Combination Effect of Temperature and Light Intensity on Lipid Productivity of *Tetradesmus obliquus*

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Abstract. Lipid enhancement is important to reduce production cost thus increasing the commercial values of microalgae biofuel production. Physical stress such as temperature and light intensity are known to increase lipid productivity because these factors might affect the phase transition of lipid, macromolecule formation and physiochemical reactions of microalgae. In this study, the effect of light intensity and temperature on lipid productivity of *Tetradesmus obliquus* was studied. *T.obliquus* UPSI-JRM02 was cultured in BG11 media at different temperature range (25–40°C) and light intensity (4000–30000 lux), respectively, within 14 days of growth period. The highest lipid productivity was obtained at temperature and light intensity of 36°C and 23500 lux. At this condition, 27 mg/L/day of lipid productivity and 23% of total lipid was successfully produced. The result shows the possibility of increasing *T.obliquus* lipid productivity by giving physical stress to the cell.

1. Introduction
In the past few years, research on alternative ways to replace the usage of fossil fuel has been conducted following the increasing concern on the decreasing stock of the resource. One of the notable discoveries is the use of microalgae biofuel as an alternative that has shown promising results. The capabilities of microalgae biofuel were attributed to the fact that it requires less area, has higher growth rate, and produces less carbon footprint, whereas biofuel from terrestrial plant is taking too much land area which is important for the food industry. Microalgae biomass is useful as feedstock for the production of bioethanol, biohydrogen, biodiesel, and bio-oil [1]. However, due to low productivity and high operation cost, the production of biofuel from microalgae is considered unaffordable [2]. In fact, the technologies for mass production of microalgae cultivation and for processing and conversion of microalgae biomass are still underdeveloped [3]. A few strategies nevertheless have been taken to increase the productivity of...
microalgae, such as by manipulating the parameter, using the right choice of species, and cultivation setup.

Microalgae growth depends on various factors, such as temperature, light intensity, salinity, nutrient content, and pH. These factors affect the growth rates of microalgae, its biomolecules composition (such as lipid, carbohydrate, and protein), and its productivity. Study by Sun et al. [4] has shown that different period of nitrogen starvation, light intensity, and iron starvation triggered the accumulation of carbohydrate and triacylglyceride (TAG) in *Neochloris oleoabundans* HK-129. Another study by Bruer [5] has demonstrated that light intensity was responsible for the accumulation of *Scenedesmus obliquus* TAG under a nitrogen-depletion condition.

Selection of productive and fast-growing strain is important for the success of mass culturing because some microalgae cannot grow under stressful condition, and certain species produce higher lipid compound compared to others. Among the species, *T. obliquus* is known to be able to (i) efficiently remove nitrogen and phosphorus in urban water and (ii) produce higher lipid content [6]. This species thus is preferred for its ability to grow faster in wastewater, and to accumulate lipid [7].

In this study, temperature and light intensity parameter were selected because both factors are able to trigger the production of lipid in microalgae. According to Goncalves [8] the effect of temperature and light on microalgae growth kinetic is important to improve the biomass productivity thus reducing microalgae cultivation cost. Light is important as a basic energy for photoautotrophic organisms. Study by Wu [9] has shown that the cellular content of lipid is proportional to the light intensity. Higher light intensity could inhibit the growth of microalgae, but would increase the total lipid content in microalgae.

The growth and biological composition of microalgae is also influenced by temperature; temperature has been found to affect the chemical reaction during photosynthesis because enzymes are sensitive towards temperature. A study by Chen [10] showed that lipid composition and fatty acid distribution was influenced by eicosapentaenoic acid (EPA) which vary when *Nitzschia laevis* grown under different temperature resulting in inhibition of cell growth and fatty acid biosynthesis at higher temperature (more than 23°C). Temperature was also selected as a variable in this study as controlling outdoor system incurs high cost particularly for high temperature microalgae cultivation [11]. Lipid is importance as a feedstock for biodiesel, a promising substitute for petroleum-based diesel fuel, while microalgae-based carbohydrates are useful for biofuel production such as bioethanol and biopropanol [12]. The combination of both effects of temperature and light intensity has been shown to have positive correlation in terms of increasing the production of lipid. The aim of this study thus is to investigate the combination effect of temperature and light intensity on the lipid productivity (daily lipid content) of *T. obliquus*.

