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The Effect of Photoperiod on Growth, and Protein, Lipid and Chlorophyll Content in *Scenedesmus Acutus*

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Abstract: The effect of photoperiod on the growth, protein, lipid and pigment content in *Scenedesmus acutus* was examined on cultures exposed to 23 ± 1 °C for ten days at the light intensity of 55 µmol photon $m^{-2}s^{-1}$. Results showed that photoperiods had an effect on cell number, and protein and chlorophyll content. The growth, protein, lipid and chlorophyll levels in *Scenedesmus acutus* cultures reached maximum in different days and photoperiods. The number of cells of the continuously illuminated cultures was highest on day seven after inoculation. The total protein and total lipid content were highest on day eight under the light/dark photoperiod of 20/4 h while total chlorophyll content was highest on day ten under the light/dark photoperiod of 24:0 h. The cultures continuously illuminated for cell growth had the highest specific growth rate (µ=0.2304). Protein content had a specific growth rate of µ=0.0725 under the light/dark photoperiod of 20/4 h while total chlorophyll content had a specific growth rate of µ=0.0890 under the light/dark photoperiod of 12:12-h.

Key words: Green algae, *Scenedesmus acutus*, photoperiod, growth, lipid

Scenedesmus acutus'un Gelişim, Protein, Lipid ve Pigment Miktarı Üzerindeki Fotoperiyodun Etkisi

Öz: Fotoperiyodun *Scenedesmus acutus*'un gelişim, protein, lipid ve pigment miktarı üzerindeki etkileri, 55 µmolphoton m-2s-1 ışık yoğunluğunda 23±1 °C sıcaklığa maruz bırakılan kültürlerde on gün süresince incelendi. Sonuçlar hücre sayısı, protein ve klorofil miktarı üzerinde fotoperiyodun etkili olduğunu gösterdi. *Scenedesmus acutus* kültürlerinde gelişme, protein, lipid ve klorofil miktarlarının farklı gün ve fotoperiyotta maksimuma ulaştığı tespit edildi. Sürekli aydınlatılan kültürlerde hücre sayısı inokülasyonu takip eden yedinci günde maksimuma ulaşırken, toplam protein ve total lipid miktarının 20:4-h aydınlık/karanlık fotoperiyodunda sekizinci günde, toplam klorofil miktarının da 24:0-h aydınlık/karanlık fotoperiyodunda onuncu günde maksimum olduğu görüldü. Spesifik büyüme oranı hücre gelişimi için sürekli aydınlatılan kültürlerde µ=0.2304 ile maksimum olurken protein miktarındaki spesifik büyüme oranı 20:4-h aydınlık/karanlık fotoperiyodunda µ=0.0725 ve toplam klorofil miktarındaki spesifik büyüme oranı ise 12:12-h aydınlık/karanlık fotoperiyodunda µ=0.0890 olduğu tespit edildi.

Anahtar kelimeler: Yeşil alg, *Scenedesmus acutus*, fotoperiyot, büyüme, lipit

1. Introduction

The growing demand for alternative energy sources results in an increased interest in biofuel production. Biofuel production from microalgae is one of the most interesting research topics. Microalgal can be converted into various biofuels such as biomass, biomethane, bioethanol and bio-oil. Due to lipids, microalgae are the most useful sources of biodiesel. Microalgal biomass is superior to other biomass-based energy resources because it grows rapidly and has high concentrations of carbohydrate and lipid. Microalgae can accumulate carbohydrates and lipid as well as secondary metabolites and are therefore useful organisms for biotechnological research. Microalgae are biotechnologically important because their biomass contains valuable components including starch, protein, lipids and a wide variety of alkanes [1]. Microalgae are used in the production of a wide variety of compounds, such as dyes, antioxidants, gelling agents, amino acids, emulsifiers and omega 3. Microalgal proteins and pigments have great potential in medical applications as well.

