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Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2021; 7(2): 90-95 Araştırma Makalesi/Research Article

Indoor Growth Performance of *Chlorella* sp. Production at Tubular Photobioreactor

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Abstract

Microalgae are known as a source of valuable biomolecules which are used in various industrial fields such as aquaculture, food, feed, pharmaceuticals, bio-fertilizers and bioenergy. *Chlorella* sp. is one of the common microalgae, cultured in the world. In this study, it was examined that growth rate and pigment contents of *Chlorella* sp. in lab-scale tubular photobioreactor at in-door conditions. Highest cell number and highest specific growth rate were determined as 155 x 10^6 cells.mL^{-1} and 0.79, respectively. Highest dry weight was measured as 4.19±0.059 g.L^{-1} and mean dry weight was found as 3.56±0.079 g.L^{-1}. Highest chlorophyll-*a* content was found at 40th day as 106.7±0.079 µg.mL^{-1}. Highest total carotenoids was 15.87±0.033 µg.mL^{-1} at the day 22. Also, 983.8 g of total biomass was harvested in last 45 days, after the exponential phase. According to the results of this study, in-door production of *Chlorella* sp. Was provided more reliable sustainability. Also, *Chlorella* sp. is photoautotrophically producible at high amounts throughout the year.

Tübüler Fotobiyoreaktörde *Chlorella* Sp. Kültürünün İç Mekanda Büyüme Performansı

Makale Bilgisi

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Anahtar Kelimeler:
- Mikroalg
- *Chlorella* sp.
- Fotobiyoreaktör
- Klorofil
- Pigmentler

Öz

Mikroalgler sahip oldukları değerli biyomoleküller ile akuakültür, gıda, yem, farmasötik, gübre ve biyo-enerji gibi farklı endüstriyel alanlarda kullanılan bir kaynaktır. *Chlorella* sp., dünyada kültürü yapılan en yaygın mikroalglerden biridir. Bu çalışmada *Chlorella* sp.'nin laboratuvar ölçekli tübüler fotobiyoreaktörde kapalı alan kültüründe büyüme ve pigment değerleri araştırılmıştır. En yüksek hücre sayısı ve spesifik büyüme oranı 155 x 10^6 hücre.mL^{-1} ve 0.79 olarak ölçülmüştür. En yüksek kuru ağırlık 4.19±0.059 g.L^{-1} ve ortalama kuru ağırlık 3.56±0.079 g.L^{-1} olarak belirlenmiştir. En yüksek klorofil a miktarı 40. günde 106.7±0.079 µg.mL^{-1} olarak ortaya çıkmıştır. En yüksek toplam karotenoid değeri ise 15.87±0.033 µg.mL^{-1} olarak 22. gün gerçekleşmiştir. Ayrıca üstel fazın ardından, son 45 gün içerisinde 983.8 gram toplam biyokütle elde edilmiştir. Çalışma, *Chlorella* sp.'nin kapalı alan üretiminin daha güvenilir bir sürdürülebilirlik sağladığını göstermiştir. *Chlorella* sp., yıl boyunca yüksek miktarlarda fotoototrofik olarak üretilebilirdir.

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INTRODUCTION

Sustainability become much more important than ever in last recent years. Need of higher amounts of raw materials and depletion of natural resources are the main reasons of that result. However, certain organisms such as microalgae, might be the solution of this problem. High productivity, sustainability and valuable metabolites make these organisms unique resources (Gouveia and Oliveira, 2009; Ruiz et al., 2016).

Microalgae are used as resources in various industrial applications such as aquaculture, food industry, agriculture, cosmetics, feed and bioenergy (Yaakob et al., 2014; Sirakov et al., 2015; Mourelle et al., 2017; Palabıyık et al., 2018; Durmaz et al., 2020). Essential and polyunsaturated fatty acids, high amounts of protein and pigments make microalgae biomasses and their products more demanded raw materials. *Chlorella* sp. is one of the most produced microalgae species after *Spirulina*. Their

cellular composition, high productivity and ability of heterotrophic and mixotrophic growth are the main reasons related with this high demand.

