Thesis for the Degree of Master of Engineering

Productivity improvement by optimizing culture conditions of microalgae under light emitting diodes irradiation

by

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(미세조류 LED배양에서 배양조건 최적화를 통한 생산성 향상)

Advisor: Prof. Sung-Koo Kim and Prof. Gwi-Taek Jeong

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A dissertation

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이의훈

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요약

최근 미세조류는 2세대 바이오매스에 비해 많은 장점이 있고, 식량자원인 1세대 바이오매스와 경쟁하지 않는 3세대 바이오매스로 연구되고 있다. 본 연구에서는 미세조류의 생산성 향상을 위해 배양 매개변수를 최적화하고 조건에 따른 변화를 분석하는 것을 목적으로 하였다. 이를 위하여 *Chlamydomonas hedleyi*의 바이오매스 및 지질 생산을 최대화하기 위하여 2단계 배양의 매개변수를 최적화하였다. 첫 번째 단계에서 바이오매스 생산을 최대화하기 위해 명암주기, 광도를 최적화 하였다. 두 번째 단계에서 지질 생산을 최대화하기 위해 스트레스 조건을 조성하였고, 질산염 고갈 유지일과 명암주기, 광도를 최적화하였다. 첫 번째 단계에서 0.95g DCW/L의 최대 바이오매스가 240 mg/L의 질산염 농도, 24:0 h의 명암주기, 500 μ $molm^{-2}s^{-1}$의 광도에서 생산되었다. 두 번째 단계에서는 12:12 h의 명암 주기와 400 $\mu molm^{-2}s^{-1}$의 광도와 2일 동안의 질산염 고갈 조건에서 최대 지질 함량이 41.3% 였다. *C. hedleyi*의 주요 지방산은 32.24%의 팔미트산 (C16:0), 27.42%의 올레산 (C18:1), 16.76%의 아라키드산 (C20:0)이었다. C16-18의 함량은 전체 지방산의 80.38%로 바이오디젤로 전환 시 높은 세탄가를 달성할 수 있다고 판단된다.

1. Introduction

Recently, microalgae are being studied as third-generation biomass that does not compete with food resources (first-generation biomass) that have many advantages compared to second-generation biomass. Microalgae can be used as a source of next-generation biofuels and useful high-value products because of their high oil yield compared to currently available crops and their ability to grow in uncultivated water (Greenwell et al., 2010).

Biodiesel is attractive as a sustainable, carbon-neutral, and economically viable alternative to fossil fuels (Rodolfi et al., 2009). Because biodiesel use can cope with fossil fuel depletion and climate change, many countries have taken steps to increase biodiesel use by establishing relevant regulatory and incentive systems (Hoekman et al., 2012). Biodiesel is generally extracted from edible crops such as soybeans. However, considering that large-scale arable land is required and the potential conflict between biodiesel production and food production, alternative raw materials are needed (Chisti, 2007).

The total lipid content of microalgal species can reach 30 - 60% of dry weight. Microalgae has high adaptability to stress conditions, which can contribute to increased lipid production (Paliwal et al., 2017). Therefore, when microalgae are cultured under continuous stress conditions, the accumulation of lipids in these microalgae may increase (Pancha et al., 2015).

Microalgae are photosynthetic organisms with a light-harvesting

antenna (LHA) that collects light energy at visible wavelengths of 400 to 700 nm (Kumar et al., 2021). However, as the range of wavelengths that can be absorbed by each species of microalgae is different, culture efficiency can be increased if the wavelength of light emitted from the light source is matched with the wavelength that can be absorbed by the microalgae (Johnson et al., 2018). Therefore, high biomass productivity and quality improvement can be achieved with an appropriate artificial light source capable of controlling the wavelength. In addition, it is possible to improve the quality of the product by controlling the photoperiod and light intensity (Carvalho et al., 2011). However, as maintenance is a necessary cost when using an artificial light source compared with sunlight, cost-effective cultivation under optimized conditions is crucial.

Light-emitting diodes (LEDs) are digital illumination devices that can precisely control the wavelength and intensity of light, which are essential parameters for microalgal growth (Yan et al., 2016). When natural light is used, light may be insufficient when it is cloudy or at night, and the growth of microalgae may be suppressed because illuminance cannot be controlled (Pilon et al., 2011). In comparison with the white light of conventional artificial light sources such as incandescent and fluorescent lamps, LEDs can only irradiate a single wavelength and have a very long life of up to 50,000 h (Olle and Viršile, 2013). Therefore, LEDs are a suitable light source for microalgal cultivation because of their higher energy efficiency compared with that of conventional light sources; the desired wavelength can be selected

with the fine control of various parameters (de Queiroz Fernandes Araújo et al., 2015).

