by optimizing conditions and performing large scale production. Astaxanthin production from *H. pluvialis* is mainly carried out in open ponds because the applied stress conditions reduce the probability of contamination. However, the first green stage is highly susceptible to the different contaminants and thus, PBRs are better choices for the mass production of *H. pluvialis* due to the ease of process control (Deniz, 2020; Wang et al., 2013). The applications of different types of PBRs for *H. pluvialis* production can be enhanced, integrating the proper scale-up strategy for the transition from lab-scale to commercial systems.

Effective scale-up is an essential and complex procedure for the successful mass cultivation of microalgae. It is highly important to choose the most suitable operation conditions depending on the cultivation system, microalga species, optimal growth conditions, and economic feasibility. According to these points, several scale-up procedures, including volumetric power consumption rate, impeller tip speed, light energy, stirrer rate, specific oxygen transfer rate, etc., are carried out during the transition from lab-scale to the industrial level. The aim of the scale-up process is enhancement of biomass and target-product yield, avoiding high cost and time consumption in the industrial scale (Aslanbay Guler et al., 2019). A number of studies have been conducted to improve the PBR production of *H. pluvialis* by integrating scale-up strategies, but more experiments are needed to understand and design an effective, large-scale cultivation system.

In this paper, the biomass production of microalgae *Haematococcus pluvialis* was studied in a 2-L airlift PBR by conducting different scale-up strategies for the transition from lab scale to the pilot scale. In this context, the main objective of this study was to investigate the use of constant mixing time (t_m), volumetric power consumption rate (P/V), and oxygen mass transfer coefficient (k_La) as scale-up methodologies under laboratory conditions for the scale-up process from 1-L aerated cultivation bottle to the 2-L airlift PBR, considering whether an increase in the cell concentration and total carotenoid amount can be achieved. According to the literature, this is the first report that compares three different scale-up strategies for biomass production from *H. pluvialis* microalgae in an airlift PBR and investigates suitable conditions for higher biomass productivity than obtained in an aerated cultivation bottle.

MATERIAL AND METHODS

Microalgae and inoculum preparation

H. pluvialis (EGE MACC-32) was provided from the Ege-MACC from University of Ege, Izmir, Turkey. Stock culture was maintained in BG11 medium (Rippka et al., 1979) under light intensity of 65 µE/m^2 s at 24 ± 2 °C in a 2-L aerated sterile bottle for 15 days. At the end of the 15th day, the cells from the stock culture were harvested and inoculated to a 250 mL Erlenmayer flask containing BG11 medium to use as inoculum in experiments. The cells were incubated under the continuous illumination of 65 µE/m^{2}s in an orbital shaker at 120 rpm at 26 ± 2 °C for five days and this culture was used as inoculum for all experiments.

Biomass production in cultivation bottle

H. pluvialis cells were cultivated in a 1-L aerated sterile bottle (8.2 cm internal diameter and 12.5 cm height) for 8 days. The bottle was continuously illuminated with the white LED downlight lamps (10 W CT-5254) and light intensity was adjusted to 65 µE/m^{2}s. Cultivation was maintained at 26 ± 2 °C in a temperature-controlled cabinet and sterile air was fed into the system at the aeration rate of 3 vvm.

Biomass production in airlift PBRs

A 2-L internal loop airlift PBR was used for the scale-up productions, with the following specifications: 1.6-L working volume, 6.4 cm diameter, 55.0 cm height, and a ratio of the cross-sectional area of the downcomer zone to the riser zone (A_d/A_r) of 5.4. More detailed design parameters and hydrodynamic properties were reported in a previous study (Aslanbay Guler et al., 2020). The PBR was constructed with transparent glass with an illuminated surface area of 0.088 m^2. Mixing and aeration were achieved by bubbling sterile air through a sparger with 6 nozzles located in the base of the column. The PBR was illuminated with a fluorescent daylight lamp along the vessel from one side, applying a light intensity of 70 µE/m^{2}s. Prior to inoculation, the system was sterilized at 121 °C and 1 atm for 15 min using an autoclave. Then *H. pluvialis* culture was inoculated into the PBR and it was operated at batch mode for 8 days at 26 ± 2 °C.