Microalgae are single cell photosynthetic, microscopic organisms which are consume CO_2 as a carbon source. Flasks, bags, ponds, photobioreactors (PBRs) and fermenter systems are used for cultivation of these microorganisms. Every cultivation system has both advantages and also disadvantages. For instance, while ponds require low installation and operational advantages, photobioreactors provide higher yields and reliable sustainability. Also, outdoor and in-door production systems varies by each other with advantages/disadvantages such as illumination costs. Outdoor production benefits from sunlight and no need artificial illumination and therefore electricity costs may be lower than the other systems. However, this production is highly dependent on environmental conditions. Consequently, sustainable production of microalgae requires fully controlled cultivation environment and production process (Olaizola, 2003). Through this, in-door production emerges as the optimum solution for year round sustainable production of microalgae. In this study, in-door production of *Chlorella* sp. at laboratory scale tubular PBR was investigated.

MATERIALS and METHODS

Cultures

Chlorella sp. strain was provided from Ege University, Faculty of Fisheries (Izmir, Turkey). Stocks were cultured in 5 L flasks with BG-11 medium. Cultures were maintained at room temperature (20-22 °C) and illuminated by fluorescent lamps with 70 $\mu mol.m^{-2}.s^{-1}$. BG-11 medium was used for both stocks and tubular PBR experiment. Medium was prepared at Kastamonu University, Faculty of Fisheries (Kastamonu, Turkey) (Table 1 & 2).

Table 1. BG-11 medium

Solution 1	**0.5 L**
$NaNO_3$	75.0 g
Solution 2	**0.5 L**
K_2HPO_4	2.0 g
$MgSO_4.7H_2O$	3.75 g
$CaCl_2.2H_2O$	1.80 g
Citric acid	0.30 g
Ammonium ferric citrate	0.30 g
$EDTANa_2$	0.05 g
Na_2CO_3	1.00 g

Table 2. Trace elements solution of BG-11 medium

Trace elements solution	**1 L**
H_3BO_3	2.86 g
$MnCl_2.4H_2O$	1.81 g
$ZnSO_4.7H_2O$	0.22 g
$Na_2MoO_4.2H_2O$	0.39 g
$CuSO_4.5H_2O$	0.08 g
$Co(NO_3)2.6H_2O$	0.05 g

Properties of Tubular PBR

Tubular PBR system (Model, Producer, City, Country) consists of 2 parts; transparent tubes and the reservoir tank. Total system volume is 140 L. System was illuminated by fluorescent lamps (45 $mmol.m^{-2}.s^{-1}$) between the tubes and also low CRI led lights with 130 $mmol.m^{-2}.s^{-1}$ were placed in front of the tubular system (4 x 100 W) for supporting the illumination (Figure 1). Light intensities were measured by Apogee MQ-620 quantum meter. System was operated at room temperature (20-22 °C) during the experiment period. System pH and temperature were tracked by sensors of JBL ProFlora pH/CO_2 controller. Also, system pH was controlled by the same device with automatic injection of pure CO_2 gas and was held at 8.00±0.05.

Figure 1. *Chlorella* sp. production at tubular PBR

Tubular PBR system was disinfected by adding sodium hypochlorite. After first 24 hours, sodium thiosulfate was added into the system for neutralization of the chloride. Then, *Chlorella* sp. Stocks were inoculated to the Tubular PBR system.

Growth and Dry Weight

Cell density was determined under microscope by using Neubauer haemocytometer. Growth rate was calculated with the formula given below.

$$\mu = \frac{\ln(N_t) - \ln(N_0)}{t - t_0}$$

0.45 µm filter papers were dried in the oven at 105 °C for 2 hours and were weighted. After that, 5 mL samples were filtered and were dried in the oven at same temperature until there was no change at weights (Zou and Richmond, 1999).

Pigment Analysis

Chlorophyll-*a* and total carotenoid contents were determined spectrophotometrically. 5 mL of samples were centrifuged, and supernatant was discarded. After that, 5 mL methanol and glass beads were added to the samples and mixed by vortex. Mechanical agitator was used for cell disruption. After this step, samples were placed into the ultrasonic bath. Lastly, samples were centrifuged, and supernatants were taken to determine absorbance values at defined wave-length by using a spectrophotometer (Hach DR 6000). Chlorophyll *a* and total carotenoids were calculated according to the Equation 1 and 2, respectively (Sanchez et al., 2005; Zou and Richmond, 2000);

Chlorophyll *a* (µg/ml) = 13.9 A_{665}^{*} (Eq. 1)

$^{*}A_{665}$; absorbance value at 665 nm

Total carotenoids (µg/ml) = 4.5 A_{475}^{*} (Eq. 2)

