Thesis for the Degree of Master of Engineering

Studies on biomass and oil production from eight microalgae using Light-Emitting Diode (LED)

by

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Department of Biotechnology

The Graduate School

Pukyong National University

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발광다이오드 (LED)를 이용한 8종의 미세조류 바이오메스 및 오일 생산에 관한 연구

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요 약

화석연료사용의 증가로부터 대기 중의 CO_2 농도가 증가하여 지구온난화 현상이 심각하게 대두되고 있으며, 에너지 원료의 고갈로 인해 대체에너지 자원의 필요가 증가하고 있다. 이 두 문제를 해결할 수 있는 대체에너지 자원 원료로서 미세조류가 각광받고 있다. 미세조류는 빛에너지와 대기 중의 CO_2를 이용해 성장하는 광합성독립영양생물이다. 미세조류는 3세대 바이오매스로서 성장이 빠르고, 오일을 체내에 축적가능 하며, 배양을 위한 많은 면적의 땅을 요구하지 않는다. 미세조류의 성장에서 가장 중요한 에너지원은 빛 에너지원으로, 빛 광도, 빛 파장에 영향을 받는다. 본 연구에서 사용한 빛 에너지원은 발광다이오드로서 형광등에 비해 전력량이 낮아 가격이 저렴하고, 미세조류가 원하는 파장 만을 이용할 수 있다는 장점이 있다. 따라서 본 연구에서는 *Isocrhysis galbana*, *Pheodactylum tricornutum*, *Nannochloropsis oculata*, *Nannochloropsis oceanica*, *Nannochloropsis salina*, *Dunaliella salina*, *Dunaliella teriolecta*, *Nannchloris atomus*, 8종의 미세조류의 파장, 광도, 영양분의 최적 농도를 결정했다. 이와 더불어 Two-phase 배양을 통해 체네의 오일함량을 증가 시켰으며, 바이오디젤의 특성으로 적합하다고 알려진 C16 (palmitic acid)와 C18 (stearic acid, oleic acid, linoleic acid, linolenic acid) 의 함량을 분석하였다.

바이오디젤 생산하기위해 8종 미세조류의 최적조건을 확립하기 위하여 f/2 배지

를 1.5L 의 멸균해수에 첨가하고 light and dark cycle (L:D cycle) 은 12:!2, air 공급은 2.5L/min 으로 일정하게 공급했으며, 온도는 20℃에서 배양했다. 최적 파장을 설정하기위해 광도 70 μmol/m^2/s LED 파장을 660 nm (red), 640 nm (orange), 520 nm (green), 465 nm (blue), 405 nm (violet) 파장에서 배양했다. 그 결과 *I. galbana*, *P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* 7종의 미세조류는 465 nm (blue) 파장에서 최대 바이오메스를 얻었고, *N. atomus* 는 660 nm (red)파장에서 최대 바이오매스를 얻어 이를 최적 파장으로 설정했다. 이 후 최적 광도를 설정하기위해 각 종의 최적 파장과 40, 70, 100, 130 μmol/m^2/s 의 광도에서 배양했다. 그 결과 8종 미세조류 모두 100 μmol/m^2/s 의 광도에서 최대 바이오매스를 생산했다. 형광등에서 동일한 조건으로 배양한 결과를 비교 했을 때, LED에서 배양한 미세조류의 바이오매스 생산이 형광등에 비해 약 2배이상의 바이오매스 생산을 확인하였으므로, LED가 효과적인 빛에너지 자원임을 확인 하였다. 최적 파장 및 최적 광도를 설정한 후 성장에 있어서 가장 중요한 영양원인 질소원 농도를 8, 16, 24, 32 mg/L 로 설정하여 배양했다. 이 때 *I. galbana*, *P. tricornutum* 은 16 mg/L에서 각각 0.93, 1.08 g dcw/L 의 최대 바이오매스를 생산했고, 오일함량은 30, 36% of dry cell weight의 함량을 얻었다. 또한 *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta*, *N. atomus* 는 24 mg/L 의 질소원 농도에서 1.01, 0.92, 0.88, 0.79, 0.75, 0.84 g dcw/L 의 바이오매스 함량과 28, 23, 22, 25, 26, 25% of dry cell weight 의 오일함량을 각각 생산했다.

최적 파장, 광도, 영양분의 농도를 설정해 최대 바이오매스를 생산한 후 오일함량 및 지방산 함량을 증가시키기 위한 배양으로 파장을 변환시켜 배양하는 two-phase 배양을 실시하였다. 최적조건하에서 배양한 미세조류가 정지기에 도달했을 때 배양하고 있는 모든 미세조류의 빛 파장을 660 nm (red) 혹은 520 nm (green) 혹은 465 nm (blue) 파장으로 변환하여 3일간 배양했다. 그 결과 8종의 미세조류에서 520 nm (green)에서 가장 많은 오일을 축적했다. 이때 오일함량과 C16 (palmitic acid)와 C18 (stearic acid, oleic acid, linoleic acid, linolenic acid)함량을 분석했을 때 *I. galbana*, *P. tricornutum*, *N. oculata*, *N.*

oceanica, *N. salina*, *D. salina*, *D. tertiolecta*, *N. atomus* 종은 60, 62, 56, 53, 50, 52, 54, 52% of dry cell weight의 오일함량을 생산했고, 이때의 지방산함량을 분석한 결과 C16 (palmitic acid)는 435.4, 360.0, 382.4, 381.5, 322.8, 326.4, 350.4, 252.4 mg/g oil 의 함량을, C18 (stearic acid, oleic acid, linoleic acid, linolenic acid)는 443.9, 542.7, 416.0, 374.1, 343.5, 449.1, 435.8, 367.8 mg/g oil 의 함량이 측정되었다.

이 결과로서 미세조류는 성장을 위해 특정파장을 흡수하여 성장하고, 이는 미세조류가 가지고 있는 chlorophyll a, β-carotene 혹은 phycocyanin의 보조색소로 인한 것임을 알 수 있었고, 최적조건에서 배양한 후 two-phase 공정을 실시 했을 때, 전체적인 오일함량 및 지방산 함량을 증가시켰다. 이 결과들은 미세조류를 LED같은 적절한 빛 에너지원을 이용했을 때 낮은 전력량으로 전체적인 바이오매스, 오일, 지방산 함량을 증가시킴으로써 차세대 미세조류 빛 에너지원으로 이용할 수 있음을 확인하였다.

1. Introduction

The search for alternative fuel source has received attention due to the limited supply of crude oil and increasing greenhouse gas emissions by using fossil fuels. (Shafiee et al., 2010). Therefore, there has been many study in using microalgae as a potential source of biodiesel.