Numerous factors such as nutrients, pH, temperature, light and salinity affect on microalgal growth. Light is the main factor controlling the biochemical and physiological processes of microalgae [2]. Intensity, wavelength and photoperiods are of paramount significance for photosynthesis. Light/dark photoperiods significantly affect algal growth and biomass. Light intensity and regime constantly change in natural environments [2]. Changes in light/dark photoperiods change the carbohydrate, protein and lipid concentrations of microalgae cells [3-6]. Krzeminska et al. investigated the effect of photoperiod on the growth rate and biomass productivity of *Botryococcus braunii*, *Scenedesmus obliquus*, *Neochloris conjuncta*, *Neochloris Terrestris* and *N. texenis* [1]. Wahidin et al. examined the effect of light intensity and photoperiod on the growth and lipid content in

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Nannochloropsis sp. and reported that algae species exhibited different growth models [7]. Numerous studies have investigated the effect of photoperiod on cell growth and lipid and protein content. Algae species differ in light requirements, reproduction patterns and life cycles. *S. acutus* is a cosmopolitan green microalgae composed of two, four or eight ellipsoid or oval cells arranged in one or two rows. *S. acutus* has been extensively studied concerning valuable chemicals and healthy nutrition. *S. acutus* is an important member of the aquatic food chain and is widely used in aquaculture as an alternative source of protein for food and feed purposes [8].

This study investigated the effect of photoperiod on the growth, and total protein, lipid and pigment content in *S. acutus*, which was grown under three photoperiod cycles for 10 days. Cell count was performed, and lipid, protein and pigment content was determined in the cultures every day.

2. Material and Methods

S. acutus samples were collected from Keban Dam Lake and isolated in the algal biotechnology laboratory of Fırat University and cultivated in Jaworski's medium, which contained 80 mg $NaNO_3$, 36 mg $Na_2HPO_4.12H_2O$, 20 mg $Ca(NO_3)_2.4H_2O$, 12.4 mg KH_2PO_4, 50 mg $MgSO_4$. $7H_2O$, 2.25 mg EDTAFeNa, 2.25 mg $EDTANa_2$, 2480 µg H_3BO_3, 15.9 mg $NaHCO_3$, 1390 µg $MnCl_2.4H_2O$, 1000 µg $(NH_4)_6Mo_7.4H_2O$, 40 µg biotin, 40 µg cyanocobalamin (B_{12}) and 40 µg thiamin (B_1). The medium was sterilized at 121 °C for 1 min at 1 atmosphere pressure. *S. acutus* samples were inoculated into 250 mL Erlenmeyer with 100 mL Jaworski's medium. The Erlenmeyers were incubated in an air conditioning unit for 10 d at 23 ± 1 °C and light intensity of 55 µmol photon $m^{-2}s^{-1}$. 10 mL of the cultures that reached a certain density was sampled and placed in the Erlenmeyers, which were then placed in the air conditioning unit for incubation at 23 ± 1 °C and light intensity of 55 µmol photon $m^{-2}s^{-1}$ under different photoperiods. The Erlenmeyers were shaken three times a day. Samples were collected from the Erlenmeyers exposed to different photoperiods. The samples were analyzed for ten days to determine the cell number, and total protein, lipid and total chlorophyll content in *S. acutus*.

Growth Measurement

S. acutus was counted under a microscope using a plankton counting chamber. Algal biomass concentration was measured at an optical density of 680 using visible density spectrophotometer. Spectrophotometer measurements were compared with microscopic counts. A standard curve was plotted with optical density to calculate the number of individuals. All calculations were performed in triplicate.

Total Protein Measurement

Total protein content was determined using Lowry method. 0.1-ml DOC solution was added onto a 1-ml sample, which was then kept at room temperature for 10 min. Afterwards, 0.1 mL TCA was added onto the sample, which was then centrifuged at 7500 rpm for 10 min. Following the removal of the supernatant, 1 mL Lowry solution was added onto the precipitate, which was then kept at room temperature for 20 min. Later on, 1 mL fooling reagent was added onto the sample, which was then kept for 30 min. Lastly, absorbance was measured at 750 nm and results were evaluated based on the standard curve [9].

Total Lipid Measurement

Total lipid content was determined using the Bligh and Dyer method [10]. A mixture of 40 mL methanol and 80 mL chloroform was added onto a 0.2-g sample, and then, 20 mL $CaCl_2$ (4%) was added onto it. The mixture was filtered through filter paper and allowed to stand overnight in the dark. The following day, methanol and water were separated using a separatory funnel, and chloroform was evaporated in a water bath at 60 °C. Chloroform was completely evaporated by allowing the remaining part to stand for 1 h at 90 °C. Afterwards, the sample was weighed.