$^{*}A_{475}$; absorbance value at 475 nm

RESULTS

Starter cell density of tubular PBR experiment was 0.925 x 10^6 cells.mL^{-1}. Cells were profilirated rapidly and cell number was reached to 41 x 10^6 cells.mL^{-1} at 13th day. Specific growth rate was calculated as 0.316 in that growth phase. After that, culture cell number was varied between 93.5-155 x 10^6 cells.mL^{-1} until the end of the experiment. Highest cell number was determined as 155 x 10^6 cells.mL^{-1} while highest specific growth rate was calculated as 0.79. Mean cell number and specific growth rate were calculated as 93.2 x 10^6 cells.mL^{-1} and 0.078 (Figure 2).

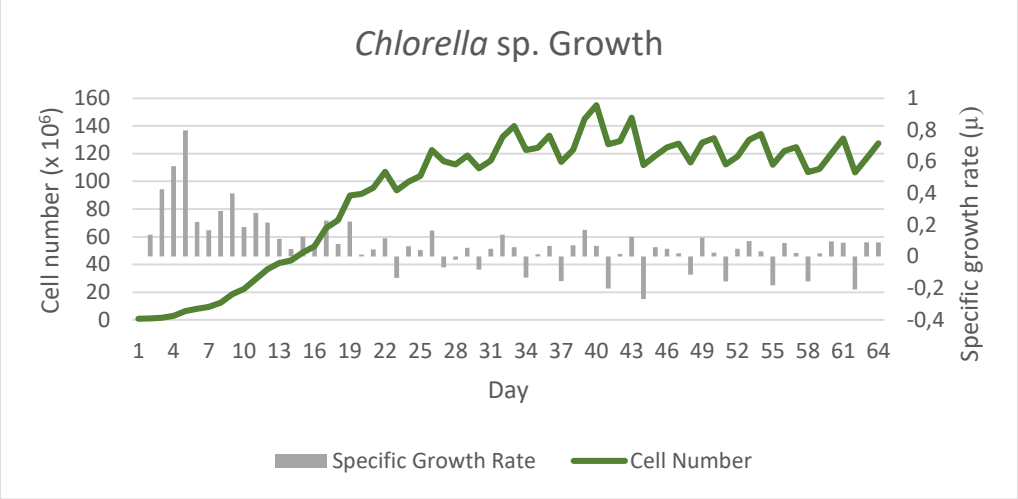

Figure 2. *Chlorella* sp. cell numbers and specific growth rates at lab scale indoor tubular PBR.

Harvest regimen was started at 5th day between 5% and 15% harvested regimes in the total culture. 275 L culture was harvested and 8.5 L medium was added, during the whole experiment. Highest dry weight was measured as 4.19±0.059 $g.L^{-1}$ and mean dry weight was found as 3.56±0.079 $g.L^{-1}$. Totally, 983.8 g *Chlorella* sp. was harvested in 64 days of culture period (Figure 3).

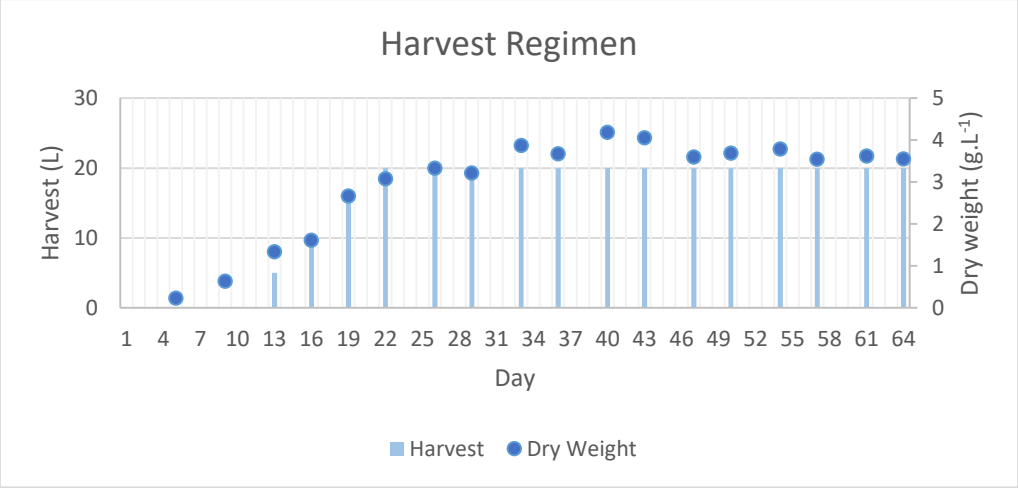

Figure 3. *Chlorella* sp. harvest amounts and dry weights at lab scale indoor tubular PBR.