Microalgae are known for their metabolic flexibility by varying the cultivation conditions of algae, thus regulation in biochemical composition of the biomass can be achieved (Pascal et al., 2012). In addition, compared to terrestrial oil crops, microalgae exhibit high productivity, short generation time and high oil content, thus large scale microalgae oil production is possible (Malcata, 2011). In microalgae culture of photoautotrophic mode, photons of light are one of the major energy sources for the growth of cells. Since, the photons of light could be absorbed by the microalgae cells as nutrients, the properties of light source, such as wavelength and intensity are definitely critical for photoautotrophic microalgae. The growth of microalgae can be controled culture conditions such as temperature, light intensity, pH, nitrogen and phosphorus in culture medium. Among these factors, the nitrogen composition of medium concentration can affect of intracellular lipid accumulations (Pruvost et al., 2009;

Rodifi et al., 2009).

Light intensity and wavelength play very critical roles in photosynthesis process which are consequently reflected in the growth of organisms. Microalgae also require light and dark phases for its productive photosynthesis. During the light phase ATP and NADPH are produced and produced during the dark phase, cellular protein, carbohydrates, nucleic acids and lipids are biosynthesized (Cheirsilp and Torpee, 2012). The specific growth rate of algae could be influenced by the light source (Chojnacka et al., 2004). Light Emitting diodes (LEDs) can produce cost effective low wattage irradiance. Photosynthetic active radiation (PAR) and the wavelength range of 400-700 nm are effective for photosynthesis (Fig. 1). Energy absorption by photosynthetic organism is thus dependent on the chemical natural of their constitutive pigments (Carvalho et al., 2011).

All chlorophyll (green pigments) have two major absorption bands: blue or blue-green (450-475 nm) and red (630-675 nm). The growth of microalgae can be improved using either red light or blue light (Korbee et al., 2005). Chlorophyll a is the major of reaction pigment, while accessory pigments; chlorophyll b, c and d extend the range of light absorption (Richmond, 2004). Light intensity should be delivered evenly over the illuminating surface of culture vessel. Adequate amount of photosynthetically active

radiation (PAR) reaching photons enables the cell in culture (Lee, 1999). Excessive light intensity may lead to photo oxidation and photo inhibition, however low light level may limit cell. Studies have been focused on microalgae oil and fatty acid composition using two-stage culture (Aflalo et al., 2007; Go et al., 2012). Two-stage culture requires complex and inconvenient process. However, two-phase process is easily convenient process because of only changing the LED wavelength as stress factor against two-stage process which twice the harvesting process after reached to stationary phase. Therefore, this study was implemented for the optimization of eight microalgae growth through various LED wavelength, light intensity and nitrate concentration and the increase of oil contents by two-phase cultivation with wavelength stress.

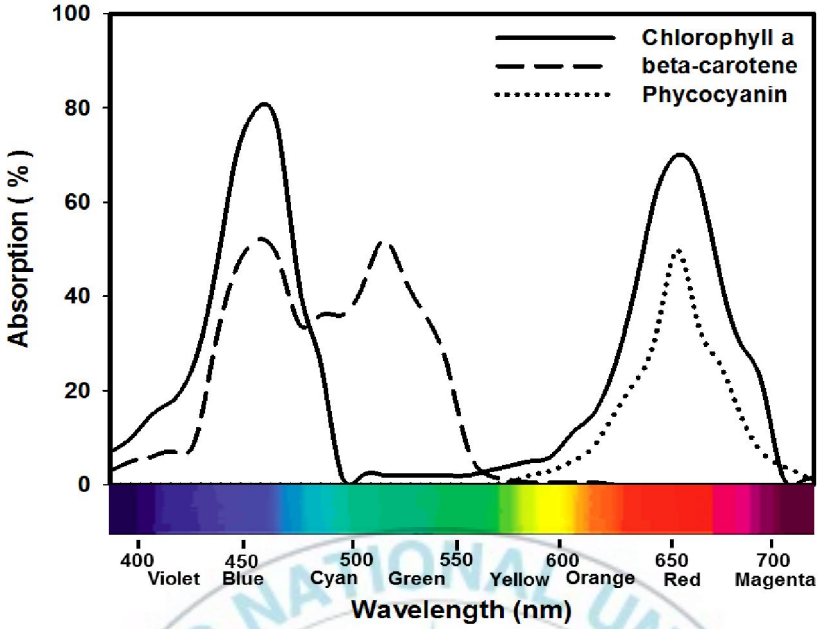

Fig. 1. The absorption spectrum of chlorophyll a, β-carotene and phycocyanin

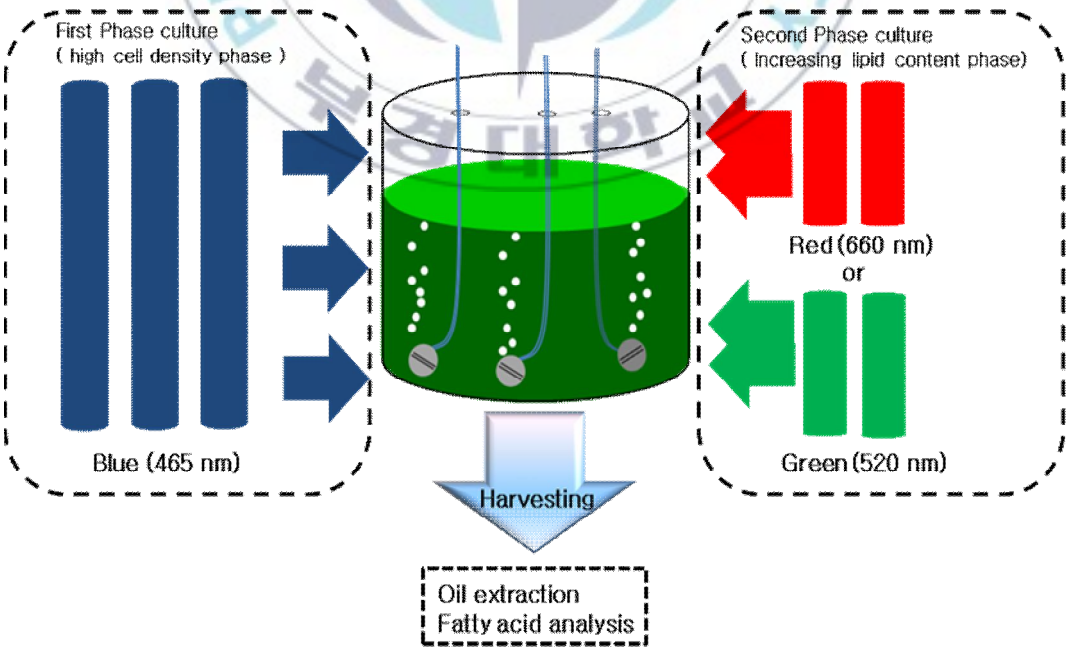

Fig. 2. Two-phase culture system

2. Material & Method

2.1. Microalgae & Culture medium

Eight microalgae, *Isochrysis galbana*, *Pheodactylum tricornutum*, *Nannochloropsis oculata*, *Nannochloropsis oceanica*, *Nannochloropsis salina*, *Dunaliella salina*, *Dunaliella tertiolecta* and *Nannochloris atomus* were obtained from NLP Co. (Busan, Korea). All microalgae were cultured at 20 ± 1°C in f/2 medium (Guillard and Ryther, 1962).