Chlorophyll analysis

Chlorophyll content was determined using the method of Strickland and Parsons [11]. A 5-mL culture was filtered through GF/C filter papers to determine pigment concentrations. Each filter paper was placed in 90% acetone at +4 °C, kept in the dark for 24 h, centrifuged at 3500 rpm for 5 min and analyzed for chlorophyll content by measuring the absorbances at 630, 645, 665 and 750 nm using the spectrophotometer.

2. Results

This study investigated the growth, and protein, lipid and pigment content in *S. acutus* at the light intensity of 55 µmol photon $m^{-2}s^{-1}$ and under four photoperiods (24:0, 20:4, 18:6 and 12:12-h light:dark). Figure 1 shows the effect of photoperiod on the growth in *S. acutus*. *S. acutus* had maximum growth at the light intensity of 55 µmol photon $m^{-2}s^{-1}$ under the light/dark photoperiod of 24:0 h. The number of cells increased from 850730 cell/ml (onset of inoculation) to 6.7 $x10^6$ cell/ml (day 7). Maximum growth under the light/dark photoperiods of 12:12 h and 20:4 h was, respectively, 6.4 $x10^6$ cell/ml and 5.3 $x10^6$ cell/ml on day eight (Figure 1). The highest (µ=0.2304) and lowest (µ=0.1939) specific growth rates in *S. acutus* were observed under the light/dark photoperiods of 24:0 h and 18:6 h, respectively.

