Investigation of Different Light Sources and Cycles on the Growth and Lipid Production Mechanisms for Various Microalgae Species

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ABSTRACT: *Microalgae are used for various purposes, mainly as food supplements by people because of the protein, carbohydrate, fatty acids, vitamins, minerals, pigments, and many other compounds they contain. Efforts to utilize microalgae as a raw material for the production of biodiesel are increasing, especially in European countries. In this study, the effects of different light sources (Led, Incandescent, Green, Yellow, Blue, Red) and cycles 10:14,12:12,14:10,0:24(night: day) on the growth and lipid production of Chlorella protothecoides and Chlorella ESP-6 species were investigated. All of the experiments were conducted in 100ml flasks that contained culture medium BG 11. The results showed that Chlorella ESP-6 reached 0.25 gdw/L maximum cell concentration under the incandescent lamp (3.16 Klux), whereas 0.39 gdw/L maximum cell concentration under the 14:10 light cycle. Chlorella protothecoides attained a maximum cell concentration of 0.18 gdw/L, and 0.26 gdw/L under the led lamp (3 Klux) and 0:24 light cycle respectively. No significant effects of different light sources on the microorganism lipid content were observed. The average lipid content of microorganisms each for applied light intensity was determined to be 45% and 17% for Chlorella protothecoides and Chlorella ESP-6 respectively. On the other hand, with the effect of the light cycles on the microorganism lipid content, it was seen that the lightness phase for Chlorella ESP-6 increased considerably. There was no significant effect on Chlorella protothecoides. The highest lipid contents were determined as 17% and 48% for both microorganisms respectively.*

KEYWORDS: *Algae oil; Chlorella; Light cycle; Light intensity; Microalgae.*

INTRODUCTION

Microalgae can grow using sunlight and carbon dioxide as land plants. Because of their rapid and stable proliferation, organisms are more productive than black plants. These photosynthetic organisms produce oxygen by photosynthesis. They may also change their metabolism in response to adverse environmental conditions. *Chlorella* and *Neochloris* species were found to have a 60% fat ratio in dry cell weights when cultured in a nutrient-deficient environment [1]. Microalgae are advantageous compared to other oil sources because they can be produced quickly and widely. Besides, algae contain more oil in the unit biomass than oil plants and they contain lipids of approximately 20–50% dry cell weight [2]. For this reason, microalgae oils can be useful

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as a raw material for one of the most sustainable energy sources called biodiesel an alternative to using vegetable oils for biodiesel production [3,4].

Microalgae require light, carbon dioxide, water, and inorganic salts to grow. The temperature should not rise above 30 ºC or below 20 ºC. About 50% of microalgae dry weight is carbon. All of this carbon is covered by carbon dioxide [3]. Light is a mandatory parameter for sustaining the vitality of microalgae. Photosynthetic growth is proportional to the light energy used by each cell. Therefore, the light must be kept at the optimum level. The light intensity is related to the depth and intensity of the culture medium. In a culture with high depth and cell concentration, the light intensity is increased [3]. At the same time, the photoinhibition effect of light intensity on microorganisms is also present. Various studies have been reported in the literature about different light source effects on growth with different types of microalgae mechanisms. In the study conducted by *Wang et al*., *Spirulina platensis* was grown in the highest red led light, while the lowest growth was in blue led light [5]. *Hultberg et al*. investigated the effects of 6 different light sources (yellow, red, blue, green, white, and purple light) on the growth of microalgae of *Chlorella vulgaris* [19]. They reported that growth under the influence of yellow, red, and white light reached its highest growth rate in white light [4]. *Das et al*, investigated the effects of different wavelengths of red (680 nm), white, blue (470 nm), and green LED (550 nm) lights on the growth of *Nannochloropsis oculata* microalgae. Experimental results indicate that the most effective light source is blue>white>green>red the less efficient light source [6]. *Teo et al*. investigated blue (457 nm), red (660 nm), red-blue LED lights and white fluorescent lamps, *Nannochloropsis sp*. and *Tetraselmis sp*. type microalgae on the growth rate. They found that the best growth was in a culture with a blue wavelength [7].