2.2. Culture conditions

For optimization of culture conditions, Eight microalgae, *I. galbana*, *P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* were cultured in various LED wavelength of 660 nm (red), 640 nm (orange), 520 nm (green), 465 nm (blue), 405 nm (violet) with light intensity of 70 μmol/m^2/s in 2-L flask with a working volume of 1.5-L sterilized sea water. All cultures were performed with 12:12 L:D cycle. After the determination of optimal wavelength, all microalgae cultured in different light intensity of 40, 70, 100 ,130 μmol/m^2/s for determination of optimal light intensity. and then, to compare of biomass and oil contents in fluorescent light and LED, eight microalgae, *I. galbana*, *P. tricornutum*, *N. oculata*, *N.*

oceacnia, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* were cultured in fluorescent light and LED with optimal culture conditions. For two-phase culture, eight microalgae were cultured with optimal conditions. When cultures reached to early stationary phase, LED wavelength was changed from blue wavelength (465 nm) to red (660 nm) or green wavelength (520 nm) then cultured for 4 days. All of the cultures were sampled every day and biomass, oil contents and fatty acid were analyzed.

2.3. Measurement of dry cell weight and nitrate concentration

Nitrate concentration and dry cell weight were determined using UV-Vis spectrophotometer (Ultraspec 6300 pro, Amersham Biosciences, Sweden) at OD_{220}(Collosetal,1999) and OD_{680}, respectively. The samples were centrifuged at 9,940 x g, 4℃ for 15 min. The supernatant was used for the measurement of nitrate concentration. Culture samples (30 mL) were centrifuged at 9,940 x g, 4℃ for 10 min. Cells were washed twice with distilled water and dried at 80℃ for 24 hour. A standard curves were plotted and used for the determination of nitrate concentration and dry cell weight.

2.4. Cell harvest and oil extraction

The microalgae were harvested by using centrifugation at 9,940 x g, 4℃ for 15 min The pellet was washed using distilled water three times. The sample was dried at 80°C for 24 hour. The oil was extracted by mixing extraction and solvent dried cells using a homogenizer (Ultra-Turrax T8, IKA®-WERKE, Germany). The oil from dried cell was extracted using chloroform:methanol (1:2, v/v) as extraction solvent (Bligh and dyer, 1959) by shaking incubator. Extraction solution was centrifuged at 620 x g for separation. The organic solvent was evaporated, and extracted oils were dried at 80℃ to constant weight. Total oil content was obtained as % of dry cell weight. Oil contents calculated by equation below:

$$\text{Oil content (\% DCW)} = \frac{W\ (g) - W_0\ (g)}{DCW\ (g)} \times 100$$

Where W_0 and W are the weights of the extractor tube before and after extracting oil, respectively. DCW is the dry cell weight of microalgae.

2.5. Fatty acid analysis

In order to determine fatty acids composition, oil extracted by solvent was methylated. Ten mg of lipid sample and 1 mL of 14% boron trifluoride methanol (Sigma-Aldrich, St. Louis, MO,

USA) were added into a screw-cap glass tube purged by nitrogen gas. The reaction in the tube was occured in a heating block (100°C) for 90 min and subsequently cooled to room temperature. One mL of distilled water and 2 mL of pentane were added to the reactant. The tube was vigorously vortexed for 2 min. After 9,940 x g centrifugation, the supernatant sample was dried by nitrogen gas in order to remove the pentane. Then, the reactant was mixed with 50 μL of n-hexane for the detection of fatty acids using gas chromatography. The analysis, on standard fatty acid compounds C14:0, C16:0, C18:0, C18:1, C18:2, C18:3, C20:0 and C22:0 using F.A.M.E. mix C14-C12 kit (Sigma-Aldrich, St. Louis, MO, USA), were carried out using the YL 6100 gas chromatography (YoungLin Inc., Korea) using a fused silica capillary column (HP-INNOWAX (30 m x 0.32 mm x 0.5 μm), Agilent Technologies, USA). The initial temperature was 100°C followed by a temperature increase program of 5°C/min to a final oven temperature 200°C. The injector and detector temperature were 200 and 250°C. The quantity of fatty acid was calculated by peak areas with standard curves of myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), behenic acid (C22:0) which were obtained from Sigma-Aldrich (St. Louis, MO, USA).

3. Result and Discussion

3.1. Optimization of wavelength

Dry cell weight with cultured five different light wavelengths (660 nm, 640 nm, 520 nm, 465 nm, 405 nm) and constant light intensity (70 μmol/m^2/s) are shown in Fig.1. Optimal wavelength was determined by cell growth of eight microalgae. High biomass was obtained at 0.45, 0.54, 0.42, 0.41, 0.43, 0.39, 0.40 g dcw/L from *I. galbana*, *P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina* and *D.tertiolecta* under wavelength of 465 nm (blue) and *N. atomus* obtained high biomass concentration of 0.41 g dcw/L at wavelength of 660 nm (red) in light intensity of 70 μmol/m^2/s, respectively. Kiang et al. (2007) reported eight microalgae microalgae, *I. galbana*, *P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* were phytoplankton using photosynthesis for cell growth. Stomp et al.(2007) reported that *I. galbana* and *P. tricornutum* as golden-green algae and diatom belong to chrysophyta and *N. oculata*, *N. oceanica*, *N. salina*, *D. salina* and *D. tertiolecta* as green algae belong to chlorophyta containing chlorophyll a, b and β-carotene. And *N. atomus* include chlorophyll a and phycocyanin. Chlorophyll a, b and carotenoid were pigment for photosynthesis. Blue area (400-500 nm) and red area (600-700

nm) were absorbed by chlorophyll a, blue area (400-500 nm) was absorbed by Chlorophyll b and blue-green are (400-600 nm) was absorbed by carotenoid. And C.Y. Wang et al (2007) reported that phycocyanin was absorbed by orange-red (600-700 nm). Therefore, optimal biomass production of eight microalgae, *I. galbana*, *P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina* and *D.tertiolecta* were obtained at 465 nm, *N. atomus* was obtained at 660 nm (red) because 465 nm (blue) wavelength was absorbed by chlorophyll a and β-carotene pigments, phycocyanin absorbed 660 nm (red) wavelength as shown in Fig. 3

