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The effects of photoperiod on the growth, protein amount and pigment content of *Chlorella vulgaris*

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Abstract

The well-known correlation between algae and renewable energy sources and in this fact has brought about a great interest for seeking growth conditions of algae. Light is the one of the most important factor for growth and contents of algae. In this study, the effect of different light regimes on the growth rate, chlorophyll and protein amounts were determined. And the results were indicated significant varieties in culture at different light/dark period. The maximum growth rate was 16/8 h light/dark period. Also, when we have investigated chlorophyll and protein contents, we obtained remarkable diversity under different regimes. These observations suggest that the maximum chlorophyll and protein amounts (235, 53; 386, 5 µg/mL) were 20/4 h periods.

Keywords: *Chlorella vulgaris,* Light regime, Cell numbers, Chlorophyll, Protein

Chlorella vulgaris'in gelişimi, protein miktarı ve pigment içeriği üzerinde fotoperiyodun etkisi

Özet

Algler ve yenilenebilir enerji kaynakları arasındaki ilişki, alglerin büyüme şartlarının araştırılmasının ana nedenini oluşturmaktadır. Işık, alglerin gelişim ve içeriğine etki eden en önemli faktörlerden birisidir. Bu çalışmada, farklı ışık rejimlerinin *Chlorella vulgaris*'in büyüme oranı, protein ve klorofil miktarına olan etkisi incelenmiştir. Araştırmadan elde edilen sonuçlar farklı aydınlık ve karanlık peryotlarda geliştirlen kültürlerde, hücre sayısı, klorofil ve protein miktarında önemli değişimlerin olduğunu ortaya koymuştur. Maximum büyüme oranı 16/8 saatlik peryotta gerçekleşmiştir. Aynı zamanda protein ve klorofil miktarlarının da farklı ışık peryotlarında dikkate değer değişimler gösterdiği belirlenmiştir. Maximum protein ve klorofil miktarına (235, 53; 386, 5 µg/mL) 20/4 saatlik peryotta ulaşıldığı gözlemlendi.

Anahtar Kelimeler: *Chlorella vulgaris*, Işık Rejimi, Hücre sayısı, Klorofil, Protein

1. Introduction

Global warming, CO_2 release, over-utilizing of petroleum fuels and other problems have appeared in the last years that convince us to think completely for green future, renewable energy and to improve public cognizance. It is well known that fossil fuels will not remain for a long time because of the dangerous accumulations of "greenhouse gas" CO_2 and due to consume resources, depending on that it's very important to explore renewable energy sources that are not harmful to the environment such as; biofuels and solar wind energy [1].

Algae have the force to transform the energy industry, providing a solution to transform the present systems for biofuels production and permit new application of present technologies, provided that one can develop its production cost to a point competitive with fossil fuels [2]. It is a known fact that, physical and chemical variability are important structural forces in communities.

Especially, fluctuations in physical, chemical factors and resources should have a strong effect on community structure and life in aquatic ecosystems, due to the strict connection between physical forcing and biota. Light is a major resource for algae and has a complex pattern of spatial and temporal variability in aquatic ecosystems.

The culture of microalgae requires a rigorous control of all growth factors: CO_2, O_2 and light. The main factor in mass culture technology of microalgae is optimization of the yield. Light situations effect directly photosynthesis and the cells growing of microalgae. Algae need in appropriate photoperiod for efficient photosynthesis, it is need light for a photochemical phase to produce (ATP)

adenosine triphosphate, NADP-oxidase and also need for biochemical phase synthesize that are necessary molecules for growth [1].

In order to optimize algal growth in mass culture, the effects of photoperiod on the growth and pigment amount of *Chlorella vulgaris* was investigated in cultures.