On the other hand, constant illumination for photosynthesis leads to negative results on microorganisms according to some studies It is emphasized that microalgal cells that photosynthesize to prevent this negative condition are needed in the dark period so that they can regenerate themselves [8]. Due to this reason, microalgae grow in dark periods ranging from 6 to 18 h, and short-lived flashing lights are used. It is stated that biomass yield will decrease as microalgae will consume oxygen and carbohydrates if exposed to long periods of darkness. A specific night/day cycle must be specified for each microalgae species [9]. Continuous illumination for some types of microalgae is important for high biomass yield [10]. *Renaud et al. , Isochrysis sp., Chaetoceros sp., Rhodomonas sp.*, and *Cryptomonas sp*. cultivating the species by applying a 12:12 light-dark period; chemical composition and fatty acid composition. 25°C, *Chaetoceros sp.* The highest lipid ratio was found at 16.8%, while the highest lipid ratio at 27-30 ° C was found in Isochrysis sp. with 21.7% [11]. *Zhila et al*., observed growth and lipid composition by applying a 10:14 light-dark period in the presence of *B. braunii* microalgae at 75% nitrogen stress. The daily biomass decreased from 6.8% to 2.9%, while the lipid ratio increased to 21% according to the cultivated culture at the end of a twenty-day cultivation [12]. *Eduardo et al*., studied that *Aphanothece microscopica Nageli* species microalgae 0:24, 2:22, 4:20, 6:18, 8:16, 10:14, 12:12, 14:10, 16: 8, 18: 6, 20 : 4, 22: 2 and 24: 0 (night: day) cycles on the biomass were applied. According to the experimental results, they reached the highest microorganism concentration (0,770 g/L) in 24-hour light cycle [13]. The effects of the night-day cycle on microorganism growth in two different microalgae species (*Palmellopsis muralis Dunaliella salina sp.)* were examined. As a result of experimental studies, the highest biomass amount in both species at 24 hours of continuous light condition was achieved [14].

In this paper, the effects of different light sources (Led, Incandescent, Green, Yellow, Blue, Red) and cycles (night: day) on growth and lipid production of *Chlorella protothecoides* and *Chlorella ESP-6* species were investigated for assessment of utilization as a raw material for biodiesel production.

EXPERIMENTAL SECTION

Materials

Microalgae species used were *Chlorella ESP-6* and *Chlorellaprotothecoides* Microalgae were cultivated in Blue Green Medium (BG 11) prepared with tap water. The chemical composition of this medium was (gdw/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 0.0029; Na_2CO_3, 0.02; Fe(III)citrate, 0.006; citric acid 0.006. The chemicals used in the study were of analytical purity. Osram brand LED and General Electric brand Incandescent, Philips brand green, yellow, blue, and red with

light intensity values of 3, 3.16, 0.81, 0.22, 0.32, 0.82 klux respectively, were used for the light source.

Microalgae cultivation under different light sources and cycles

Experiments were carried out in 100ml open flasks. Microalgae inoculating from stock cultures to 5% of the growth medium. Microalgae cultures are settled in isolated rooms illuminated by different light sources and cycles specially prepared so as not to be affected by each other. Fujika's mechanical time-controlled plugs are used to provide a light cycle. The ambient temperature of the cultures is set at 25 ± 2 ºC. Temperature (using digital thermometer probes) and pH (Mettler Toledo seven Easy 20 model pH meter) were measured with a relative accuracy of ± 0.5 ºC and ± 0.001 pH. The pH value of the medium was initially measured at 7.76. The cultures were aerated from the surface. The cell concentration and growth were determined spectrophotometrically at 600 nm using Jenway 6800 UV Vis. Spectrophotometer.
The growth rate was determined as

$$ln\frac{X}{Xo}=\mu_{net}.torX=X_0e^{\mu_{net.\ t}} \quad (1)$$

Where μ_{net}net specific growth rate, X, and X_o are cell concentration at t, and t = 0. The exponential growth is characterized by a straight line on a semilogarithmic plot of lnX versus time. The time required to double the microbial mass is given by:

$$\tau_d = \frac{\ln 2}{\mu_{net}} = \frac{0.693}{\mu_{net}} \quad (2)$$

Where τ_d is the doubling time of cell mass.