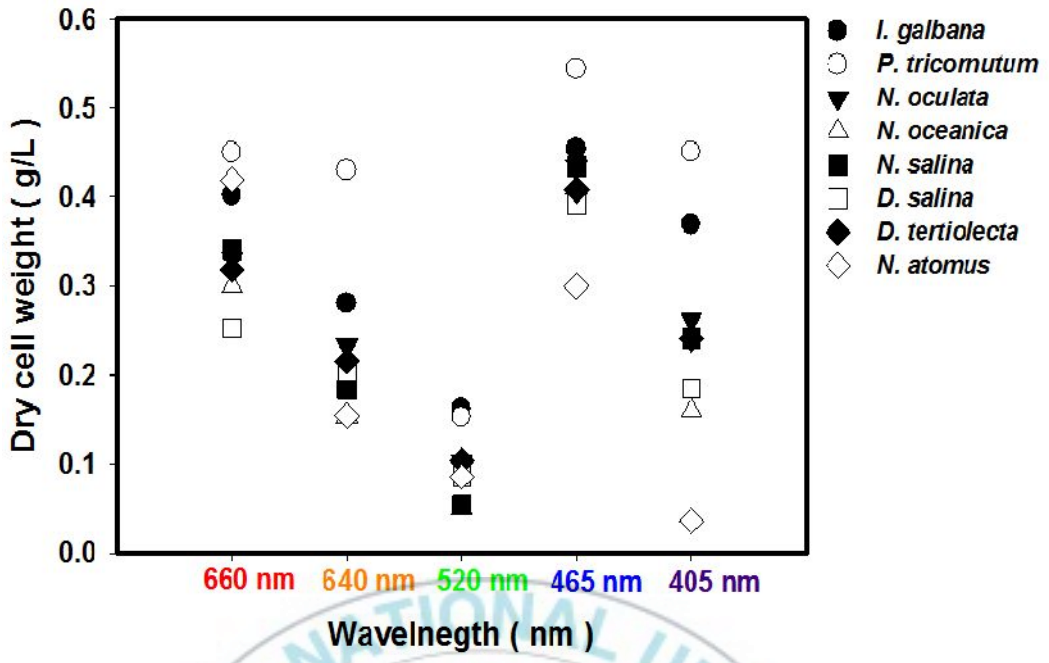

Fig. 3. Biomass production of eight microalgae under various LED wavelength, 660, 640, 520, 465, 405 nm in light intensity of 70 μ mol/m^2/s.

3.2. Optimaization of light intensity

Fig. 4 shows the optimization of light intensity for eight microalgae under optimal wavelength. High biomasses were obtained at 0.58, 0.64, 0.68, 0.60, 0.58, 0.55, 0.51, 0.51 g dcw/L in 100 μmol/m^2/s of light intensity from *I. galbana, P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* under optimal LED wavelength, respectively. However, light intensity of 130 μmol/m^2/s did not increase

biomass production comparing to the light intensity of 100μ mol/m^2/s. Zou et al. (2000) reported that it would be due to the photoinhibition which results from over extraction of electrons on photosynthetic system. And *I. galbana* CCMP 1324 grown with light intensities of over 106 μmol/m^2/s showed low biomass production because of photoinhibition .Lin et al. (2007) reported that *I. galbana* CCMP 1324 with high light intensities showed bleach and lysis of the cell. Therefore, 100 μmol/m^2/s was selected as optimal light intensity for the growth of *I. galbana, P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus*.

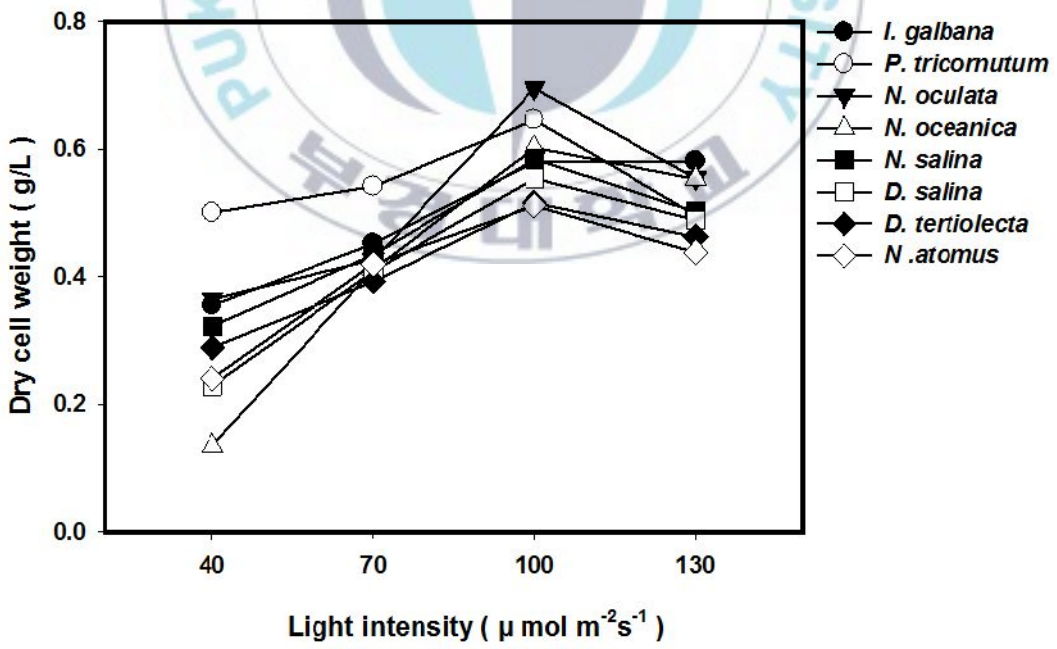

Fig. 4. Biomass production of eight microalgae in various light intensity, 40, 70, 100, 130 μmol/m^2/s under optimal LED wavelength.

3.3. Comparison of biomass and oil contents in fluorescent light and LED

To compare of biomass and oil contents in fluorescent light and LED wavelength as light source, eight microalgae, *I. galbana, P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* were cultured in optimal conditions.

