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EFFECT OF LIGHT INTENSITY ON THE GROWTH OF *CHLORELLA VARIABILIS*

(IŞIK ŞİDDETİNİN CHLORELLA VARIABILIS'İN BÜYÜMESİNE ETKİSİ)

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ABSTRACT

Microalgae are increasingly employed for production of a wide range of industrial chemicals. Light intensity is an important parameter affecting microalgae cell growth. Analysis of light penetration into the algae culture broth is important for an efficient photobioreactor design. In this study, effect of light intensity on the growth of microalgae species Chlorella variabilis was investigated in a compartmentalized photobioreactor system. The optimal range of light intensity for highest growth rate (0.037 h^{-1}) and cell concentration (0.30 gdw/L) was found to be 17 - 36 klux, lower light intensities yielded in significantly slower growth and lower cell concentrations. Photoinhibition effect was clearly observed at light intensities above 36 klux. Maximum photobioreactor depth which can provide 95% of the maximum growth rate was determined to be 8 cm.

Keywords: Chlorella variabilis, Photobioreactor, Light intensity

ÖZ

Mikroalgler çeşitli endüstriyel kimyasalların üretiminde artarak kullanılmaktadır. Işık şiddeti mikroalg hücre büyümesini etkileyen önemli bir değişkendir. Işığın alg kültürüne nüfuzunu incelemek verimli bir fotobiyoreaktör tasarımı için önemlidir. Bu çalışmada, mikroalg türü olan Chlorella Variabilis'in büyümesine ışık şiddetinin etkisi bölümlendirilmiş bir fotobiyoreaktör sisteminde incelenmiştir. En yüksek büyüme hızı (0.037 h^{-1})ve hücre derişimi (0.30 gdw/L) için ideal ışık şiddeti aralığı 17-36 klüks olarak bulunmuştur, daha düşük ışık düzeyleri büyüme hızı ve hücre derişimini önemli ölçüde düşürmektedir. Fotoinhibisyon 36 klüks üzerindeki ışık şiddetlerinde açıkça gözlenmiştir. En yüksek büyüme hızının %95'ini sağlayabilecek en yüksek fotobiyoreaktör derinliği 8 cm olarak belirlenmiştir.

Anahtar Kelimeler: *Chlorella variabilis, Fotobiyoreaktör, Işık şiddeti*

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1. INTRODUCTION

Microalgal biotechnology is one of the emerging fields in biotechnology era. Recently, there has been a great interest using microalgae as sunlight-driven cell factories that convert carbon dioxide to, foods, feeds, high-value bioactives, andbiofuels such asbiodiesel derived from microalgal oil [1-2].

Light is an essential substrate for photoautotrophic growth of microalgae. A major limitation of microalgae cultivation is that the photons cannot penetrate deeply into the culture broth due to the self-shading of the cells even when enough photons are supplied at illumination surface [2]. Longer light penetration depth will increase the overall light utilization efficiency and thus algal productivity. The light penetration depth is a critical parameter for a successful photobioreactor [3].

Algae can be grown with exposure to natural or artificial light. Artificial lighting techniques have provided insight into how algae respond to varying light conditions [4]. The level of light intensity is critical because at a certain level algae experience light saturation and dissipate the excess energy as heat [5]. Light saturation can be mitigated by the spatial dilution of light, which also reduces mutual shading of cells in the culture, which results in higher growth rates and lower content of accessory pigments [6]. On the other hand, the biomass density affects both the light intensity and the light penetration. Optimal cell density is specific to each strain and needs to be maintained in order for light intensity and light penetration to remain at optimal levels [7].

Above a certain value of light intensity, a further increase in light level actually reduces the biomass growth rate. This phenomenon is known as photo inhibition. Microalgae become photoinhibited at light intensities only slightly greater than the light level at which the specific growth rate peaks. Photoinhibition results from generally reversible damage to the photosynthetic apparatus, as a consequence of excessive light. Elimination of photoinhibition or its postponement to higher light intensities can greatly increase the average daily growth rate of algal biomass [1].

Analysis of light penetration into the algae culture broth is therefore important, and change of light intensity inside a photobioreactor as a function of depth needs to be shown for an efficient photobioreactor and illumination system design.