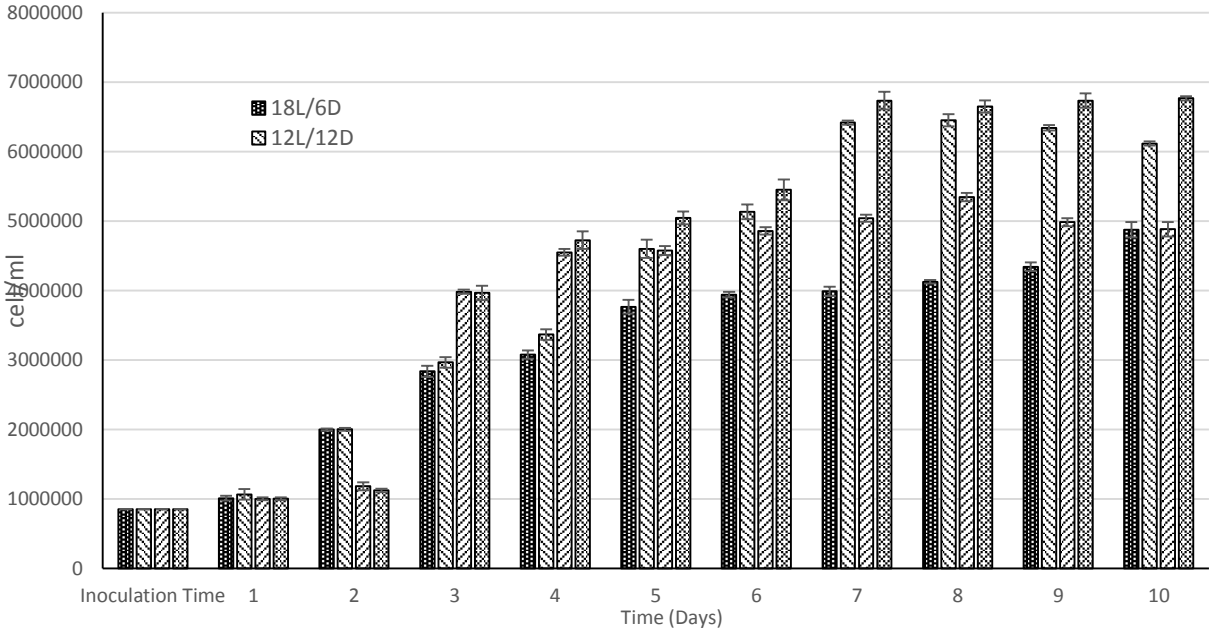

Figure 1. Cell number of *S. acutus* cultured at different photoperiods

Figure 2 shows the effect of photoperiod on the protein content of *S. acutus*. The protein content increased from 39.473µg/ml (onset of inoculation) to 65.610µg/ml (day eight under the light/dark photoperiod of 20:4 h). The highest protein content (65.486µg/ml) under the light/dark photoperiods of 24:0 h and 18:6 h was, respectively, 65.486µg/ml and 65.030 µg/ml on day ten. The highest protein content (64.356 µg/ml) under the light/dark photoperiod of 12:12 h was observed on day eight. The rate of increase in protein content in *S. acutus* cultures exposed to different photoperiod cycles also differed. The rate of increase in protein content under the light/dark photoperiods of 20:4 h and 12:12 h was, respectively, µ=0.0725 and µ=0.0698. The protein content under the light/dark photoperiod of 24:0 h was highest on day ten, and the rate of increase was µ=0.0562. The protein content under the light/dark photoperiod of 18:6 h was highest on day ten, and the rate of increase was µ=0.554. The rate of increase in protein content was highest under the light/dark photoperiod of 20:4.

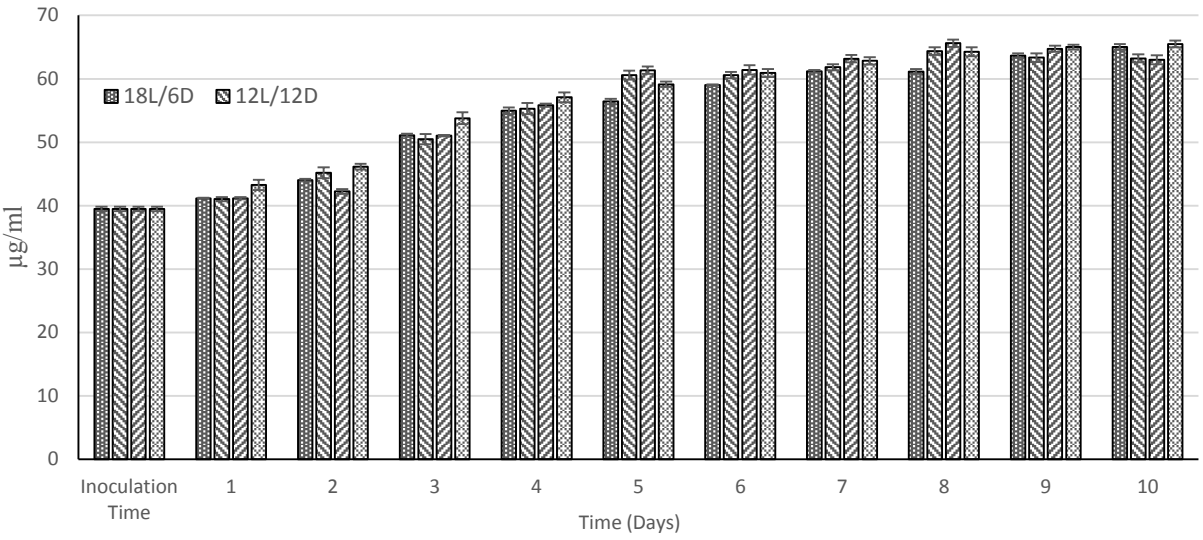

Figure 2. Protein contents of *S. acutus* cultured at different photoperiods

Figure 3 shows the effect of photoperiod on the lipid content of *S. acutus*. The total lipid content continuously increased from 4% (onset of inoculation) to 10.6% (day nine under the light/dark photoperiod of 18:6 h). The highest lipid content under the light/dark photoperiods of 12:12 h, 20:4 h and 24:0 h was, respectively, 10.77%, 11.5% and 11.71% on day eight. The highest increase in lipid content was observed in 24:0 h cultures on day eight.