As shown Fig. 5(a), the comparision of LED to fluorescent light under optimal conditions. Biomass production was increased to 2-fold when cells cultured in LED comparing to fluorescent light under optimal conditions. As shown Fig. 5(b), oil contents of eight microalgae using LED and fluorescent light using optimal conditions. Oil contents of 30, 36, 28, 23, 25, 25, 26, 22 % *I. galbana, P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* of dry cell weight were obtained at optimal LED wavelength. Therefore, LED could be used for the microalgae culture as proper light source comparing to fluorescent light.

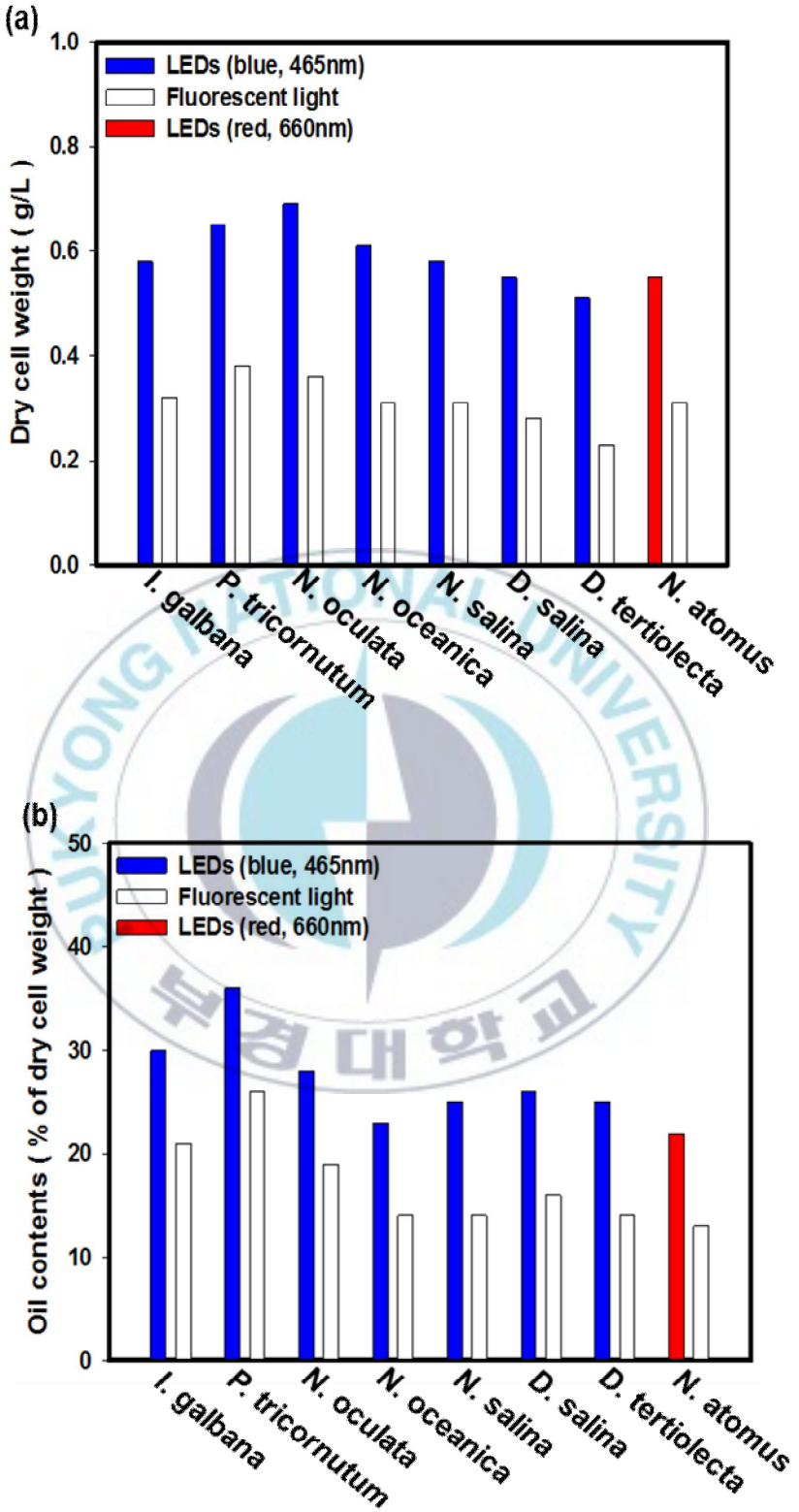

Fig. 5. Comparison of fluorescent and LED light culture

3.4. Optimal conditions of nitrate concentration

Nitrate concentration for the growth of eight microalgae was optimized. Various nitrate concentrations of 8, 16, 24, 32 mg/L were applied to the culture with light intensity of 100 μmol/m^2/s under optimal LED wavelength. As shown in Fig. 6, high biomasses were obtained at 0.92, 1.08 g dcw/L from *I. galbana* and *P. tricornutum* with nitrate concentration of 16 mg/L, respectively and reached to stationary phase on 18 day. *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* showed cell densities of 1.01, 0.92, 0.88, 0.79, 0.75, 0.84 g dcw/L with nitrate concentration of 24 mg/L and reached to stationary phase on 18, 19 day under optimal wavelength and light intensity, respectively. The culture of *I. galbana* and *P. tricornutum* with nitrate concentration of 16 mg/L showed high biomass production comparing to that with nitrate concentration of 24, 32 mg/L, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* with nitrate concentration of 24 mg/L showed high biomass production comparing to nitrate concentration of 32 mg/L. Thus, it can be concluded that after a certain level of tolerance, higher nitrate concentrations become toxic for algal survival. In previously study, this is because there is increase in the activity of nitrate reductase at higher concentrations of nitrate leading to enhanced production of nitrite

and ammonia that are accumulated in vivo. Therefore, the accumulated nitrite and ammonia act as toxins, resulting in decrease in biomass production. (Jeanfils et al., 1993).

Oil contents and fatty acid composition of microalgae were varied by nitrogen source concentration. The increase of the urea concentrations in the medium decreased the lipid contents in microalgae, *Chlorella* sp. culture (Hsieh et al., 2009). Therefore, proper nitrogen sources and concentrations are important factors for both biomass and oil production in microalgae culture.