2. Material and Methods

Chlorella vulgaris was collected from Keban Dam Lake. *C. vulgaris* was separated from water samples by micropipettes in the laboratory. *C. vulgaris* samples were inoculated to Jaworski medium [4]. The medium consisted of the following components (per liter of distilled water): 36 mg $Na_2HPO_4.12H_2O$, 80 mg $NaNO_3$, 12.4 mg KH_2PO_4, 20 mg $Ca(NO_3)_2.4H_2O$, 50 mg $MgSO_4.7H_2O$, 15.9 mg $NaHCO_3$, 2.25 mg EDTAFeNa, 2.25 mg $EDTANa_2$, 2480 µg H_3BO_3, 1390 µg $MnCl_2.4H_2O$, 1000 µg $(NH_4)_6Mo_7.4H_2O$, 40 µg cyanocobalamin (B_{12}), 40 µg thiamin (B_1), 40 µg biotin. The culture medium was sterilized at 121 °C temperature and 1.05 kg cm^{-2} for 30 min. *Chlorella vulgaris* was inoculated in the liquid media. The algae were cultured in Erlenmeyer flask (250ml) containing 100 ml of medium. The experimental cultures were grown in the same liquid medium at a temperature 23±1 °C and a light intensity of 2000 lux on five different photoperiod (16:8, 17:7, 18:6, 19:5, 20:4 h). The experimental sets were run in triplicate, and all cultures were hand shaken twice daily. The number of *Chlorella vulgaris* cell was determined by direct counts of cells in the growth medium using Tahoma slide inverted microscope. The content of photosynthetic pigments was measured at 630 nm, 645 nm, 665 nm and 750 nm with spectrometers [5].

3. Results and Discussion

The objective of this study was, to determine the effects of photoperiods on growth, pigment content and protein amount of *Chlorella vulgaris.*

The effects of photoperiod were investigated when algae *C. vulgaris* has been exposed to different photoperiod (16:8, 17:7, 18:6, 19:5, 20:4 h). Figure 1 shows the population growth of the cultures was exposed to different photoperiod. The highest increase in algal cell number was observed on twentieth day in 16:8 h photoperiod.

The observation of effects of different photoperiods on *C. vulgaris* indicated a different increase in population growth. On the first day, *C. vulgaris* in culture exposed to 16:8, 17:7, 18:6, 19:5 and 20:4 h of photoperiods, was counted as 60, 125, 200, 245, 360 cells/mL, respectively. On the second day, *C. vulgaris* was counted in increase by 365, 470, 580, 500, 560 cell/mL at 16:8, 17:7, 18:6, 19:5 and 20:4 h of photoperiods. This increase in the cell number of *C. vulgaris* continued until the fifth day. At the end of the first week, cell numbers of algae reached 10 times compared to the first days and the highest number of cells was determined at 16:8 h period on the day ninth cell count.

When investigation reached the end of the second week; 16:8, 17:7, 18:6, 19:5, 20:4h at these periods, the cell number was found to be; 21.500 ,14.400, 18.000, 23.040, 12.960 cells/mL. The operation of the last day of the cell counting was found to be 54.000 cell/mL and an increase in maximum number of cells was observed. When cell counting study completed, in terms of increase in the number of cells determined that stage of 16:8 h may be quite favorable and at different light intensities, increase in light period was associated with the increased specific growth rate.

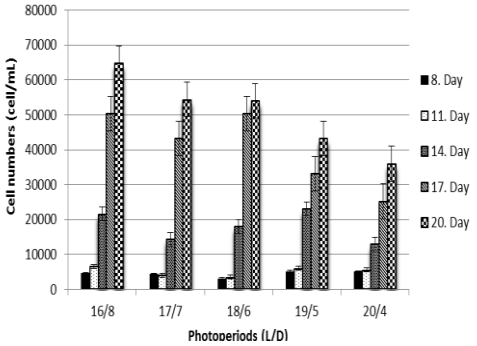

Fig.1 Cell numbers of *C. vulgaris* cultured at different photoperiods (16:8, 17:7, 18:6, 19:5, 20:4 h. light/dark)

C. vulgaris samples analyzed for the seventeen days and the increase of the chlorophyll amounts was found to be dependent on the photoperiod time. The first measurements were performed all day for five days and then, the process of the study continued for three days interruption until the seventeenth day. The

content of chlorophyll significantly varied under various light regimes.

As a result of this study, Chlorophyll a, chlorophyll b and total chlorophyll values were calculated separately and these results were shown in Fig. 2. Calculated from the first day of inoculation that the amount of total chlorophyll 105.37 mg/ ml was found to 16:8 period. On the first day cell count of other periods; 133.86, 139.77, 128.52, 138.42 µg/mL. And cultures exposed to different light duration showed small variations in chlorophyll content during the first day of the light period. Until the end of the first week, the amount of the chlorophyll continued to increase. During the measurements performed on the eighth day, the amount of chlorophyll was increased to 174.41 mg/ml at 16:8 h period. During this time, chlorophyll amounts of 16:8 h period showed maximum increase and maximum concentration of chlorophyll amount were sampled on 17th day in all cultures. On the last day of the investigation, the increase in chlorophyll amount continued and studies showed that light regime had an effect on chlorophyll amount of *C. vulgaris.* And this investigation about chlorophyll amount show that 16:8 h is the best period of pigment content increased.