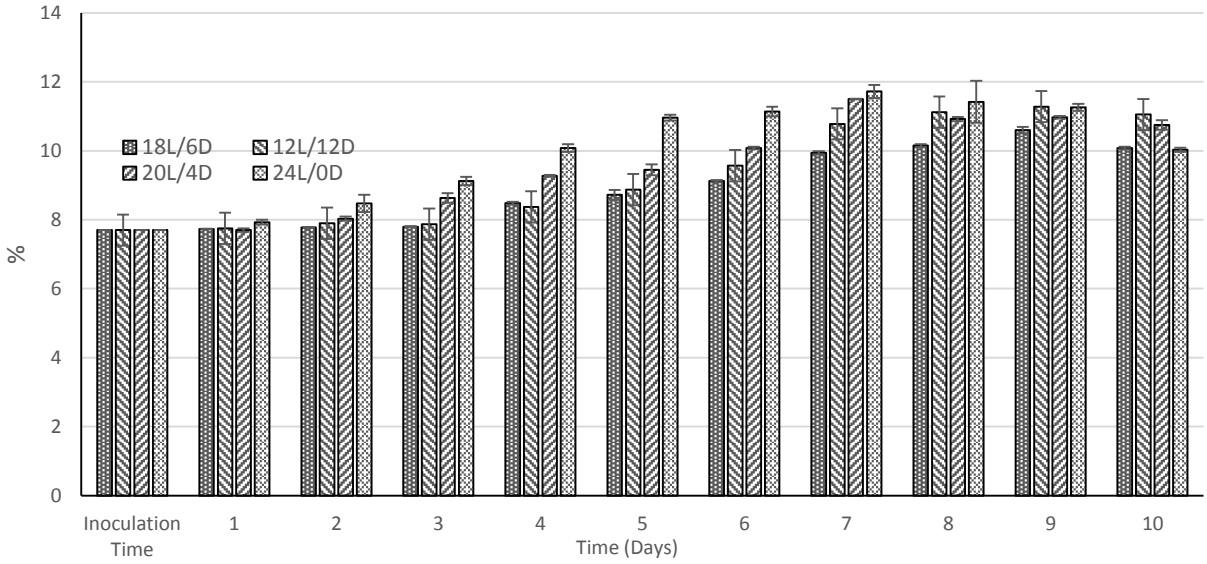

Figure 3. Lipid contents of *S. acutus* cultured at different photoperiods

This study investigated the effect of photoperiod on the total chlorophyll content of *S. acutus* for ten days. Figure 4 shows the results. The highest total chlorophyll content under the light/dark photoperiods of 24:0 h and 20:4 h was, respectively, 6.123 mg/ml and 4.970 mg/ml on day ten. The highest total chlorophyll content under the light/dark photoperiods of 18:6 h and 12:12 h was, respectively, 4.80 mg/ml and 5.550 mg/ml on day eight. *S. acutus* cultures had the highest (µ=0.0890) and lowest (µ=0.0531) specific growth rate under the light/dark photoperiods of 12:12 h and 18:6 h, respectively.