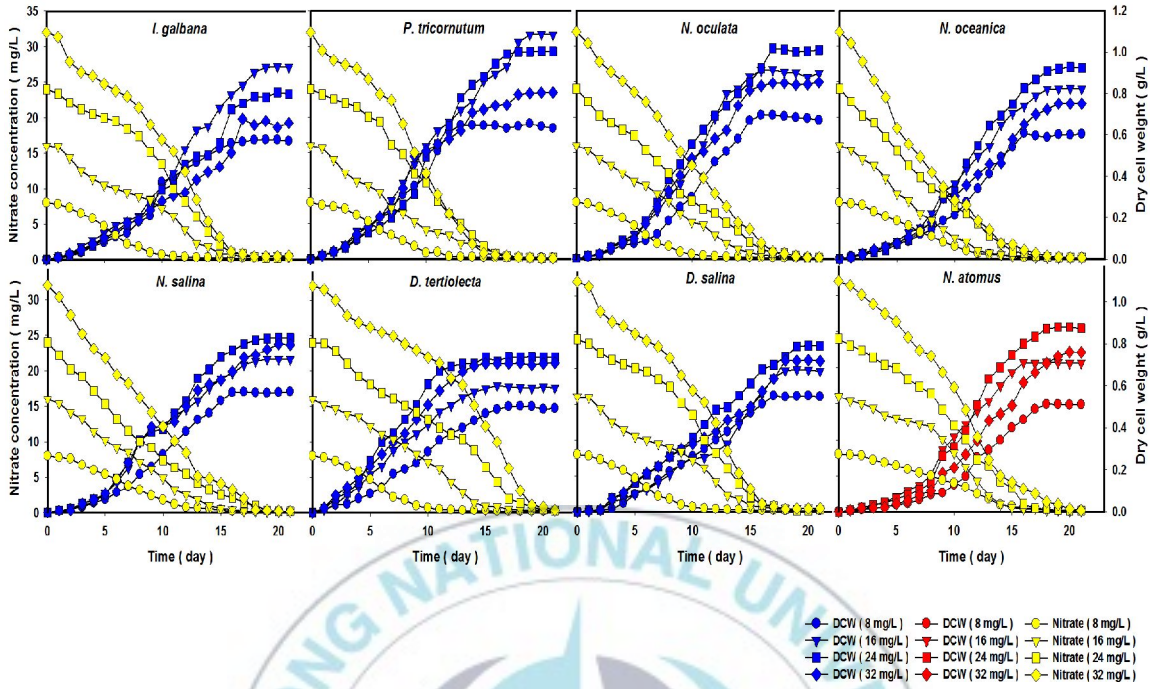

Fig. 6. Cell growth of eight microalgae in various nitrate concentration of 8, 16, 24, 32 mg/L under optimal LED wavelength and light intensity.

3.5. Oil contents and fatty acid composition with two-phase cultivation

The two-phase culture was tried for the culture of eight microalgae with the biomass production in first phase, and oil accumulation in second phase culture. The LED wavelength, of 465 nm (blue), light intensity of 100 μmol/m^2/s and nitrate concentration of 16 mg/L for *I. galbana* and *P. tricornutum*, 24 mg/L for *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* were applied for biomass production in the first phase culture. After the culture reached to stationary phase as a first phase the wavelength was changed to induce the production of oil as a second phase culture. Fig. 5 shows increasing oil contents from two-phase culture with wavelength stress for 3 day. As shown in Fig. 7, eight microalgae were cultured with optimal conditions in first phase culture. High biomasses were obtained with 0.92, 1.08, 1.01, 0.92, 0.88, 0.79, 0.75, 0.84 g dcw/L from *I. galbana*, *P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* respectively. When eight microalgal cells reached to early stationary phase, wavelength was changed from 465 nm (blue, control) to 660 nm (red) and 520 nm (green). *I. galbana*, *P. tricornutum*, *N. oculata*, *N. oceanica* and *N. salina* produced at 38, 42, 38, 36, 38% of dcw under wavelength of 465 nm (blue,

control) on 2 day of second phase culture. *D. salina* and *D. tertioelcta* produced at 32, 32% of dcw under the same wavelength on 1 day of second phase culture. Also, *N. atomus* produced at 35% of dcw with wavelength of 660 nm (red, control) on 2 day. On the same day, oil contents was obtained at 57% and 60% of dcw from *I. galbana*, 58% and 62% of dcw from *P. tricornutum*, 50% and 56% of dcw from *N. oculata* and 47% and 53% of dcw from *N. oceanica*, 46% and 52% of dcw from *N. salina*, 48% and 52% of dcw from *D. salina* and 46% and 54% of dcw from *D. tertioelcta* with 660 nm (red) and 520 nm (green) of LED wavelengths of second phase culture, respectively. Also, *N. atomus* produced oil contents of 47% and 50% of dcw with 465 nm (blue) and 520 nm (greed) of LED wavelength of second phase culture.

Table 1. Fatty acid composition of eight microalgae with optimal wavelength and light intensity using LED

Cell / Fatty acid	*I. galbana*	*P. tricornutum*	*N. oculata*	*N. oceanica*	*N. salina*	*D. salina*	*D. tertiolecta*	*N. atomus*
Myristic acid (C14:0)	5.5 ± 0.2	1.5 ± 0.3	4.15 ± 0.3	3.08 ± 0.2	3.48 ± 0.4	8.82 ± 0.4	9.12 ± 0.4	6.68 ± 0.4
Palmitic acid (C16:0)	253.52 ± 0.4	128.14 ± 0.2	135.26 ± 0.2	134.62 ± 0.3	141.15 ± 0.3	152.58 ± 0.5	168.02 ± 0.3	122.15 ± 0.5
Stearic acid (C18:0)	40.14 ± 0.2	32.22 ± 0.4	23.52 ± 0.1	21.15 ± 0.4	20.92 ± 0.2	52.48 ± 0.4	32.14 ± 0.1	15.11 ± 0.4
Oleic acid (C18:1)	32.17 ± 0.4	201.32 ± 0.2	101.15 ± 0.4	99.45 ± 0.2	105.47 ± 0.4	24.74 ± 0.1	28.95 ± 0.4	27.44 ± 0.3
Linoleic acid (C18:2)	24.12 ± 0.6	12.34 ± 0.3	20.14 ± 0.3	18.25 ± 0.4	22.15 ± 0.3	18.81 ± 0.3	27.41 ± 0.3	18.12 ± 0.2
Linolenic acid (C18:3)	102.5 ± 0.2	21.15 ± 0.4	14.14 ± 0.2	20.12 ± 0.3	30.11 ± 0.2	132.11 ± 0.5	118.14 ± 0.5	133.08 ± 0.3
Arachidic acid (C20:0)	3.1 ± 0.5	2.57 ± 0.1	1.15 ± 0.4	2.41 ± 0.2	1.54 ± 0.4	1.11 ± 0.4	2.44 ± 0.3	3.2 ± 0.2
Behenic acid (C22:0)	2.2 ± 0.3	1.44 ± 0.3	0.92 ± 0.1	1.19 ± 0.3	1.12 ± 0.5	1.08 ± 0.2	1.88 ± 0.7	1.52 ± 0.1
Total fatty acid	463.25 ± 2.8	400.5 ± 2.2	276.91 ± 2	300.27 ± 2.3	325.94 ± 2.7	391.73 ± 2.8	388.1 ± 3	327.3 ± 2.4