In this study, effect of incident light intensity on the growth of microalgae species *Chlorella variabilis* was investigated. For this purpose, a compartmentalized photobioreactor system was devised to analyze the change of light intensity inside the photobioreactor as a function of depth. As a result, the optimal range of light intensity for peak algal growth rate was found, photoinhibition region was shown, and maximum useful photobioreactor depth was determined.

2. MATERIAL AND METHODS

Microalgae strain *Chlorella variabilis* donated by Dr. Turgay Çakmak (Medeniyet University) was used in this study. The strain was grown in BG11 medium to have the following composition (g/L): $NaNO_3$, 1.5; KH_2PO_4,0.04; $MgSO_4.7H_2O$, 0.075; $CaCl_2.2H_2O$, 0.036; H_3BO_3 0.003; Fe(III)citrate, 0.006; citric acid 0.006. The pH of the medium was 6.8.

Spectrophotometer cuvettes with 2 ml working volume and 1 cm light path was used as growth chambers (photobioreactors). Illumination was provided by a 7W 2700K LED lamp.

The experimental setup was shown in Figure 1. The sides of the system were closed to prevent indirect illumination.

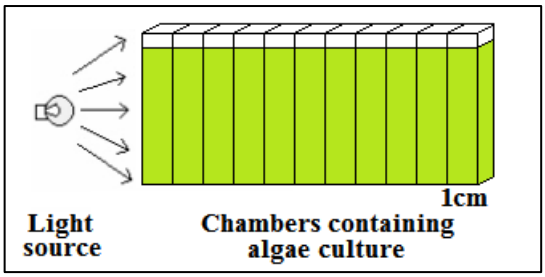

Figure 1. Experimental setup

Light intensity measurements were made by a light meter (Extech Instruments). The temperatures inside the photobioreactors were measured by using digital thermometer probes. A pH-meter (Mettler-Toledo) was used to measure pH.

The cell concentration was determined spectrophotometricaly at 660 nm using Shimadzu UV-1201 Spectrophotometer. An optical density of 1.0 at 660 nm was found to correspond to a biomass concentration of 0.21 gdw/L_c for this algae strain. Three percent inoculation from freshly grown algae was made to the culture medium.

Growth rate was calculated as $\mu = \ln(X_2/X_1)/(t_2-t_1)$ where X is the cell concentration (gdw/L), t is the time (hour). Maximum growth rate (μ_{max}) is the growth rate obtained during the logarithmic phase of the growth.

3. RESULTS AND DISCUSSION

After the inoculation, the effect of incident light intensity on the algae growth was clearly seen (Figure 2) during the run.

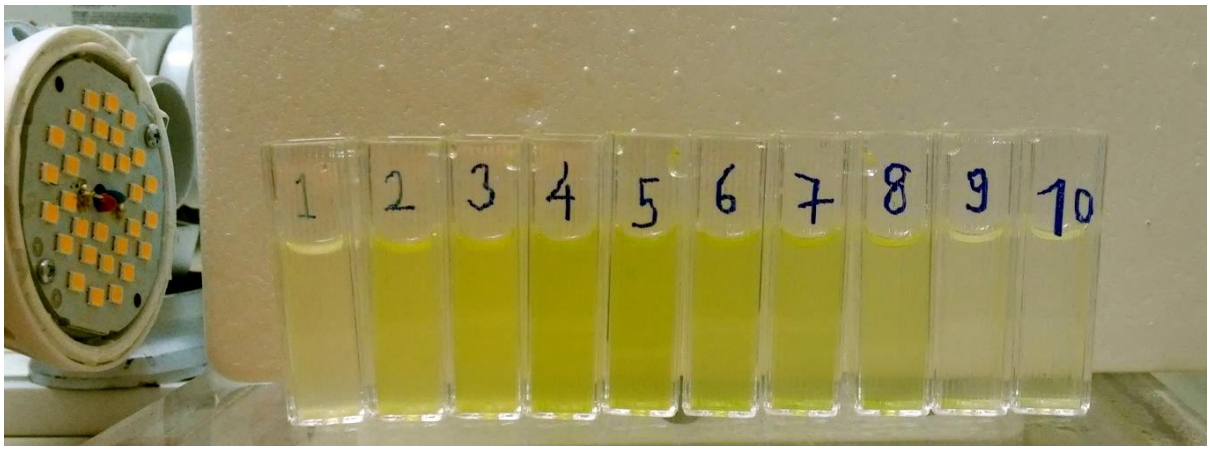

Figure 2. Growth of *C. variabilis* 6 days after inoculation

Growth data was collected on 3rd, 6th, 9th, 13th days of the process to form a growth curve, which was illustrated in Figure 3.