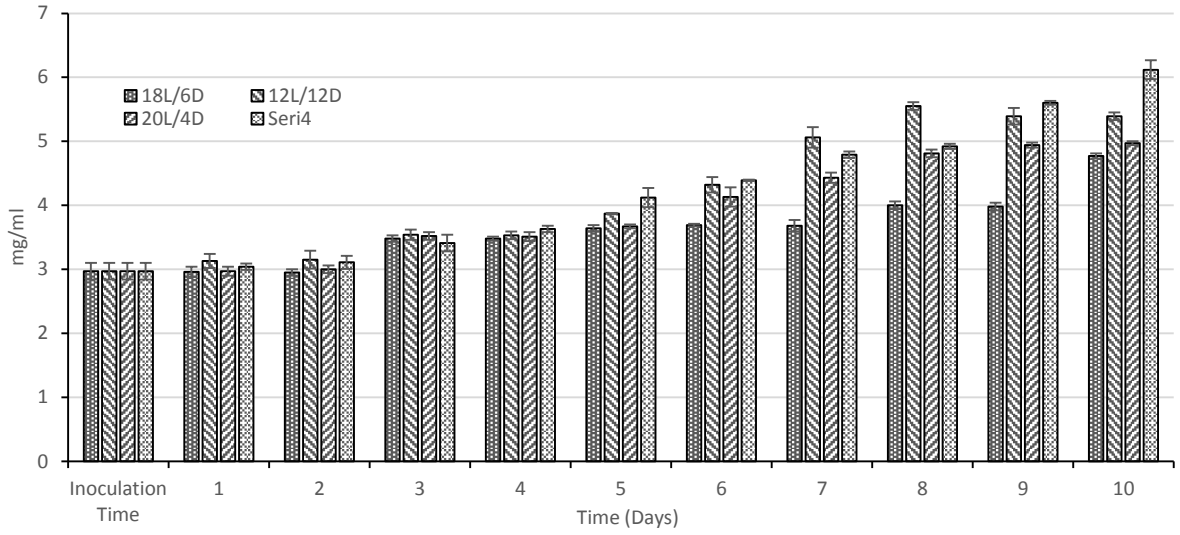

Figure 4. Chlorophyll contents of *S. acutus* cultured at different photoperiods

3. Discussion

Research shows that changes in light regimes reveal differences in the pigment and biochemical composition of microalgae [5, 12, 13, 14]. The growth rate in many microalgae occurs under high light conditions [15-17]. Photosynthetic responses to photoperiod vary across microalgae species [8, 15]. However, photoperiod affects not only the growth rate but also the composition and quantity of high-value microalgal products in algae species [2, 5, 7]. The total protein, pigment and fatty acid content in *C. vulgaris* [14], the growth and lipid production in *Porphyridium cruentum* ([18] and *B. braunii* [19] and the cell density, cell growth rate and total lipid content in *Nannochloropsis* sp [7] are affected by changes in photoperiod. Photoperiod culture studies in microalgae cultures illuminated by artificial light are cost-effective. Some studies are investigating the effect of photoperiod on microalgae biomass [2, 19, 20]. Our results show that photoperiod affect on cell growth, and protein and chlorophyll content in *S. acutus*. Khoeyi et al. reported that photoperiod is one of the most important factors affecting the biomass growth in *Chlorella vulgaris* [2]. Especially long light duration increase biomass and specific growth rate. In our study, the highest specific growth rate was observed under the light/dark photoperiod of 20:0 h, which has also been reported by previous studies. Seyfabadi et al. reported that long light duration increase in the protein content in *C. vulgaris* [2]. In our study, *S. acutus* cultures had the highest protein content and quite high specific growth rate (μ=0.0725) on day eight under the light/dark photoperiod of 20:4 h, followed by 12:12 h, 24:0 h and 18:6 h. This result indicates that light duration increases the protein content of microalgae. Numerous studies show that photoperiod at different developmental phases affect the total lipid content and lipid composition of microalgae [2, 7, 14, 21]. Raungsomboo (2012) reported that *Botryococcus braunii* had the highest lipid content under the light/dark photoperiod of 16: 8 h. In our study, the highest lipid content was observed under the light/dark photoperiod of 24:0 h on day eight. Ruangsomboon, Seyfebadi et al., Singh and Singh, reported the effect of photoperiod on pigment content [19, 14, 22] . In our study, the total chlorophyll content regularly increased from the onset of inoculation. The highest rate of increase was observed under the light/dark photoperiod of 20:4 h. However, the highest specific growth rate was observed under the light/dark photoperiod of 12:12 h.

Our results showed that an increase in photoperiod increased the cell number, and thus, biomass of *Scenedesmus acutus*. We believe that increasing cell concentrations under light/dark photoperiods can reduce production costs. The necessity of a dark phase in culture studies is accounted for by that of light and dark phase in photosynthesis. Compounds produced in a light phase (ATP, NADPH) are used in the dark phase to synthesize the metabolic molecules necessary for growth. Our results showed that photoperiod had affect the growth and chlorophyll, protein and lipid content in *S. acutus*.

References

[1] Krzemińska I., Pawlik-Skowrońska B., Trzcinska M. and Tys J. (2014). Influence of photoperiods on the growth rate and biomass productivity of green microalgae. Bioprocess. Biosyst. Eng. 37: 735-741.

[2] Khoeyi ZA., Seyfabadi J. and Ramezanpour Z. (2012). Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae, *Chlorella vulgaris*. Aquacult. Int. 20:41-49.

[3] Tzovenis I., Pauw ND. and Sorgeloos P. (1997). Effect of different light regimes on the docosahexaenoic acid (DHA) content of *Isochrysis* aff. *galbana* (clone T-ISO). Aquaculture International. 5: 489-507.