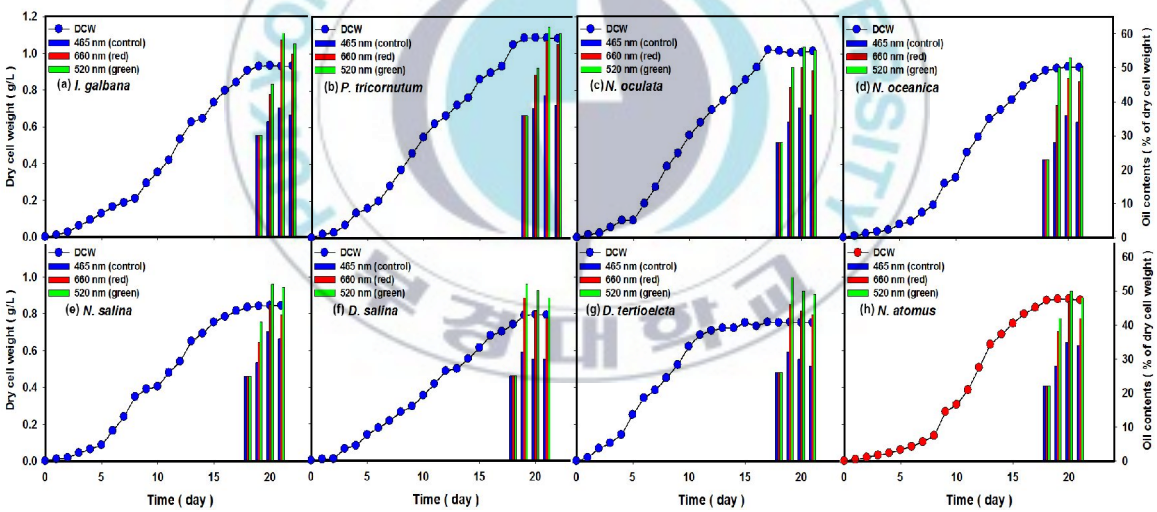

Fig. 7. Cell growth and oil contents of eight microalgae using two-phase culture under optimal conditions of LED wavelength, light intensity and nitrate concentration

Generally, nitrogen source such as nitrate depletion of microalgae culture reaches to stationary phase of microalgae growth and changes the metabolic pathway for the accumulation of oils (Su et al., 2011). Huang et al.(2010) reported the activation of diacylglycerol acyl-transferase, which converts fatty acid acyl-CoA to triacylglycerol under growth inhibition conditions. This could be the reason why wavelength change of light the inhibits growth of microalgae, thus increases the intracellular oil accumulation. The fatty acid composition of microalgae can be changed by the control of the culture condition and period (Lin et al., 2007). The changes in the oil contents and eight fatty acid composition after the stationary phases of eight microalgae culture are shown in Fig. 6. The palmitic acid contents and unsaturated fatty acid of eight microalgae increased until 2 days after stationary phases. However, farther exposure to light stress decreased oil contents and fatty acid contents after 2 days second phase culture changing wavelength from 465 nm (blue) to 520 nm (green) or 660 nm (red) as shown Fig. 8. Oil contents of 60% of dry cell weight and high palmitic acid and linolenic acid of 435.4 mg/g oil and 320.5 mg/g oil were observed in *I. galbana* under wavelength of 520 nm (green) on 2 days of second phase as shown Fig. 8a. Fig. 8b shows that oil contents of *P. tricornutum*

increased to 62% of dry cell weight and fatty acid composition of palmitic acid and oleic acid of 365.0 mg/g oil and 470.4 mg/g oil, respectively, under 520 nm (green) on 2 days of second phase culture. Oil contents of 56% of dry cell weight was obtained with light stress at 520 nm (green) and high palmitic acid and oleic acid contents were measured at 382.4 mg/g oil and 340.5 mg/g oil from *N. oculata* on 2 days of second phase culture as shown Fig. 8c.

Fig. 8d shows oil contents, palmitic acid and oleic acid of 53% of dry cell weight, 380.5 mg/g oil and 282.4 mg/g oil produced from *N. oceanica*, respectively with wavelength change from 465 nm (blue) to 520 nm (green) after stationary phase on 2 days of second phase culture. Fig. 8e shows oil contents of 52% of dcw, palmitic acid of 322.8 mg/g oil and oleic acid of 268.4 mg/g oil. Also, *D. salina* and *D. tertiolecta* produced oil contents of 52% and 54% of dcw with palmitic acid contents of 326.4 mg/g oil and 350.4 mg/g oil, linolenic acid contents of 282.5 mg/g oil and 280.1 mg/g oil with wavelength change from 465 nm (blue) to 520 nm (green) after stationary phase on 1 days of second phase culutre as shown in Fig. 8f and Fig. 8g. *N. atomus* obtained maximum oil contents of 50% of dcw with palmitic acid of 252.4 mg/g oil and linolenic acid of 301.2 mg/g oil under wavelength changed from 660 nm (red) to 520 nm (green) on 2 days after

stationary phase culture as shown in Fig. 8h..

The major saturated fatty acid is palmitic acid synthesized initially in microalgae (Harwood et al., 2009). In addition, palmitic acid and oleic acid have been recognized as the most common component of biodiesel (Knoth, 2008). In recent study, Pal et al. (2011) reported palmitic acid and unsaturated acids of oleic acid and linolenic acid increased from *Nannochloropsis* sp. under growth inhibition conditions. Similar to this results, high palmitic acid was obtained from eight microalgae after stationary phase with changing wavelength on 2 days of second phase culture. The fatty acids increase in green light which is light stress as second phase culture could have been due to a compensatory increase in the amount of chloroplasts and a rearrangement of the thylakoid structure within the chloroplast as a response to the low absorbance of the wavelength (Zhang et al., 2011). This is the reason for the increase of oil contents, saturated and unsaturated fatty acid composition second phase culture by changing wavelength from 465 nm (blue) to 520 nm (green) or 660 nm (red) in second phase culture as shown Fig. 7 and Fig. 8.