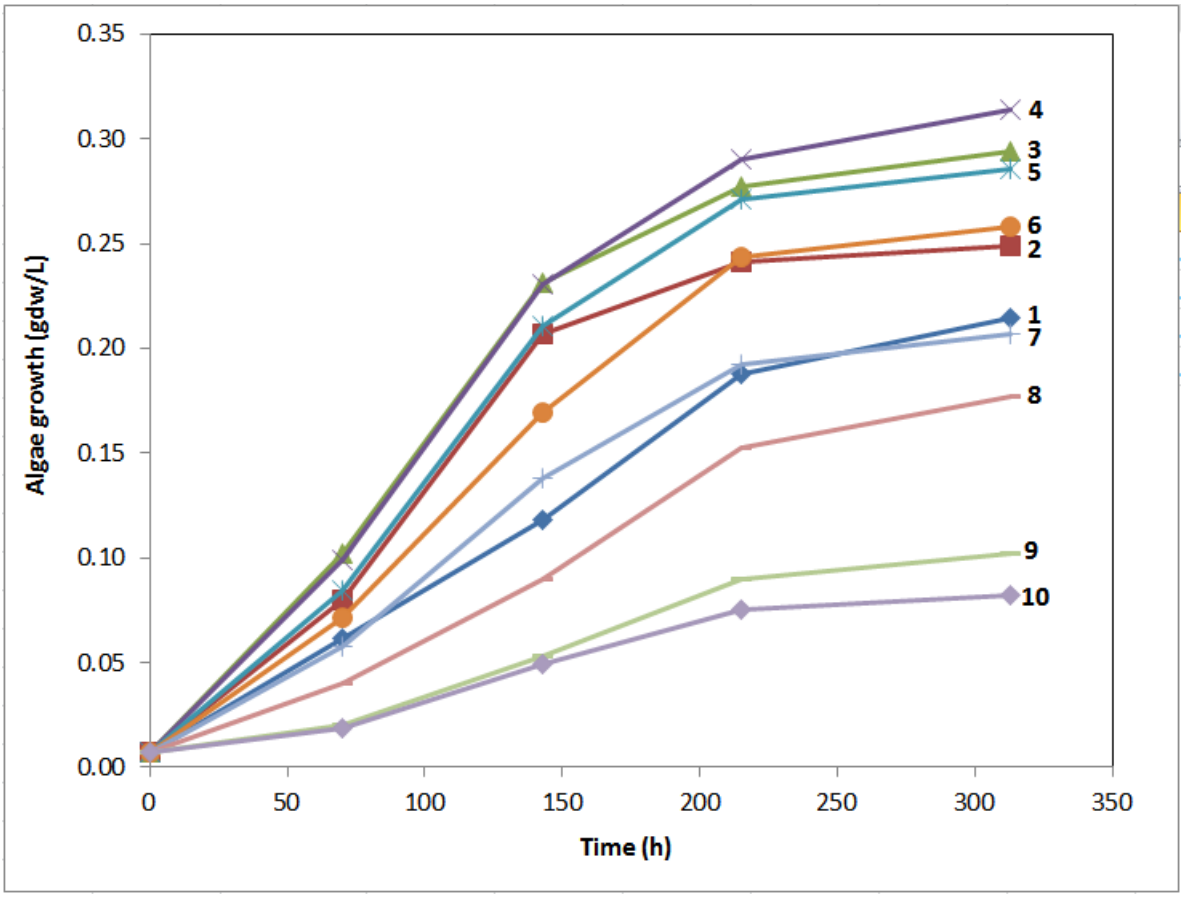

Figure 3. Growth of *C. variabilis* under different light intensities (1: 80 klux, 2: 50 klux, 3: 36 klux, 4: 25 klux, 5: 17 klux, 6: 12 klux, 7: 8.5 klux, 8: 6.4 klux, 9: 4.5 klux, 10: 3.6 klux)

Both the maximum cell concentration (X_{max}) and maximum growth rate (μ_{max}) were found to be depending on the light intensity received (Table 1). Compared to the light intensities, the difference in the temperature of the cultures is too small (Table 1), and most possibly can not explain the difference in the growth alone.