2. Materials and Methods
2.1 Microalgae strain and culture maintenance.
*T. obliquus* UPSI-JRM02 was isolated from Jeram Sanitary Landfill, Selangor, Malaysia, in our previous study [13]. The strain was characterized and identified based on 18s rRNA and 28s rRNA molecular technique [13]. *T. obliquus* culture was maintained in 1L Erlenmeyer flask, which contained BG11 medium of NaNO₃ (1.5 g), K₂HPO₄·3H₂O (4.0 g), MgSO₄·7H₂O (7.5 g), CaCl₂·2H₂O (3.6 g), citric acid (0.6 g), ferrie ammonium citrate (0.6 g), Na₂EDTA·2H₂O (0.1 g), Na₂CO₃ (0.2 g), and 1 mL of trace metal solution per liter. The trace metal solution contained H₃BO₃ (2.86 g), MnCl₂·4H₂O (1.8 g), ZnSO₄·7H₂O (0.22 g), CuSO₄·5H₂O (0.08 g), CoCl₂·6H₂O (0.05 g), and Na₂MoO₄·2H₂O (0.391 g) per liter. The culture was illuminated with cool white fluorescent lamp at 4000 lux light intensity and 12:12 light:dark photoperiod. The aeration was
performed by air pump connected with 0.2µm PTFE in line air filter (Sartorius Midisart 2000, USA). The pH and temperature were controlled at 7.5 and 26 ± 1°C, respectively.

2.2 Microalgae cultivation.
The culture was initially grown in an Erlenmeyer flask to the middle of the logarithmic phase in BG11 medium at day 6 to 7. The pre-cultured cells were collected by centrifugation (4000 rpm × 5 min) and resuspended in nitrogen-free BG11 to completely remove the nitrogen. The pre-cultured microalgae containing 5.5x10⁶ cells/mL was inoculated in 300 mL of BG11 medium in a 500 mL Erlenmeyer flask. Filtered air was supplied continuously using air pump. A total of 10 experiments with selected factors (temperature and light intensity) in three replicates were conducted, as suggested through the Research Surface Methodology (RSM). Lipid productivity were chosen as the response to analyse the effect of the combined factors on the productivity of *T. obliquus*. Five level of temperature (25, 29, 33, 36 and 40°C) and light intensity (4000, 10500, 17000, 23500, and 30000 lux) were conducted in this experiment. The light intensities were controlled to change the power of the LED bulb (Panasonic, cool daylight), respectively. The experiments were conducted in an incubator shaker to obtain the desired temperature. The light bulbs were inserted inside the incubator for light-intensity experiment. pH and nitrogen concentration were maintained at 8.5 and 600 mg/L, respectively. The microalgae cultivation was performed for 14 days, and biochemical analyses of its growth were monitored every 2 days and on day 14.

2.3. Growth and biomass measurement.
Optical density of the microalgal cell was measured at 680 nm (OD₆₈₀) and 750 nm (OD₇₅₀) using a visible spectrophotometer (Secomam Prim Visible Light Spectrophotometer, France). Cell count was performed using a haemocytometer with appropriate dilution. The cell biomass was harvested by centrifugation before being oven-dried for 15 hours. The weights of the wet and dry samples were measured. Specific growth rates were calculated using the formula described in equation 1, where N₁ and N₂ are defined as biomass at time 1 (t₁) and time 2 (t₂) [14].

\[ \mu = \frac{\ln (N_2 - N_1)}{t_2 - t_1} \]  

(1)

The biomass productivity (mg/L/day) was calculated using Equation 2 [15].