Chlorophyll-*a* content of *Chlorella* sp. was 7.42±0.08 $\mu g.mL^{-1}$ at 9th day. Highest chlorophyll *a* amount was found at 40th day as 106.7±0.079 $\mu g.mL^{-1}$. Lastly, mean chlorophyll *a* amount was calculated as 69.2±0.051 $\mu g.mL^{-1}$ for the experiment period (Figure 4).

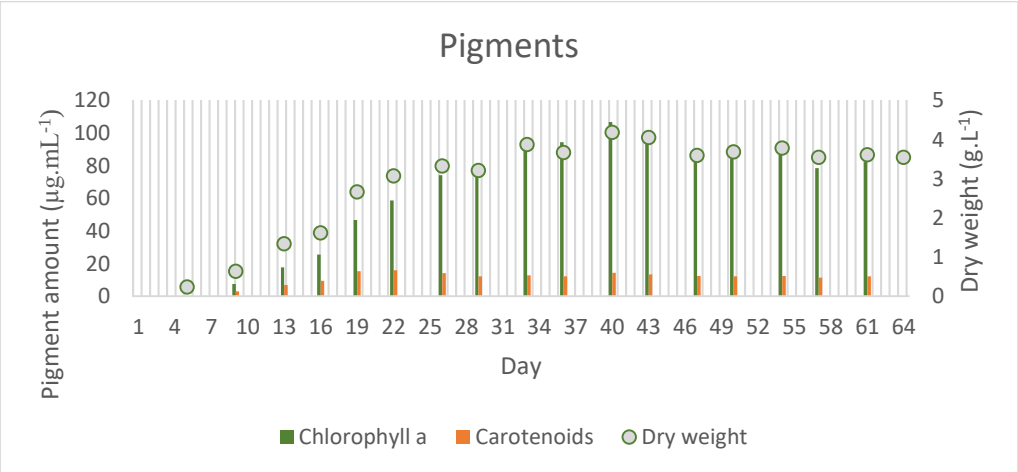

Figure 4. *Chlorella* sp. pigment amounts per mL dry weights at lab scale indoor tubular PBR.

Total carotenoid contents were also determined. According to the results, at the first measurement (day 9) total carotenoids was found as 2.91±0.009 µg.mL^{-1}. Highest carotenoid content was 15.87±0.033 µg.mL^{-1} at the day 22 and mean carotenoid content was calculated as 11.81±0.069 µg.mL^{-1} (Figure 4).

Also, cellular dry weight and cellular pigment amounts were calculated according to the dry weights, pigment amounts and cell numbers. Highest cellular dry weight was calculated for the day 5 as 36.6 pg.cell^{-1} and lowest cellular dry weight was found as 27.01 pg.cell^{-1} for the day 40. Lastly, mean cellular dry weight was found as 29.18 pg.cell^{-1}. Cellular chlorophyll-*a* amount was varied between 0.399-0.709 pg.cell^{-1}. Highest cellular chlorophyll-*a* content was found at 36th day while lowest chlorophyll *a* amount was found at the day 9. Mean chlorophyll-*a* content was determined as 0.595 pg.cell^{-1}. Total carotenoid content per cell amount was found at the highest level as 0.179 pg.cell^{-1} at 16th day. Lowest carotenoids was 0.091 pg.cell^{-1} at the day 43 and mean carotenoids was determined as 0.12 pg.cell^{-1} (Figure 5).