Scale-up procedures

The transition from aerated bottle to the 2-L airlift PBR (Figure 1) was carried out with three different scale-up strategies individually by changing the aeration rate. These strategies were constant mixing time (t_m), volumetric power consumption rate (P/V), and oxygen mass transfer coefficient (k_La). The mixing time was experimentally determined using the pH-response technique proposed by Van't Riet and Tramper (1991). The oxygen mass transfer coefficient was measured using the unsteady state method (Shuler & Kargi, 2002). The P/V value was calculated using the following equation (1) (Chisti & Jauregui-Haza, 2002),

$$\frac{P_G}{V_L} = \frac{\rho_L g U_{Gr}}{1+{}^{A_d}/_{A_r}} \quad (1)$$

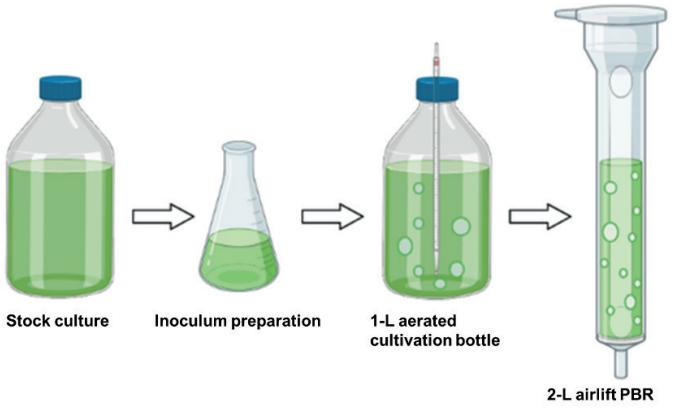

Figure 1. Scale-up process for *Haematococcus pluvialis* cultivation.

where P_G is the power input due to aeration (W), V_L is the culture volume (m^3), g is the gravitational acceleration (9.81 m/s^2), U_{Gr} is the superficial gas velocity (m/s), A_d is the cross-sectional area of the downcomer region (m^2), and A_r is the cross-sectional area of the riser region (m^2). In the aerated cultivation bottle, t_m, k_La, and P/V values were found to be 18 s, 0.01 s^{-1}, and 60 W/m^3, respectively. According to these experiments and calculations, PBR production was carried out only by varying the aeration rate while keeping the production parameters constant and to reach the calculated values in the cultivation bottle. Consequently, the aeration rates of 0.9, 1.24, and 1.8 L/min were used for the constant t_m (18 s), constant P/V (60 w/m^3), and constant k_La (0.01 s^{-1}) strategies in 2-L PBRs, respectively.

Analytical measurements and calculations

Cell growth was determined by measuring cell concentration and dry weight. Cell concentration was measured by counting samples in a Neubauer hemocytometer using an optical microscope. The dry weight content was determined by taking 5 mL aliquot and filtering it through pre-weighed GF/C filter. Then it was dried at 60°C for 12 h and allowed to cool in a desiccator before being re-weighed. The total content of carotenoids in the microalgal biomass was determined using the spectrophotometric method. Briefly, 5 ml of cells were harvested at via centrifugation (6000 rpm – 5.0 min) and extraction was carried out with 4:1 (v/v) dimethyl sulfoxide (DMSO): water at 55 °C for 1 h in the dark. The amount of total carotenoid (mg/L) was determined by measuring the light absorption at wavelengths of 480, 649, and 665 nm and calculated using the following equations (Wellburn, 1994):

$$Chlorophyll - a = 12.47\, A_{665} - 3.62\, A_{649} \quad (2)$$

$$Chlorophyll - b = 25.06\, A_{649} - 6.5\, A_{665} \quad (3)$$

$$Total\ carotenoid = (1000\, A_{480} - 1.29\, Chl\_a - 53.78\, Chl\_b)/220 \quad (4)$$

The specific growth rate (μ) (day^{-1}) of the microalgae was calculated using the equation (5);

$$\mu = \frac{lnX_2 - lnX_1}{t_2 - t_1} \quad (5)$$

where X_1 and X_2 (cells/ml) are the cell number at time 1 (t_1) (day) and time 2 (t_2) (day), respectively (Bailey & Ollis 1986). Furthermore, doubling time (DT) (day) was calculated as

$$DT = \frac{ln2}{\mu} \quad (6)$$

The biomass productivity was calculated (g/L/day) using Equation (7)

$$Biomass\ productivity = \frac{N_f - N_i}{\Delta t} \quad (7)$$

where N_i and N_f (g/L) are the initial and final biomass concentrations, respectively, and Δt (day) is the time of cultivation (Zhu et al., 2016).