Currently, more than 40,000 species of microalgae have been reported (Singh and Dhar, 2011), and research on microalgal cultivation using LEDs is ongoing (Das et al., 2010; Lunka and Bayless, 2013). However, although a large number of them have been identified, only a few species are being used for in-depth studies. *Chlamydomonas hedleyi* is a microalga belonging to the green algae plant class (Lee et al., 1974). It has been used as biomass for the production of mycosporine-like amino acids (MAAs) (Suh et al., 2014), as a UV-blocking functional molecule, and to supply lipids or carbohydrates (Burch et al., 2015). However, studies have rarely been conducted on *C. hedleyi*, and information on biomass and lipid production is still limited.

The study in Chapter II focused on optimizing parameters related to biomass and lipid production in *C. hedleyi*. In addition, the potential as a biofuel source was evaluated. Microalgae with high lipid content have low biomass production, whereas microalgae with high biomass production have low lipid content. Consequently, high lipid production can be achieved by exposing microalgal cells to stress conditions. However, it does not result in rapid growth and high biomass production (Aziz et al., 2020). Therefore, in this study, a two-phase culture system suitable for both biomass production and lipid production was used when culturing microalgae (Fig. 1).

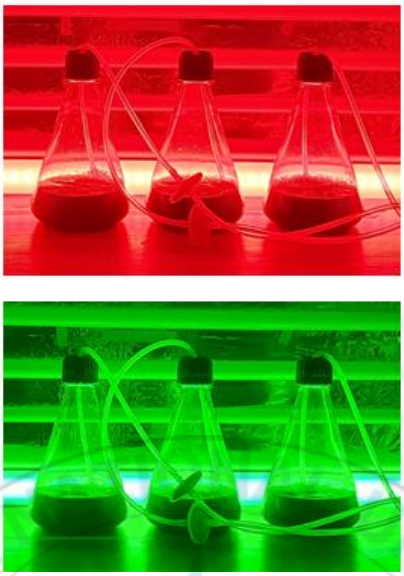

Fig 1. Photographs of the microalgae two-phase culture system. A red wavelength (625 nm) was used in the first phase, and a green wavelength (520 nm) was used in the second phase for culturing *C. hedleyi*.

2. Materials and Methods

2.1. Microalgal and culture conditions

C. hedleyi (LIMS-PS-1225) was obtained from the Korea Institute of Ocean Science and Technology (Geoje-si, Korea) and pre-cultured for 12 days in sterilized seawater with modified f/2 medium. The composition of the modified f/2 medium in 1 L of seawater was as follows: 75 mg $NaNO_3$, 5 mg $NaH_2PO_4 \cdot H_2O$, 4.36 mg Na_2EDTA, 3.15 mg $FeCl_3 \cdot 6H_2O$, 0.02 mg $MnCl_2 \cdot 4H_2O$, 0.02 mg $ZnSO_4 \cdot 7H_2O$, 0.01 mg $CoCl_2 \cdot 6H_2O$, 0.01 mg $CuSO_4 \cdot 5H_2O$, 0.006 mg $Na_2MoO_4 \cdot 2H_2O$, 30 mg Na_2SiO_3, 0.2 mg thiamine-HCl, 0.01 mg vitamin B_{12}, and 0.1 mg biotin (Guillard and Ryther, 1962). For use in experiments, *C. hedleyi* was cultured in a 2 L flask with a working volume of 1 L, and the initial cell concentration was set at an optical density of 0.2 (at 680 nm). The aeration rate and culture temperature were set at 2.5 vvm and 20℃, respectively. As the light source, a fluorescent lamp was used with a light intensity of 200 $\mu molm^{-2}s^{-1}$ under a 12:12 h light/dark cycle.

2.2. LED for microalgal culture

The LED photoperiod was set at 12:12, 18:6, and 24:0 h light/dark cycles, and light intensities of 300, 400, 500, and 600 $\mu molm^{-2}s^{-1}$ were evaluated under an optimized photoperiod. LED bars (90 × 6 × 4 cm^3; Ciel Light Co. Ltd., Korea) were placed parallel to each flask, and the light intensity was adjusted according to the number of panels. The LED chip can irradiate red (625 nm) and green (520 nm) wavelengths,

and 36 chips were arranged horizontally at 1 cm intervals (Fig. 1). The light intensity was measured at the center of the culture using a light sensor (HD2102.2; Ohm S.R.L., Italy).