Samples of 3 ml were collected regularly from culture media for pH, temperature, and absorbance measurements. After the incubation period, microalgae were harvested by centrifugation at 5000 rpm for five minutes.

Lipid content analysis of microalgae species

Lipid analyses were carried out according to the method of Dyer [15]. 20 ml of a 0.4% $CaCl_2$ solution on a 0.2 g sample was added to 120 ml of methanol /chloroform 1/2 and then stirred on a magnetic stirrer for three hours. Then, the drained samples from the filter paper (Whatman No: 1) were stored in the filter for two hours at 105 ºC and filtered through the flasks. The mouths of the balloons were closed in an air-tight manner and kept in a dark place for one night and the next day the upper layer consisting of methanol water was removed with the help of a separating funnel. The chloroform remaining in the bubbles was evaporated from the lipid to chloroform at 60 ºC in the dryer. The balloons were then allowed to stand at 90 ºC for one hour to allow the entire chloroform to evaporate and cool to room temperature in the desiccator. It was weighed on the precision balance. The following formula is used in the calculation of the fat ratio.

$$\text{Fat content}=\frac{\text{[baloon weight+fat weight]-baloon wight}}{\text{sample weight}}\text{x}100 \quad (3)$$

Lipid Fatty acid composition analysis via gas chromatography

The fatty acid composition was determined using a GC 7820 Agilent gas chromatograph equipped with a Flame Ionization Detector (FID) and a 30 m x 320 µm x 0.25 µm capillary column (CARBOWAX 20M). The detector temperature is 280 ºC and the split ratio is 1:50. The column temperature at 50 ºC after waiting for the one-minute same temperature, increases to 200ºC by 25ºC/min and increases to 230 ºC by 3 ºC/min. after waiting at the same temperature for 18 minutes, increasing to 280 ºC by 40 ºC/min., and waiting for 3 minutes at the same temperature is programmed. Helium was used as a carrier gas. For the identification of the fatty acids composition, the oils are primarily converted into methyl esters [16].

RESULTS AND DISCUSSION

Effect of different light sources

The time-dependent growth of *Chlorella ESP-6* and *Chlorella protothecoides* in photobioreactors under the influence of different light sources are shown in Fig. 1 and Fig. 2, respectively.

The results showed that *Chlorella ESP-6* reached 0.25 gdw/L maximum cell concentration (0.035 h^{-1} specific growth rate) under the incandescent lamp (3.16 klux), whereas *Chlorellaprotothecoides* attained a maximum cell concentration of 0.18 gdw/L (0.025 h^{-1} specific growth rate) under the led lamp (3 Klux). Also doubling times of microalgae was 19.8 hand 27.7h for *Chlorella ESP-6* and *Chlorella protothecoides*, respectively. No studies have been conducted on the effect of microorganism growth and fat content of different light sources for *Chlorella ESP-6* in the literature. There is a limited number of studies for *Chlorella protothecoides*, *Izabela et al*. have reported that the effect of light intensity on growth rate in cultures at 35,130,420 µmol light intensity, and consequently the lipid