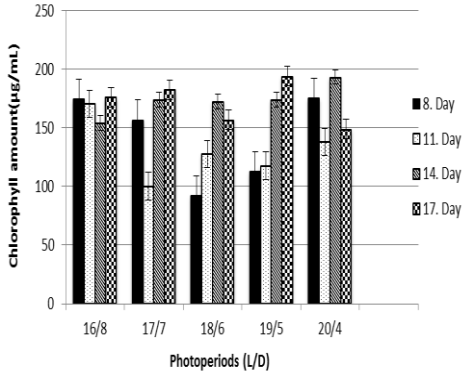

Fig.2 Chlorophyll contents of *C. vulgaris* cultured in different photoperiods.

Protein concentration was associated with different light regime and in this study determined the type of relationship between them. Protein analysis was made during the period of 5 days after that period of 15 days with two days apart. On the first day of the study, protein amounts (16:8, 17:7, 18:6, 19:5, 20:4 h periods) ; 27.2, 25.2, 11.8, 28.5, 21.8 µg/mL.

During the first five-day measurements, the maximum protein amount was observed on day 2 in the 16:8 periods. Figure 3 shows protein amounts of *C.vulgaris* cultured under different photoperiods. After a break of two days *C. vulgaris* samples exposed to different lighting period and amount of protein continued to increase at the end of the first week. As a result, according to the first day, compared to values and 16:8 h period showed an increase of about 100 times , the amount of protein was found to be 519,8 µg/mL. When reached the last day of the study, we observed significantly increased in the protein content and determinated increment in protein content associated with light period.

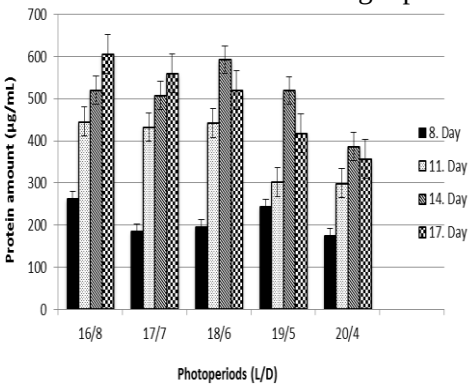

Fig.3 Protein contents of *C. vulgaris* cultured in different photoperiods

It has been reported that some physical and chemical factors such as, water temperature, salinity, pH and light affect algal growth in aquatic systems. Light regime is discontinuous and the intensity varies daily in nature. Changes in light illuminance, quality and photoperiod bring about varies in their biomass and chemical composition of algae, therefore, showing various adaptations to different environmental conditions [6-15]. These changes of the light have been shown to bring about differences in biochemical structure and pigment of microalgae [10, 11, 16]. During the investigation, we determined that chlorophyll amount increased at the different period so we said that different light periods cause of pigment differences and in the increased of chlorophyll amount is the most important at the period of 16:8h. When we look at the biochemical conditions, especially protein amount is showed too much increased in period of the 16:8h. There was also a significant increase of chlorophyll and protein content with increased in the different regimes. Algal cells

can adapt or answer responds to changes in different light regime. The most common answer is, diversity in pigment and the overall biochemical composition.

Adjustments to both light and nutritional circumstances are necessary for manipulation of the pigment composition and biomass of *C. vulgaris* [17]. To determine the best growth condition was used different light regime period and thus, we found significant increase of cell numbers in period of the 16:8 h.

One study found phytoplankton growth was a function of the total amount of light per day, and other studies have found that, depending on the species of microalgae, growth can be controlled by photoperiod [11, 12, 16, 17]. This result demonstrated that light regime was the most important factor in growth of *C. vulgaris*. Our results showed that an increased in light duration to 16:8 hour had a favorable effect on growth, protein and chlorophyll amount.

4. Acknowledgments

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5. References

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