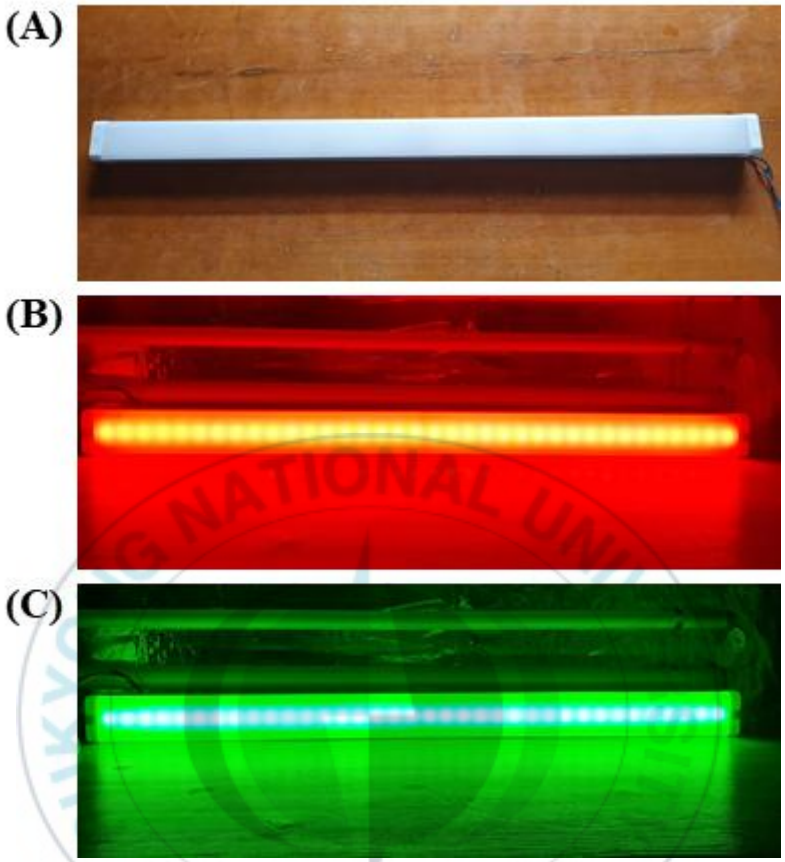

Fig 2. Photographs of the light sources used in experiment. LED bar capable of irradiation two wavelengths (A) was used, LED of red wavelength (B) and LED of green wavelength (C).

2.3. Measurement of microalgal growth

The optical density of microalgae measured with a UV spectrophotometer (UltrospecTM 6300 Pro; Biochrom Ltd., UK) was converted to dry cell weight based on a standard curve. The nitrate concentration in the culture medium was measured using an optical method at OD_{220} (Collos et al., 1999). The correlation equation (R^2=0.99) for the dry cell weight of *C. hedleyi* at OD_{680} is shown in Eq.(1):

Dry cell weight of *C. hedleyi* (g DCW L^{-1})
= 0.43 (OD_{680}) (R^2=0.99) Eq. (1)

2.4. Measurement of total lipid content

Microalgal cells were harvested by centrifugation for 10 min at 5,000 rpm (Supra R22; Hanil Scientific Inc., Korea), washed twice with distilled water, and lyophilized in a freeze dryer (SFDSM-24L; Sam Won Industry, Korea). Then, 5 mL of distilled water was added per 10 mg of lyophilized microalgal biomass, and the cells were disrupted using a sonicator (300 W, 20 kHz; KFS-300N Ultra sonicator, Korea Process Technology Co., Ltd., Korea). The total lipid content was obtained by solvent extraction using chloroform, methanol, and distilled water (Bligh and Dyer, 1959). The dry weight lipid content was determined using Eq. (2):

$$\text{Lipid content (\% of DCW)} = \frac{(W_2 - W_1)X\,100}{DCW} \quad \text{Eq. (2)}$$

where W_1 (g) is the weight of the empty 20 mL glass test tube, W_2 (g) is the weight of the extracted lipid plus the weight of the empty glass test tube, and DCW (g) is the weight of the dry cells of *C. hedleyi*.