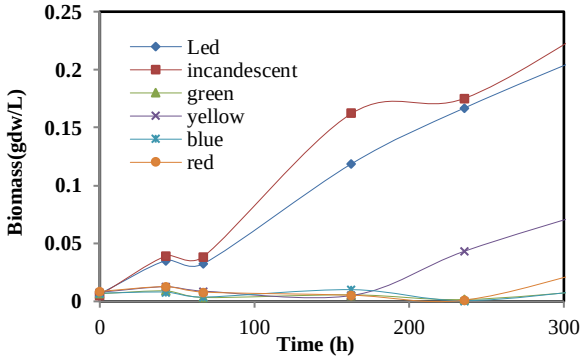

Fig. 1: Chlorella ESP-6 growth curve under different light sources

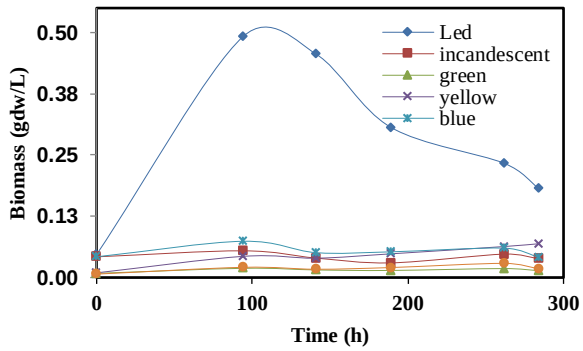

Fig. 2: Chlorella protothecoides growth curve under different light sources

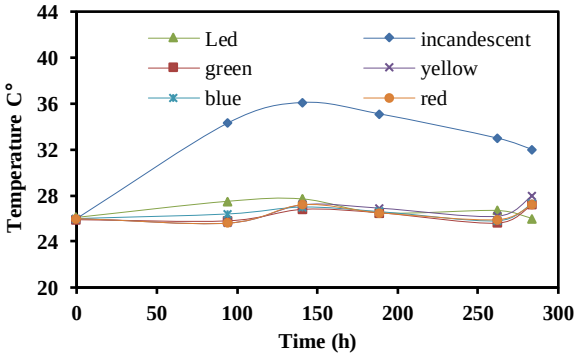

Fig. 3: Temperature change in photobioreactors containing Chlorella protothecoides under the different light sources

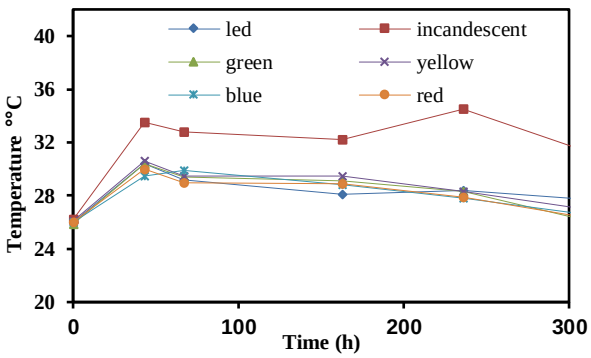

Fig. 4: Temperature change in photobioreactors containing Chlorella ESP-6 under the different light sources

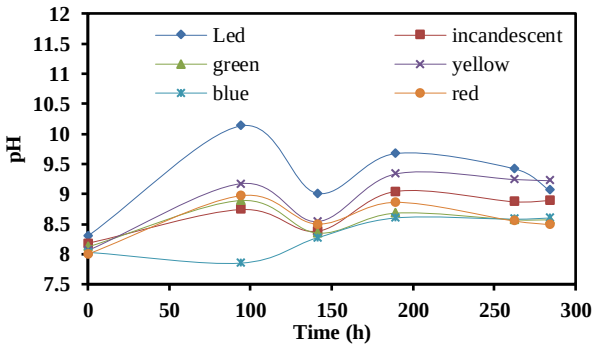

Fig. 5: pH distribution in photobioreactors containing Chlorella protothecoides under the different light sources