Table 1. Maximum cell concentration and maximum growth rate at different light intensities

Light intensity (klux)	Temperature (°C)	X_{max} (gdw/L_c)	μ_{max} (h^{-1})
80	27.8	0.22	0.031
50	26.6	0.25	0.035
36	25.0	0.29	0.038
25	24.2	0.31	0.038
17	23.3	0.29	0.036
12	23.0	0.26	0.033
8.5	22.8	0.21	0.030
6.4	22.6	0.18	0.025
4.5	22.4	0.10	0.015
3.6	22.4	0.08	0.014

At low values light intensity was the limiting factor for the growth, at high values photoinhibition was observed which also had a negative impact on the growth (seen in Figure 2, can be deducted from Figure 3, better visualized in Figure 4).

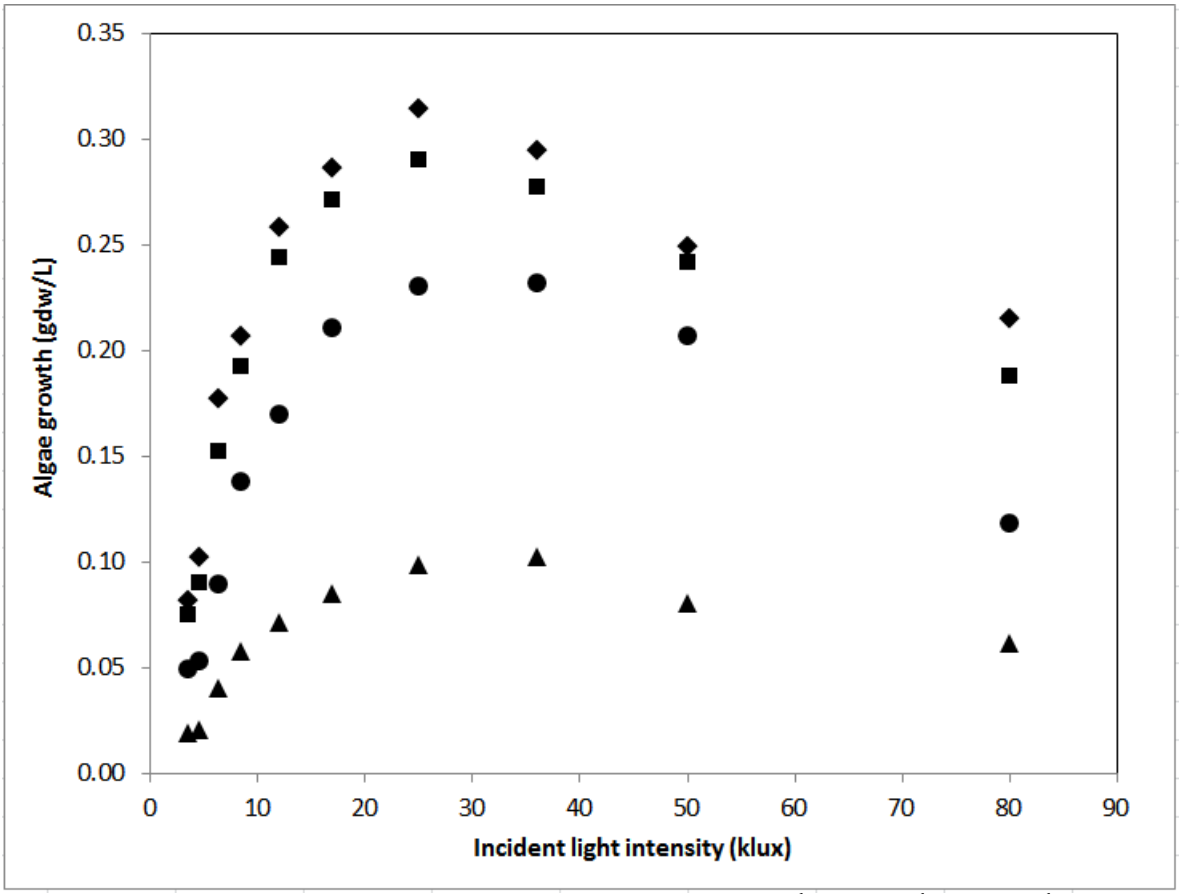

Figure 4. Growth of *C. variabilis* under different light intensities (▲ : 3rd day, ● : 6th day, ■ : 9th day, ◆: 13th day)