2.5. Fatty acid methyl ester (FAME) composition analysis

The extracted lipids were converted to FAMEs using a modified transesterification method (Griffiths et al., 2010). Next, FAME profiling was performed by gas chromatography (YL 6100; YoungLin Inc., Korea) with a flame ionization detector (FID) and silica capillary column (HP-INNOWAX; 30 m × 0.32 mm × 0.5 μm; Agilent Technologies, USA). Column temperature adjustments were as follows: 140°C for 5 min, followed by an incremental temperature increase to 240°C at the rate of 5°C/min and subsequent incubation at 240°C for 10 min. The temperatures of the injector and FID were set at 250°C. FAMEs were identified by comparing their retention times with those of authentic standards (F.A.M.E. Mix C14-C22; Methyl palmitoleate; cis-5, 8, 11, 14, 17-Eicosapentaenoic acid methyl ester; all-cis-4, 7, 10, 13, 16, 19-Docosahexaenoic acid methyl ester; Sigma-Aldrich, USA).

2.6. Statistical analyses

Each experiment was performed in triplicate. Differences in the lipid content were evaluated by one-way analysis of variance (ANOVA) and Duncan's multiple range test using SPSS software (ver. 23.0; SPSS

Inc., Chicago, IL, USA). Differences were considered statistically significant at $P < 0.05$.

3. Results and Discussion

3.1. Effects of LED photoperiod on microalgal growth and lipid and lipid accumulation

Chlamydomonas hedleyi was incubated in a 2 L flask at a working volume of 1 L. As red wavelengths have a similar effect on the growth of green algae, it has been reported that the response to red wavelengths is consistent between different microalgal species (Kim et al., 2014). Therefore, in this study, the LED wavelength was set to red, which could result in the highest light energy utilization efficiency in green algae according to previous studies (Ra et al., 2016; Kim et al., 2019).

Fig. 3(A) shows the biomass production of *C. hedleyi* according to the LED photoperiod. Other conditions were the same as those in the previous experiment, and an optimal nitrate concentration of 240 mg/L was used. Under light:dark cycles of 12:12, 18:6, and 24:0 h, biomass yields were 0.63 g DCW/L on day 16, 0.76 g DCW/L on day 13, and 0.83 g DCW/L on day 12, respectively, at reaching stationary phase. Light is the most important factor that affects the photosynthesis and biochemical composition of microalgae (Krzemińska et al., 2014). The biomass production of *C. hedleyi* was increased with an extended light irradiation period. These results were consistent with those of other studies on photoperiods (Khoeyi et al., 2012; Ruangsomboon, 2012).

Fig. 3(B) shows the lipid production of *C. hedleyi* according to the LED photoperiod. In the second phase, under nitrogen starvation conditions, a green wavelength was used to induce maximal lipid production, and the effect of the photoperiod on lipid accumulation was evaluated. Blue and red wavelengths are well absorbed by photosystems Ⅰ and Ⅱ in chloroplasts (Perrine et al., 2012). However, green wavelengths are not absorbed but reflected and thus could inhibit the photosynthesis of microalgae (Jung et al., 2019), which has been demonstrated in the literature (Yan et al., 2013).

The lipid production of *C. hedleyi*, which was cultured under light:dark cycles of 12:12, 18:6, 24:0, was the highest on day 2 of the second phase (37.6, 35.0, and 32.3%, respectively). The highest lipid content was obtained under the 12:12 h light:dark cycle. Storage lipids in the form of triacylglycerol (TAG) supply energy to metabolic processes (Thompson, 1996). Most microalgae produce TAG when cultured under adverse growth conditions. However, prolonged stress periods generally reduce the total biomass and TAG productivity (Klok et al., 2013). Therefore, irradiation for more than 12 h reduced the lipid production of *C. hedleyi*.