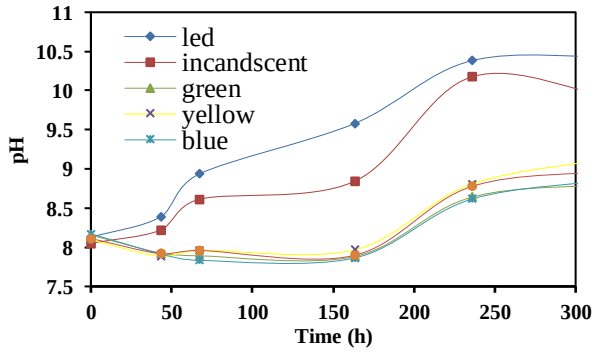

Fig. 6: pH distribution in photobioreactors containing Chlorella ESP-6 under the different light sources

content increased from 24% to 37.5%. During the incubations, the temperatures of the systems were between 26.3-28.6 °C (in the presence of led, green, yellow, blue, and led lamps) and 32.3-36.1℃ (in the presence of incandescent lamp) for *C.protothecoides* and *C.ESP-6* (Fig. 3-4 respectively). The incandescent lamp caused a high-temperature effect on the system [3].

The pH of the media was around 8.0 for both runs initially, however, it increased sharply after the start of the runs to the alkali range and stayed between 9.1-10.1for and 9.9-11.0 for *Chlorella protothecoides* and *Chlorella ESP-6* during the rest of the culture (Fig. 5-6).

No significant effect of different light sources on the microorganism fat content was observed. Tables 1 and 2 show growth rates and lipid contents of *Chlorella ESP-6* and *Chlorella protothecoides*, respectively, for different light intensities.

Table 1: Effect of a different light source on growth and lipid content for Chlorella ESP-6

	Light intensity (Klux)	X_{max} (gdw/L)	µmax (h^{-1})	Doubling Time (h)	Fat content (%)
Led	3.00	0.22	0.030	23.1	17
Incandescent	3.16	0.25	0.035	19.8	14
Green	0.81	0.01	0.007	99.0	15
Yellow	0.22	0.08	0.014	49.5	14
Blue	0.32	0.01	0.005	138.6	16
Red	0.82	0.03	0.011	63.0	13

Table 2: Effect of a different light source on growth and lipid content for Chlorellaprothocoides

	Light intensity (Klux)	X_{max} (gdw/L)	μ_{max} (h^{-1})	Doubling Time(h)	Fat content (%)
Led	3.00	0.18	0.025	27.7	45
Incandescent	3.16	0.04	0.003	23.1	43
Green	0.81	0.01	0.008	86.6	47
Yellow	0.22	0.07	0.016	43.3	43
Blue	0.32	0.04	0.006	115.5	40
Red	0.82	0.02	0.012	57.7	46

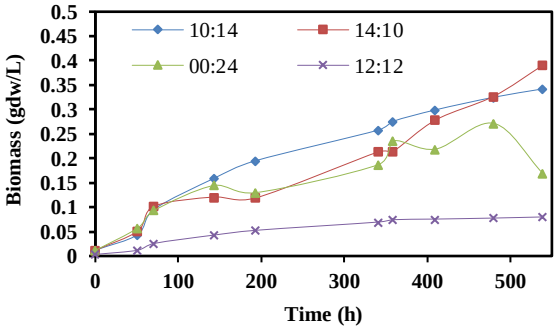

Fig. 7: Chlorella ESP-6 growth curve under different light cycles

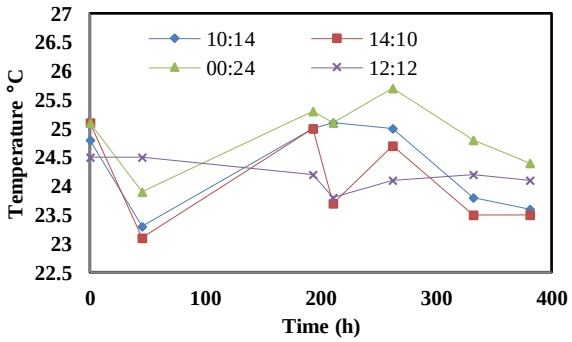

Fig. 9: Temperature change in photobioreactors containing Chlorella protothecoides under the different light cycles