Photoinhibition was clearly observed in the first two centimeters (illumination >36 klux), yielding in low growth and cell concentration. At 17-36 klux illumination range (between 3-6 centimeters), growth rate and cell concentrations obtained were quite close, 0.29-0.31 h^{-1} and 0.036-0.038 gdw/$L_c$respectively. After 7th centimeter (illumination <17 klux) both growth and cell concentration decreased considerably.

A comparable study with *Chlorella sorokiniana* at 5.5, 11.1, 16.7, 22.2 klux light intensities revealed that at 5.5 klux the growth of the algae was photo limited, whereas at the other three conditions growth patterns were similar [8].

Based on this data, the optimum panel photobioreactor depth that can be utilized for maximum algae growth on condition that 36 klux illumination is provided from both sides of the panel was calculated and illustrated in Figure 5.

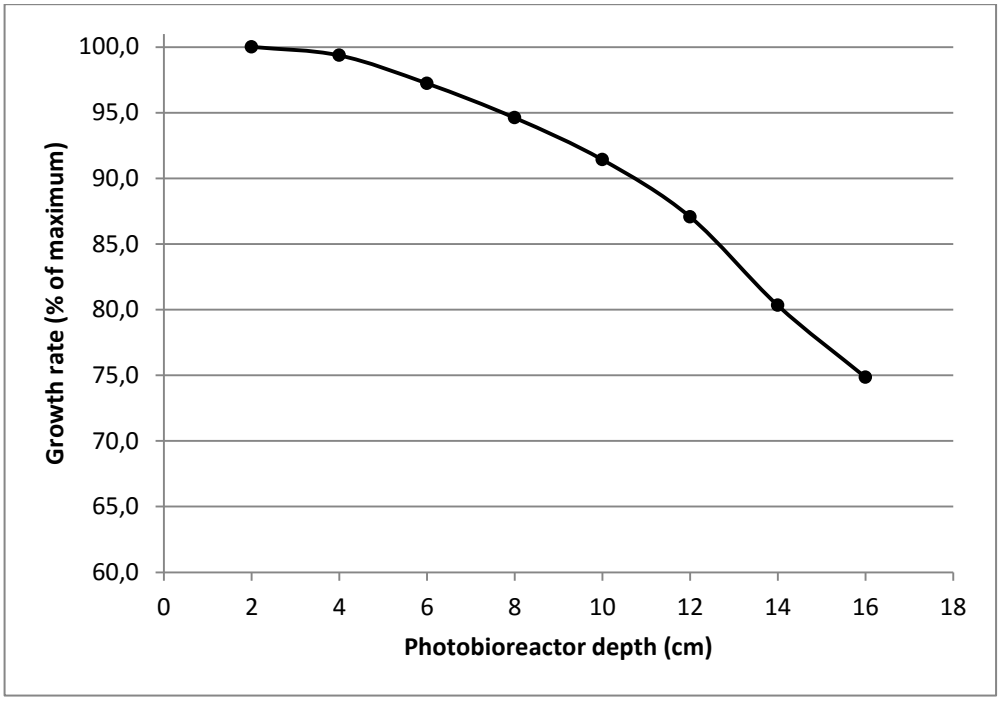

Figure 5. Effect of photobioreactor depth on growth rate (36 klux illumination from both sides of the panel)

It can be deduced that the photobioreactor depth should not exceed 8 cm if 95% of maximum growth rate is desired, the depth can be increased to 10.5 cm if 90% of maximum growth rate is enough. It should be noted that in addition to the growth rate, the volume and material cost of the photobioreactor are other factors to be considered when deciding the depth of the photobioreactor.

A comparable result was reported by Das et al (2013); when *Chlorella sorokiniana* was used, light was found mostly within 3 cm of the culture suspension irrespective of incident light intensity and cell concentration [8], which suggests 6 cm panel depth when illuminated from both sides.

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