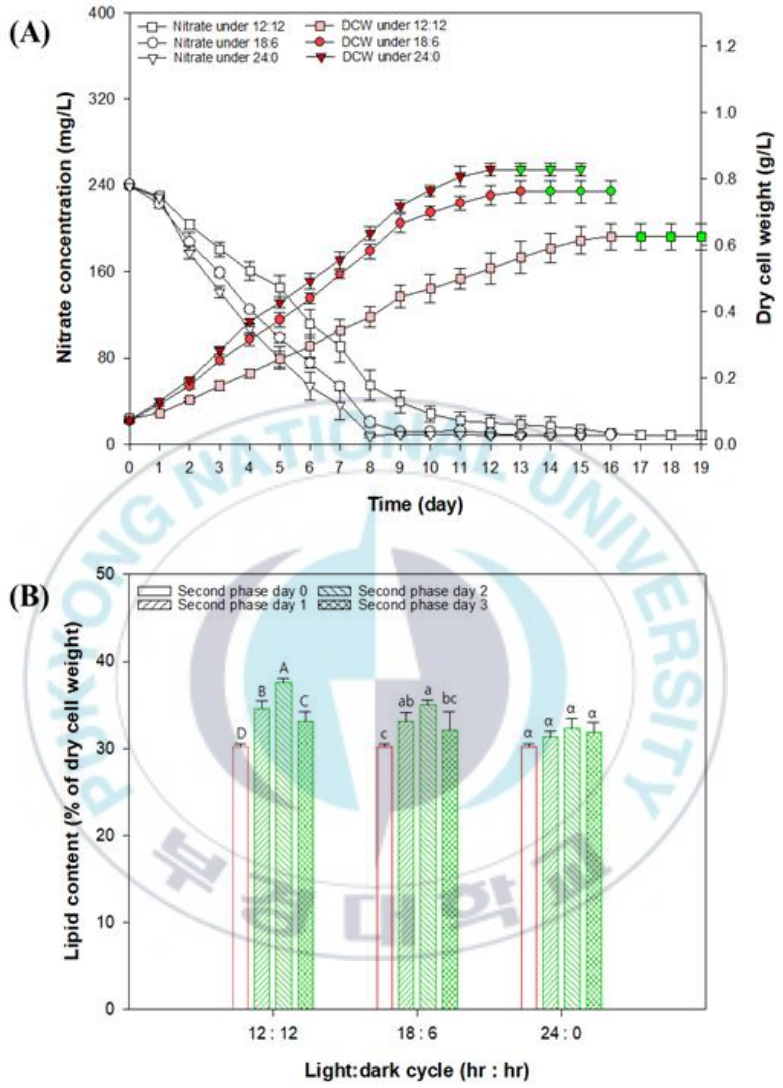

Fig 3. Effect of the LED photoperiod on the biomass (A) and lipid production (B) of *C. hedleyi* ($P < 0.05$, Duncan's test). The green symbols in (A) indicate the second phase of conversion from red wavelength (625 nm) to green wavelength (520 nm)

3.2. Effect of LED light intensity on microalgal growth and lipid accumulation

Fig. 4(A) shows the biomass production of *C. hedleyi* under various LED light intensities. The nitrate concentration and photoperiod were set at 240 mg/L and a 24:0 h light:dark cycle, respectively, which were optimal conditions. *C. hedleyi* produced 0.87, 0.90, 0.95, and 0.92 g DCW/L biomass under light intensities of 300, 400, 500, and 600 $\mu molm^{-2}s^{-1}$, respectively. As the light intensity was increased, the biomass production of *C. hedleyi* was increased; however, the biomass was decreased under a light intensity of 600 $\mu molm^{-2}s^{-1}$.

In nature, the light intensity continuously changes over time, and the light:dark cycle is not constant. Microalgae have developed adaptation mechanisms to cope with these changes (Ramanna et al., 2017). When exposed to light, microalgae absorb some of the light through pigments and scatter some, and the rest pass through cells without interactions (Nikolaou et al., 2015). When microalgae are exposed to high light intensity, excessive light causes the photo-oxidation of the photosystem II component, resulting in photosystem II inactivation. This phenomenon ultimately reduces microalgal productivity by damaging essential proteins required for electron transfer during photosynthesis (Natali and Croce, 2015). A light intensity of 600 $\mu molm^{-2}s^{-1}$ was a high intensity that could cause photo-oxidation in the photosystems of *C. hedleyi*, as demonstrated in similar studies (Liao et al., 2018).

Fig. 4(B) shows the lipid production of *C. hedleyi* according to the LED light intensity. The effect of the light intensity of the LEDs on

the lipid production of *C. hedleyi* was evaluated using a light:dark cycle of 12:12 h under nitrogen starvation conditions. The lipid production of *C. hedleyi* cultured under light intensities of 300, 400, 500, and 600 $\mu molm^{-2}s^{-1}$ was the highest on day 2 of the second phase (40.2, 41.3, 37.8, and 36.7%, respectively). Under a light intensity of 400 $\mu molm^{-2}s^{-1}$, the lipid production of *C. hedleyi* was the highest, and under light intensities of 500 and 600 $\mu molm^{-2}s^{-1}$, lipid production was decreased.