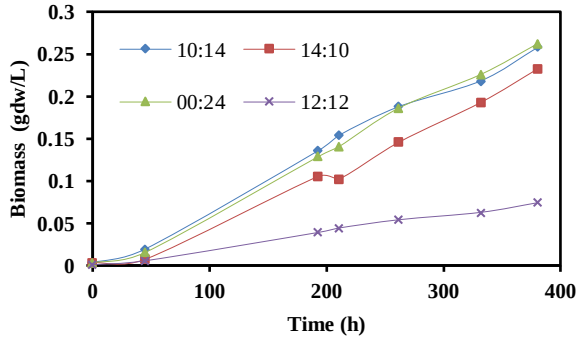

Fig. 8: Chlorella protothecoides growth curve under different light cycles

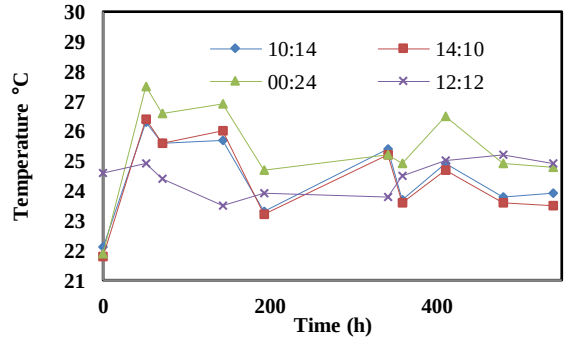

Fig. 10: Temperature change in photobioreactors containing Chlorella ESP-6 under the different light cycles

Effect of different light cycles

The time-dependent growth of *Chlorella ESP-6* and *Chlorella protothecoides* under the influence of different light cycles are shown in Fig. 7 and Fig. 8, respectively.

As can be seen from the experimental results, the maximum lipid content (17%) was reached in the 24-hour light phase, even though the maximum cell concentration was reached in the 14:10 cycle for *Chlorella ESP-6* (0.39gdw/L).

Table 3: Effect of different light cycles on growth and lipid content for Chlorella ESP-6

	X_{max} (gdw/L)	µmax (h^{-1})	Doubling Time (h)	Fat content (%)
14:10	0.39	0.028	24.7	12
12:12	0.08	0.011	63.0	14
10:14	0.34	0.022	31.5	13
0:24	0.27	0.030	23.1	17

Table 4: Effect of different light cycles on growth and lipid content for Chlorella protothecoides

	X_{max}(gdw/L)	µmax (h^{-1})	Doubling Time(h)	Fat content (%)
14:10	0.23	0.018	38.5	43
12:12	0.074	0.013	53.3	39
10:14	0.24	0.013	53.3	45
0:24	0.26	0.014	49.5	48

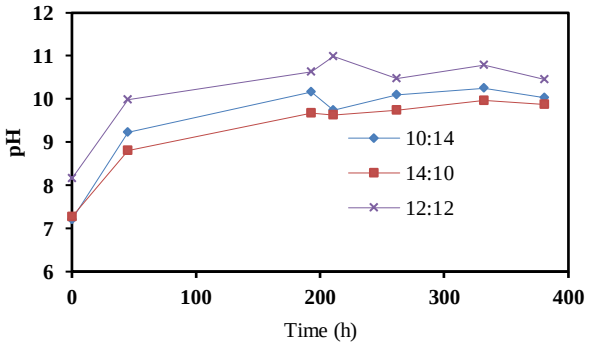

Fig. 11: pH distribution in photobioreactors containing Chlorella protothecoides under the different light cycles

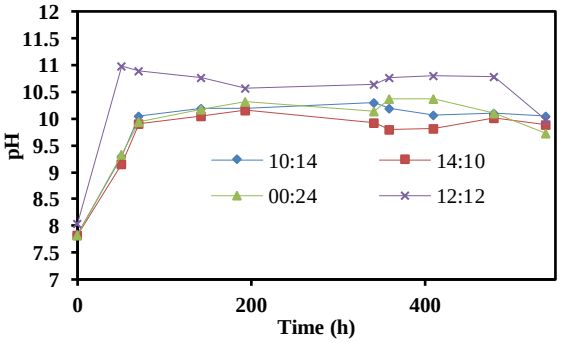

Fig. 12: pH distribution in photobioreactors containing Chlorella ESP-6 under the different light cycles