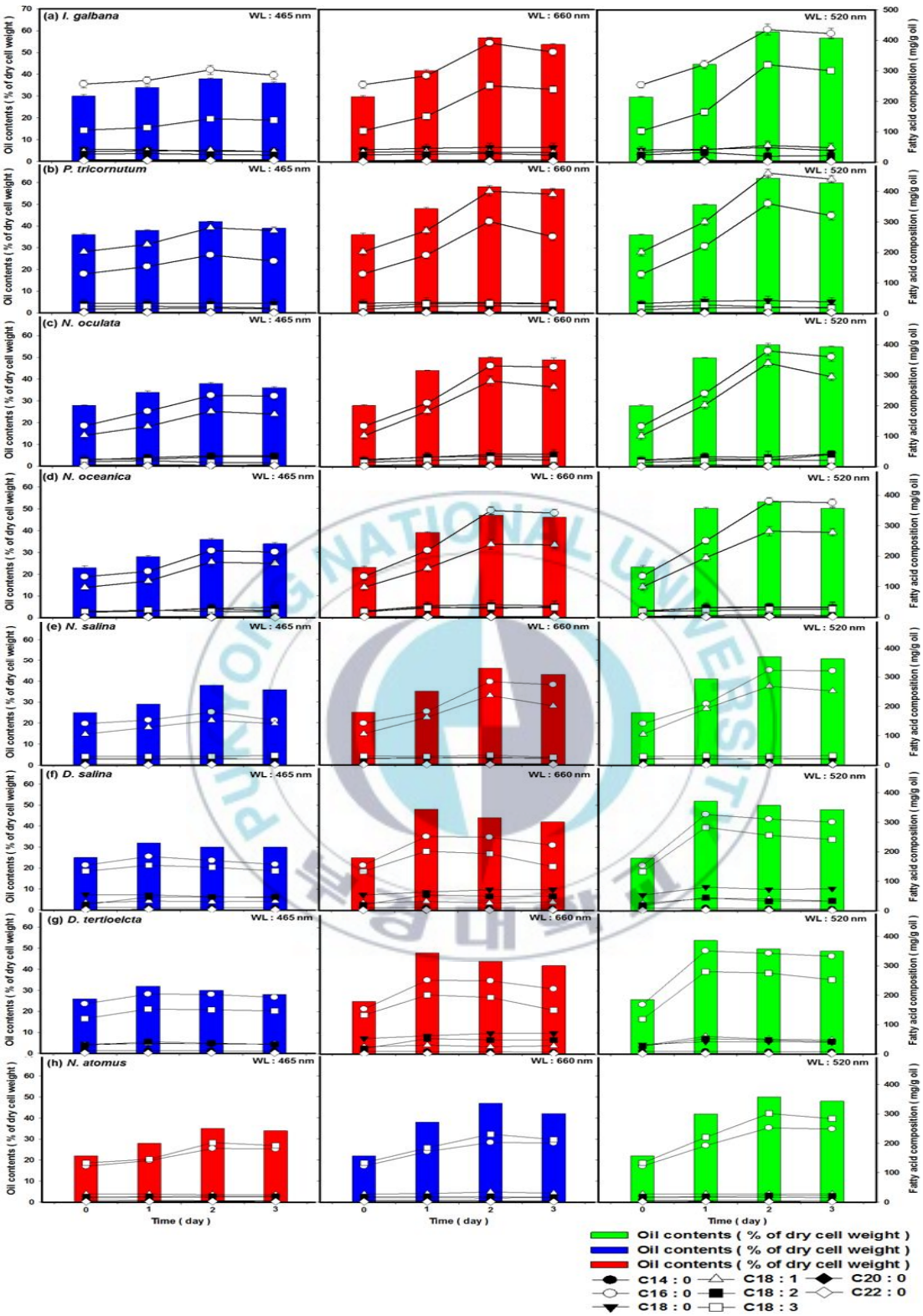

Fig. 8. Oil contents and fatty acid composition from eight microalgae using two-phase culture with changed wavelength.

4. Conclusion

Eight microalgae, *I. galbana, P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* were cultured in various wavelength, light intensity and nitrate concentration for the evaluation of optimal culture conditions. The optimal wavelength and light intensity were determined as wavelength of 465 nm (blue) and 100 μmol/m^2/s from *I. galbana, P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, *D. salina* and *D. tertiolecta*. And *N. atomus* determined optimal wavlength of 660 nm (red) and light intensity of 100 μmol/m^2/s. The maximum biomass of *I. galbana* and *P. tricornutum* were obtained at 0.92, 1.08 g dcw/L in nitrate concentration of 16 mg/L, respectively. *N. oculata*, *N. oceanica*, *N. salina*, *D. salina*, *D. tertiolecta* and *N. atomus* were obtained at 1.01, 0.92, 0.88, 0.79, 0.75, 0.84 g dcw/L, respectively in nitrate concentration of 24 mg/L. After, eight microalgae reached to stationary phase, the second phase culture was implemented for increasing oil contents. After the maximum biomass was obtained in first phase, wavelength was changed from 465 nm (blue) to 660 nm (red) or 520 nm (green), 660 nm (red) to 465 nm (blue) or 520 bn (green) in second phase. As the result, oil contents were measured at 60, 62, 56, 53, 50% of dry cell weight from *I.*

galbana, *P. tricornutum*, *N. oculata*, *N. oceanica*, *N. salina*, respectively in wavelength of 520 nm (green) on 2 days. *D. salina* and *D. tertiolecta* obtained maximum oil contents of 52% and 54% of dry cell weight in wavlength of 520 nm (green) on 1 days. And *N. atomus* produced maximum oil contents of 50% of dry cell weight when wavelength changed from 660 nm (red) to 465 nm (blue) or 520 nm (green). This study also showed the high accumulation of palmitic acid during second phase of two-phase culture from eight microalgae under 520 nm (green) on 2 days of second phase. *I. galbana*, *D. salina*, *D. tertioelcta* and *N. atomus* showed high accumulation of linolenic acid and *P. tricornutum*, *N. oculata*, *N. oceanica* and *N. salina* showed high accumulation of oleic acid as unsaturated fatty acid with changing wavelength from 465 nm (blue) to 520 nm (green) or 660 nm (red) to 520 nm (green) in second phase culture. Therefore, the present results suggested that the two-phase culture could increase cell mass of microalgae and overall oil production with palmitic acid and unsaturated fatty acid such as oleic acid and linolenic acid.

5. Acknowledgment

학부 4학년 1학기에 졸업논문발표를 하기위해 생물고분자 공학 실험실에 들어와 석사 2년 과정을 거쳐 졸업을 할 수 있게 끊임없이 지도를 해 주신 저의 지도교수님이신 김성구 교수님과 사모님께 진심으로 깊은 감사를 드립니다. 또한 학교생활을 하면서 많은 도움을 주신 홍용기 교수님, 이형호 교수님, 김중균 교수님, 박남규 교수님과 저에게 실험에 대해 아낌없이 조언을 해 주신 정귀택 교수님께 감사드립니다.

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