When stressed due to variations in the light intensity, the nutrient profile of microalgae changes significantly. At high light intensities, microalgae could synthesize TAG as a defense mechanism; TAG levels have been reported to increase in several microalgal species (Breuer et al., 2013; He et al., 2015). However, at a high light intensity of 500 $\mu molm^{-2}s^{-1}$ or more, the lipids of *C. hedleyi* could be photo-oxidized, resulting in a decrease in production.

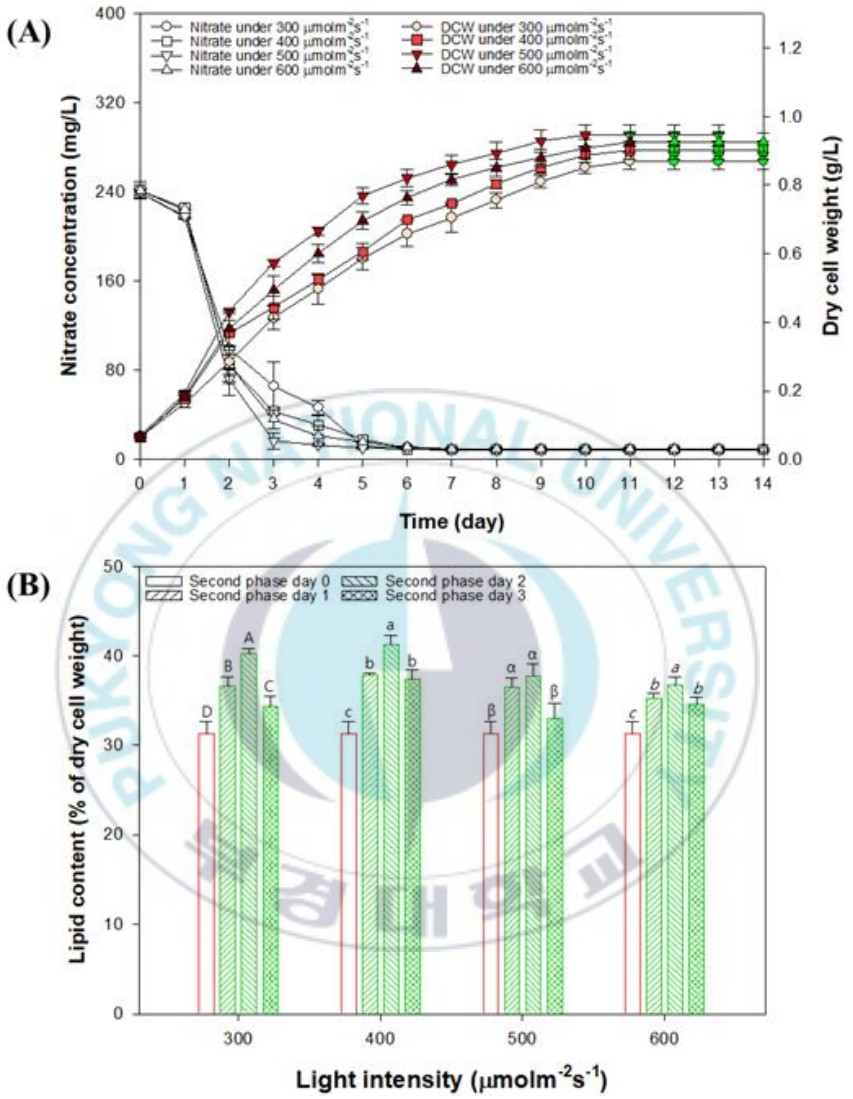

Fig 4. Effect of the LED light intensity on the biomass (A) and lipid production (B) of *C. hedleyi* ($P < 0.05$, Duncan's test). The green symbols in (A) indicate the second phase of conversion from red wavelength (625 nm) to green wavelength (520 nm)

3.3. Fatty acid composition

Table 1 shows the composition of fatty acids of *C. hedleyi* during the second phase under light intensities of 300, 400, 500, and 600 $\mu molm^{-2}s^{-1}$. The main fatty acids of *C. hedleyi* were palmitic acid (C16:0), oleic acid (C18:1), and arachidic acid (C20:0), which were 35.57, 25.77, and 16.84% on day 0 of the second phase, respectively. The content of saturated fatty acids including palmitic acid was decreased as the light intensity was increased. On the other hand, the content of unsaturated fatty acids including oleic acid was increased. Under optimized conditions with 400 $\mu molm^{-2}s^{-1}$ light intensity on day 2 of the second phase, the contents of palmitic acid (C16:0), oleic acid (C18:1), and arachidic acid (C20:0) in the microalgae were 32.24, 27.42, and 16.76%, respectively, with a lipid content of 41.3%.