All the experimental analyses were repeated at least two times and are presented in the figures and tables with the average values.

RESULTS AND DISCUSSION

One of the most critical aspects concerning mass cultivation of microalgae is the possibility of transition from lab-scale to the industrial level, and the first step in this process is performing an efficient scale-up strategy to enhance cultivation yield over that obtained at the lab-scale in terms of product and biomass yield. In this study, three different scale-up strategies, including mixing time (s), volumetric power consumption rate (W/m^3), and oxygen mass transfer coefficient (s^{-1}) were used for the biomass production from *H. pluvialis* cells in an airlift PBR. In order to provide high amount of green biomass, cultivation was transferred from 1-L cultivation bottle to the 2-L airlift PBR by taking into account geometric similarity.

Once the cells were cultivated in the aerated cultivation bottle, cell concentration significantly increased from the 4th day of cultivation and reached the highest amount (7.90 ± 0.15 × 10^5 cells/mL) at the end of production (Figure 2a). Among the PBR cultivations, the maximum cell concentration, 4.60 ± 0.20 × 10^5 cells/mL, was achieved in the constant P/V experiment. The cell concentration of 2.10 ± 0.20 × 10^5 cells/mL in constant t_m experiment was much lower than other strategies, and this result may be an effect of the lowest aeration rate of 0.9 L/min. This result was also sup-

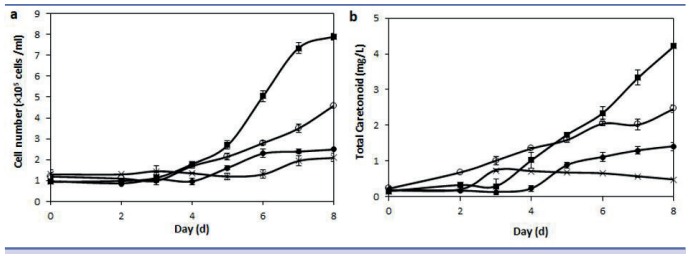

Figure 2. Growth profile and total amount of carotenoids for *H. pluvialis* cells in aerated cultivation bottle (■) and 2-L airlift PBR for constant volumetric power consumption rate (o), constant oxygen mass transfer coefficient (●) and constant mixing time (×), a) cell number; b) total carotenoid amount.

ported by the observation that *H. pluvialis* cells formed aggregates and collapsed at the bottom of the airlift PBR during cultivation in the constant t_m experiment because of insufficient aeration. According to the results obtained, the moderate aeration rate of 1.24 L/min in the constant P/V experiment led to higher cell concentration than was obtained in the other two scale-up strategies. This was due to the fact that a homogenous culture medium could not be provided because of low aeration in the constant t_m experiment, and during the constant k_La experiment, cells might have been exposed to shear stress and lost their flagella through bubble burst at the liquid-gas interface because of the higher aeration rate of 1.8 L/min. A similar result was observed for *Haematococcus alpinus*, an alpine strain of *Haematococcus*, where the moderate aeration rates did not affect cell growth negatively but further increases in airflow slowed cell growth and caused cell enlargement (Mazumdar et al., 2019). In addition, the transformation of vegetative green cells into red cysts was observed towards the end of culture in most PBR productions (results not shown). This may be associated with the fact that some cells became inactive and no further cell growth was achieved.

Total carotenoid amount showed similar changes with the cell concentration, as expected (Figure 2b). In the cultivation bottle, the total carotenoid amount obtained was 4.21 ± 0.11 mg/L at the 8th day of production. However, all of the PBR cultivations resulted in a decrease in total carotenoid amount and concentrations were found to be between the ranges of 0.5 - 2.5 mg/L. Among the PBR productions, maximum carotenoid concentration of 2.47 ± 0.10 mg/L was obtained in constant P/V criterion. It was an expected result because the chlorophyll and carotenoid contents of *H. pluvialis* cells show parallel changes with the growth profile during the green phase. Also, it is important to note that carotenoid concentration is strongly related to the lighting efficiency of cultivations due to the light harvesting role of the pigments during photosynthesis (Shah et al., 2016). Although airlift PBRs have a high illuminated surface area to volume ratio and thus more efficient lighting, cells may be exposed to light heterogeneously due to ineffective mixing. Homogenous cell distribution can be achieved by the increase of gas flow rate, but excessive hydrodynamic forces by aeration may cause shear stress to cells (Choi et al., 2018). In order to provide effective mixing, enhance the mass transfer, and prevent mechanical stress, air flow rate should be adjusted carefully together with light intensity considering biomass yield and accumulation of target product.