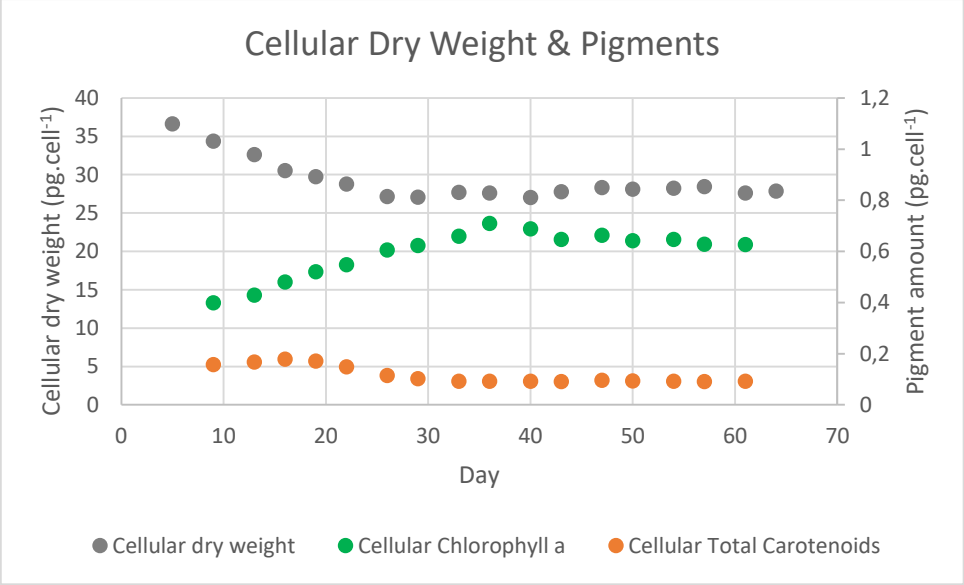

Figure 5. *Chlorella* sp. cellular dry weights and pigment amounts per cell.

DISCUSSION

In a recent study, nutrient reclamation for Chlorella vulgaris was investigated. Culture was done with artificial illumination and it was found that *Chlorella vulgaris* concentration was reached to 1.1 g.L^{-1} level (Chang et al., 2018). It is stated that maximum dry weight was 1.84 g.L^{-1} of *Chlorella vulgaris* produced at membrane PBR (Gao et al., 2019). A study shows that *Chlorella sorokiniana* was reached to 146 x 10^6 cells.mL^{-1}. Also, it was indicated that maximum dry weight was found as 3.45 g.L^{-1} with high level of NO_3 concentration (Ziganshina et al., 2020). Wong et al., (2016) was determined that *Chlorella vulgaris* may reach to 0.8 g.L^{-1} at column bubbling PBR. It was stated that 3.66 g.L^{-1} dry weight of *Chlorella vulgaris* was maximum at PW-PBR (Liao et al., 2017). In this study *Chlorella* sp. was produced at in-door lab scale tubular PBR for 2 months. According to the results, *Chlorella* can be growth in-door intensively much as 4.2 g.L^{-1}. Culture density was not fluctuated after exponential phase and mean cell number was calculated as 121.2 x 10^6 cells.mL^{-1} for stationary phase. Also, 3.63 g.L^{-1} mean dry weight was found for that period.

Chlorophyll-*a* concentration of *Chlorella vulgaris* at PBR was determined as 15.46 ± 1.05 mg.L^{-1} by Chang et al., (2018). In another study, Liao et al., (2017) was determined that highest chlorophyll-*a* accumulation of *Chlorella vulgaris* was 99.29 mg.L^{-1} at PW-PBR. Lower chlorophyll-*a* content of *Chlorella vulgaris* was found at flat-plate PBR experiment. It is stated that chlorophyll-*a* yield was 4.5 mg.L^{-1} (Lakaniemi et al., 2011). In this study, maximum chlorophyll-*a* content was determined as 106.7±0.079 mg.L^{-1}. It is concluded that culture density was the main factor of variations of maximum chlorophyll-*a* content between various studies. Cellular accumulation amounts might be more useful when tracing the changes in cellular composition. In our study, cellular chlorophyll *a* amount was increased while cellular dry weight and cellular total carotenoids content were decreased. Chlorophyll-*a* per cell increase might be the result of cellular response to culture density. It is expected that mutual shading will increase as the cell number increases. Thus, cells will have lower oppurtinity to get energy from the illumination for photosynthetic reactions. Decrease in cellular carotenoids level can be explained with low stress conditions during the experiment period. Also, decrease in cellular dry weight supports the inverse ratio between cell number and cellular dry weight was reported by Zou & Richmond, (1999).

2 months of in-door production of *Chlorella* sp. at tubular photobioreactor was done in this study. After exponential phase 3.63 g.L^{-1} dry weight was obtained without any stress conditions. Study shows that in-door production of *Chlorella* sp. provided more reliable sustainability.

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