It has been reported that five C16 - C18 fatty acids are the most common feedstocks suitable for biodiesel production, which are palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) (Knothe, 2009). *C. hedleyi* contained large amounts of C16:0 and C18:1; however, the polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6), which are not suitable for biodiesel production due to poor oxidation stability, were present in very small amounts. C18:1 in *C. hedleyi* may provide the best compromise between oxidative stability and low temperature fluidity (Hoekman et al., 2012).

The two most common quality standards for biodiesel are EN 14214 in Europe and ASTM D6751 in the United States. A cetane number of

at least 51 for Europe and at least 47 for the United States must be satisfied (ASTM, 2008; CEN, 2003). The cetane numbers of C18:0 and C18:3 are 101 and 22.7, respectively (Knothe, 2009). The cetane number is high when there are many carbons with single bonds, and the cetane number is low when the degree of unsaturation is high. In *Chlorella vulgaris* and *Phaeodactylum tricornutum*, the proportion of C16-18 is 64.15 and 74.50%, respectively. Both microalgae contain significant amounts of C16-18 and satisfy the standard range of the cetane number in Europe and the United States (Song et al., 2013). In this study, *C. hedleyi* contained large amounts of C16-18 (80.38%) under optimal conditions and almost no EPA and DHA, which are long-chain unsaturated fatty acids. Therefore, *C. hedleyi*, which has a high content of C16-C18 fatty acids, may be satisfied with a cetane number in the standard range. In addition, as the content of PUFAs with three or more double bonds is low and thus results in a low degree of unsaturation, the lipids of *C. hedleyi* would have high oxidative stability.

Table 1. Composition of the fatty acids of *C. hedleyi* during the second phase under light intensities of 300, 400, 500, and 600 μmolm^{-2}s^{-1}.

Fatty acid (% of total fatty acid)	Day 0	Day 1			
		300	400	500	600
Myristic acid (C14:0)	0.84 ± 0.02	1.53 ± 0.03	0.91 ± 0.03	0.60 ± 0.02	0.62 ± 0.01
Palmitic acid (C16:0)	35.57 ± 0.3	34.43 ± 0.5	33.94 ± 0.4	34.03 ± 0.1	32.22 ± 0.2
Palmitoleic acid (C16:1)	6.97 ± 0.1	9.25 ± 0.2	7.88 ± 0.2	6.36 ± 0.3	6.40 ± 0.1
Stearic acid (C18:0)	5.10 ± 0.3	6.88 ± 0.2	4.76 ± 0.1	3.87 ± 0.2	4.02 ± 0.4
Oleic acid (C18:1)	25.77 ± 0.4	25.03 ± 0.1	28.52 ± 0.3	28.41 ± 0.1	28.90 ± 0.2
Linoleic acid (C18:2)	6.39 ± 0.2	4.62 ± 0.3	5.02 ± 0.2	5.90 ± 0.1	6.68 ± 0.3
Linolenic acid (C18:3)	0.42 ± 0.06	0.31 ± 0.04	0.35 ± 0.04	0.32 ± 0.02	0.39 ± 0.02
Arachidic acid (C20:0)	16.84 ± 0.5	15.77 ± 0.4	16.27 ± 0.3	18.31 ± 0.5	18.16 ± 0.1
Eicosapentaenoic acid (C20:5)	0.12 ± 0.02	0.17 ± 0.01	0.25 ± 0.01	0.22 ± 0.03	0.24 ± 0.04
Behenic acid (C22:0)	1.07 ± 0.03	1.21 ± 0.02	1.08 ± 0.01	1.00 ± 0.01	0.66 ± 0.04
Docosahexaenoic acid (C22:6)	0.92 ± 0.02	0.79 ± 0.03	1.03 ± 0.02	0.98 ± 0.01	1.71 ± 0.02
Unsaturated fatty acid	40.59 ± 0.8	40.18 ± 0.68	43.06 ± 0.77	42.19 ± 0.56	44.32 ± 0.68
Saturated fatty acid	59.41 ± 1.15	59.82 ± 1.15	56.94 ± 0.84	57.81 ± 0.83	55.68 ± 0.75