Table 1 shows the calculated kinetic parameters of cultivation bottle and airlift PBRs operating with three different scale-up strategies for green *H. pluvialis* cells. During the bottle experiment, cells showed significant growth, with the dry mass value of 0.62 g/L within eight days and the specific growth rate reaching 0.38 day^{-1}, which corresponds to a culture doubling time of 1.79 days. Among three scale-up strategies, maximum biomass amount (0.49 g/L) and maximum growth rate (0.31 day^{-1} (doubling time of 2.27 days)) were found in the constant P/V criterion. In a similar study, the effect of the airflow rate on the growth of *H. pluvialis* cells in an internal-loop airlift PBR was investigated and

Table 1. Kinetic parameters for the cultivation of *Haematococcus pluvialis* cells.

Cultivation	Aeration rate (L/min)	Dry weight (g/L)	Biomass productivity (g/Lday)	Specific growth rate (day^{-1})	Doubling time (day)
Cultivation bottle	3	0.62	0.077	0.38	1.79
t_m	0.9	0.17	0.021	0.07	9.35
P/V	1.24	0.49	0.061	0.31	2.27
k_La	1.8	0.35	0.044	0.17	3.99

four different aeration rates, including 0.25, 0.5, 0.75, and 1.1 vvm, were used. Cell growth decreased when aeration was increased above a certain value and maximum specific growth rate of 0.23 day^{-1} was obtained at 0.5 vvm airflow rate (Vega-Estrada et al., 2005). In another study, *H. pluvialis* cells were cultivated in an airlift PBR supplemented with CO_2 and gave a maximum specific growth rate of 0.317 day^{-1} (Haque et al., 2017), which is comparable to that reported in the present study. Biomass productivity values obtained were in parallel with the dry mass amount and the minimum productivity of cells was recorded in the constant t_m strategy as 0.021 g/L day. Overall, obtained kinetic values were in parallel with cell growth profile and carotenoid accumulation where constant P/V strategy was the most effective cultivation than other scale-up procedures.

CONCLUSION

Mass cultivation of microalga in controlled PBR provides remarkable advantages compared with open ponds, considering process control, contamination risk, and operation conditions. From the engineering and biological points of view, a systematic scale-up procedure is essential to selecting the most suitable conditions depending on the cultivation system, microalga species, optimal growth conditions, and economic feasibility during transition to the industrial scale. In the present study, scaling up from an aerated cultivation bottle to the airlift PBR was evaluated for the biomass production of *H. pluvialis* using three different scale-up strategies: constant t_m, constant P/V, and constant k_La. According to the findings obtained, constant P/V strategy provided the most efficient production for biomass production and total carotenoid accumulation due to enhanced mixing, mass and heat transfer, and dispersion of light. However, enlarging the system from 1-L to 2-L caused a decline in performance in terms of biomass and carotenoid productivity. The decrease in growth rate and carotenoid amount in PBR cultivation might be related to the insufficient aeration rate, high shear stress due to bubble coalescence, or improper reactor configuration in terms of the column and draft tube length, which led to uneven densities of fluid flow. These data were also supported by the observations of *H. pluvialis* cells forming a conglomeration and collapsing at the bottom of the PBR, and the transformation of vegetative green cells into red cysts towards the end of culture. The results ob-

tained in this study will be considered in order to design optimal PBR configuration and determine more suitable operation conditions for the large-scale production of *H. pluvialis* biomass. In this context, draft tube structure, illuminated surface area to volume ratio, and A_d/A_r value may be modified and different scale-up strategies may be applied to find the proper aeration rate in order to achieve more effective cultivation.

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Ethics committee approval: There was no need for ethics committee approval.

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