The maximum cell concentration for *Chlorella protothecoides* (0.26 g / L) was reached in a 0:24 light cycle culture and the lipid content was determined as 48%. The effect of the light-dark cycle on cell growth differs according to microalgae. The temperature and pH distributions of both microalgae cultures are also shown in Fig (9-12). The temperature distributions have been reduced or increased due to the opening and closing of the LED lamps. Also, The pH of the media was around 8.0 for both runs initially, however, it stayed between 9.1-10.1 for and 9.9-11.0 for *Chlorella protothecoides* and *Chlorella ESP-6* during the rest of the culture.

There is no study about the light cycle effect on *Chlorella ESP-6* and *Chlorella protothecoides* was not found in the literature. Tables 3 and 4 show growth rates and lipid contents of *Chlorella ESP-6* and *Chlorella protothecoides*, respectively, for different light cycles.

Also, the average fatty acid compositions of the lipids were listed in Table 5 and Table 6 for *Chlorella protothecoides* and *Chlorella ESP-6*, respectively. The Average molecular weight of the fatty acids was higher for *Chlorella protothocoides* lipids (870 g/mol) compared to *Chlorella ESP-6 lipids (*849 g/mol). Saturated fatty acids (SFA) are a very important factor for biodiesel fuel properties. The cetane number (CN) increases in fuels with high amounts of SFA [17,18]. Therefore, both saturation and unsaturation of FAMEs must be an optimal ratio for high biodiesel quality. The ratio of saturated fatty acids for each microalgae was higher than unsaturated fatty acids. On the other hand, The cetane number is an indication of the ignition quality of the fuel. high fuel is easy to ignite and can burn quickly. The cetane number of diesel fuel ranges from 55 to 60. As the carbon number of the fatty acids in the biodiesel increases, the number of the cetane increases accordingly. The number of cetane in biodiesel ranges from 55 to 75, as the number of biodiesel cetane exceeds that of diesel [19-21].

Table 5: Fatty acid compositions of Chlorella protothecoides

Type of fatty acid	Percentage %
C14:0 Myristic	1.55
C15:0 n-Pentadecanoic	13.7
C15:1 Pentadecenoic	2.8
C16:1Palmitoleic	25.4
C18:0 Stearic	5.3
C18.1 Oleic	7.3
C18:3 a-Linoleic	11.6
C20:0 Arachidic	32.1

Table 6: Fatty acid compositions of Chlorella ESP-6

Type of fatty acid	Percentage %
C6:0 Hexanoic	10.8
C15:0 n-Pentadecanoic	15.6
C17:0 Margaric	22.3
C20:0 Arachidic	51.3

Also, the cetane numbers of both microalgae oils were determined according to Equation (4), 66.9 and 74.9 for *Chlorella protothecoides and Chlorella ESP-6* respectively.

$$CN=58.1+\frac{2.8\ (n-8)}{2}-15.9 \qquad (4)$$

In the formula, n refers to the number of carbons.

CONCLUSIONS

This paper clarifies that lipids obtained from different microalgae species under different illumination factors can be used as a raw material for biodiesel production.

From the obtained experimental data, it is possible to draw some conclusions from this study;

- ✓ The average fat content of microorganisms each for applied light intensity and cycles were determined to be 48% and 17% for *Chlorella protothocoides*and *Chlorella ESP-6* respectively.
- ✓ No significant effects of different light sources and cycles on the microorganism lipid content were obtained.
- ✓ For both high and fast oil production, it is appropriate to use continuous LED light sources for both types of microorganisms.
- ✓ Both microalgae oils are obtained from different cultivation conditions, suitable for biodiesel production as a raw material.

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