Day 2				Day 3			
300	400	500	600	300	400	500	600
1.01 ± 0.03	0.92 ± 0.04	0.73 ± 0.01	0.73 ± 0.02	1.16 ± 0.03	0.91 ± 0.04	0.69 ± 0.02	0.66 ± 0.03
34.32 ± 0.3	32.24 ± 0.5	32.36 ± 0.4	32.36 ± 0.3	27.50 ± 0.4	29.06 ± 0.2	29.69 ± 0.3	35.77 ± 0.5
8.07 ± 0.2	8.69 ± 0.1	8.48 ± 0.3	6.52 ± 0.4	8.82 ± 0.2	6.37 ± 0.3	8.29 ± 0.2	6.53 ± 0.3
4.81 ± 0.3	4.15 ± 0.2	5.72 ± 0.2	4.27 ± 0.3	5.75 ± 0.2	3.85 ± 0.3	6.77 ± 0.4	4.18 ± 0.2
27.70 ± 0.5	27.42 ± 0.6	30.05 ± 0.5	30.43 ± 0.4	30.56 ± 0.4	32.33 ± 0.6	32.03 ± 0.5	30.09 ± 0.4
6.44 ± 0.4	7.49 ± 0.2	6.02 ± 0.3	6.33 ± 0.3	5.95 ± 0.1	5.95 ± 0.2	5.93 ± 0.2	6.64 ± 0.3
0.36 ± 0.03	0.39 ± 0.01	0.27 ± 0.02	0.20 ± 0.01	0.39 ± 0.01	0.87 ± 0.02	0.32 ± 0.01	0.43 ± 0.02
15.16 ± 0.3	16.76 ± 0.2	13.94 ± 0.5	18.78 ± 0.4	17.92 ± 0.4	18.34 ± 0.3	14.13 ± 0.3	13.66 ± 0.1
0.18 ± 0.01	0.23 ± 0.04	0.25 ± 0.01	0.26 ± 0.02	0.16 ± 0.01	0.19 ± 0.02	0.17 ± 0.01	0.18 ± 0.02
0.93 ± 0.02	0.63 ± 0.02	1.03 ± 0.01	0.60 ± 0.03	1.00 ± 0.02	1.31 ± 0.01	1.17 ± 0.01	0.82 ± 0.01
1.02 ± 0.01	1.08 ± 0.03	1.16 ± 0.02	1.19 ± 0.03	0.78 ± 0.01	0.82 ± 0.01	0.81 ± 0.01	1.04 ± 0.01
43.77 ± 1.15	45.30 ± 0.98	46.23 ± 1.15	44.93 ± 1.16	46.68 ± 0.73	46.53 ± 1.15	47.55 ± 0.93	44.91 ± 1.05
56.23 ± 0.95	54.70 ± 0.96	53.77 ± 1.12	55.07 ± 1.05	53.32 ± 1.05	53.47 ± 0.85	52.45 ± 1.03	55.09 ± 0.84

4. Conclusion

A two-phase culture system was successfully used to maximize the biomass and lipid production of *C. hedleyi*. A sodium nitrate concentration of 240 mg/L, a light:dark cycle of 24:0 h, and a light intensity of 500 $\mu molm^{-2}s^{-1}$, which were optimized in the first phase, produced a maximum biomass of 0.95 g DCW/L. In the second phase, a stress environment combined with nitrogen starvation, a light:dark cycle of 12:12 h, and a light intensity of 400 $\mu molm^{-2}s^{-1}$ produced a maximum lipid content of 41.3%. The main fatty acids of *C. hedleyi* were palmitic acid (C16:0), oleic acid (C18:1), and arachidic acid (C20:0) under optimal conditions. The C16-C18 fatty acid content was 80.38%, which may be associated with a high cetane number, and 8.69% of palmitoleic acid (C16:1) and 27.42% of oleic acid (C18:1) may provide the best compromise between the oxidation stability and low temperature fluidity of biodiesel. As the fatty acid content of *C. hedleyi* could meet the biodiesel standard requirements of Europe and the United States, it may be considered as a raw material for biodiesel production.

5. References

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