[4] Price LL., Yin K. and Harrison PJ. (1998). Influence of continuous light and L:D cycles on the growth and chemical composition of *Prymnesiophyceae* including coccolithophores. J Exp. Biol. Ecol. 223: 223-234.

[5] Fabregas J., Maseda A., Dominquez A., Ferreira M. and Otero A. (2002). Changes in the cell composition of the marine microalga, *Nannochloropsis gaditana*, during a light:dark cycle. Biotechnoll. Let. 24: 1699-1703.

[6] Kendirlioglu G., Agirman N. and Cetin A.K. (2015). The Effects of Photoperiod on The Growth and Pigment Content of *Chlorella vulgaris*. F.U.Turkish Journal of Science & Technology, 10 (2), 7-10.

[7] Wahidin S., Idris A. and Shaleh SRM. (2013). The influence of light intensity and photoperiod on the geowth and lipid content of microalgae *Nannochloropsis* sp. Bioresour. Technol. 129: 7-11.

[8] Richmond A. (2004). Biological principles of mass cultivation. In Richmond A (ed) Handbook of microalgal mass, culture: biotechnology and applied phycology. CRC Press, Blackwell Publishing Company, Oxford, pp 566.

[9] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol regent. J. Biol. Chem. 1951; 193:265-75.

[10] Bligh EG, Dyer WJ. A rapid method for total lipid extraction and purification. Can. Biochem. Physiol. 1959; 37:911-917.

[11] Strickland J. and Pearson TR. (1972). A Pratical Handbook of Seawater Analysis, second ed. Fish Res. Can. Bull.

[12] Renaud SM., Parry DL., Thinh LV, Kuo C., Padovan A. and Sammy N. (1991). Effect of light intensity on the proximate biochemical and fatty acid composition of *Isochrysis* sp. and *Nannochloropsisi oculata* for use in tropical aquaculture. J. Appl. Phycol. 3: 43-53.

[13] Sanchez-Saavedra MP. and Voltolina D. (2002). Effect of photon fulence rates of hite and blue-green light on growth efficiency and pigment content of three diatom species in batch cultures. Cienc. Mar. 28: (3): 273-279.

[14] Seyfabadi J. Ramezanpour Z. and Khoeyi ZA. (2011). Protein, fatty acid, and pigment content of Chlorella vulgaris under different light regimes. J Appl. Phycol. 23: 721-726.

[15] Bouterfas R., Belkoura M. and Dauta A. (2006). The effcts of irradiance and photoperiod on the growth rate of three freshwater green lgae isolated from a eutrophic lake. Limnetica, 25 (3): 647-656.

[16] Watson D., Daume S., Prince J., Beazley L. and Knott B. (2004). The influence of light intensity on the density on the density of different diatoms as fed for juvenile greenlip abalone (*Haliotis laevigata*). Aquaculture, 235: 345-359.

[17] Meseck SL., Alix JH., Gary H. and Wikfors GH. (2005). Photoperiod and light intensity effects on growth and utilization of nutrients by the aquaculture feed microalga *Tetraselmis chui* (Ply429). Aquaculture, 246: 393-404.

[18] Oh SH., Han JG., Kim Y Ha JH., Kim SS., Jeong MH., Jeong HS., NY., Cho JS., Yoon WB., Lee SY., Kang DH. and Lee HY. (2009). Lipid production in *Porphyridium cruentum* grown under different culture conditions. Journal of Bioscience and Bioenginering, 108, 5, 429-434.

[19] Ruangsomboon S. (2012). Effect of light, nutrient, cultivation time and salinity on lipid production of newly isolated strain of the green microalga, *Botryococcus braunii* KMITL 2. Bioresource Technology, 109: 261-265.

[20] Mata TM., Melo AC., Simoes M. and Caetano NS. (2012). Parametric study of a brewery effluent treatment by microalgae *Scenedesmus obliquus*. Bioresour. Technol. 107: 151-158.

[21] Kotimchenko SV. and Yakovleva IM. (2005). Lipid composition of the red alga Tichocarpus crinitus exposed to different levels of photon irradiance. Phytochemistry, 66: 73-79.

[22] Singh SP. and Singh P. (2015). Effect of temperature and light on the growth of algae species: A review. Renewable and Sustinable Energy Reviews, 50: 431-444.