\[ \text{Biomass productivity} = \frac{\text{amount of biomass (mg/L)}}{\text{number of days}} \]  

(2)

2.4. Lipid analysis.
Total lipid was extracted according to the protocol of Bligh and Dyer (1959) with some modification and measured gravimetrically [16]. For each sample, about 100 mg of microalgal powder (W) was mixed with 2 mL of chloroform and 1 mL of methanol before being incubated at room temperature for 24 h. Then, the mixture was centrifuged at 4000 × g for 10 min, and the supernatant was transferred into a pre-weighed vial (W₁). The microalgal residue was then mixed with 1 mL of chloroform/methanol (2:1, v/v) and centrifuged as described above. The supernatants were then dried in an oven at 70°C until a constant weight (W₂) was achieved. The lipid content and lipid productivity were calculated using Equation 3 and 4, respectively [16,15].

\[ \text{Total lipid} (\% \text{ dry weight}) = \frac{(W_2 - W_1)}{W_1} \times 100. \]  

(3)
Where $W_1$ is the initial weight and $W_2$ is the final weight.

\[
\text{Lipid productivity} = \frac{\text{biomass productivity (mg/L/day)} \times \text{lipid content}}{100}
\] (4)

2.5. Fatty Acid Methyl Ester (FAME) analysis.
Twenty mg of dried microalgae biomass was transmethylated with 2.5 mL of methanol mixture and 2% (v/v) $\text{H}_2\text{SO}_4$ at 80°C for 2.5 hours. 1 mL of n-hexane and 1 mL of saturated NaCl solution were added to the suspension after it cooled, forming separated layers in the tube. The upper n-hexane layer containing FAME was collected for gas chromatograph mass spectrometer (GC/MS) analysis [17]. The GC/MS analysis was conducted with an Agilent 7890A gas chromatograph (GC) directly coupled to the mass spectrometer system (MS) of an Agilent 5975C inert MSD with a triple-axis detector. The analysis was performed with DB-5MS UI column (Agilent Technologies) and 5% phenyl methylpolysiloxane at stationary phase. The MSD Chemstation was used to determine all the peaks in the raw GC chromatogram. A library search was carried out for all the peaks using the NIST/EPA/NIH version 2.0, and the results were combined in a single peak table.

3. Results and Discussion
3.1 Effects of temperature and light intensity
Enhancement of lipid productivity in microalgae is necessary to achieve sustainable microalgae biofuel to meet the current demand. This can be achieved by selection of right microalgae strain and cultivation condition, such as temperature and light intensity. *T. obliquus* was chosen because the microalgae can be easily maintained, has simple nutrient requirements, and is rich in bioactive compound [18]. Five different temperatures (25, 29, 33, 36, and 40°C) and light intensities (4000, 10500, 17000, 23500, and 30000 lux) with 10 sets of experiments were design to investigate the effect of temperature and light intensity on the lipid productivity of *T. obliquus*. The growth densities of *T. obliquus* were measured by spectrophotometer at absorbance 680 nm and 750 nm, as shown in Figure 1. The highest growth rate for this experiment was Set D at light intensity of 23500 lux and temperature of 36°C, with maximum growth density at ~1.84x10⁶ cells/mL (OD₆₈₀) and specific growth rate (SGR) of 0.22/day. The highest SGR produced was by Set I (0.25/day) while the lowest SGR was at 0.16/day (Set A and E). Set F has the lowest growth rate which the highest was only at 0.1484x10⁶ cells/mL (OD₆₈₀) and reached the stationary stage as early as day 6. This slow growth rate might be due to the limiting factor of temperature because *T. obliquus* is a mesophilic microalgae that can grow at temperature between 15 to 40°C [19].
Figure 1: Growth curve of *T. obliquus* at different combination of temperature and light intensity A) 29°C, 10500 lux B) 36°C, 10500 lux C) 29°C, 23500 lux D) 36°C, 23500 lux E) 25°C, 17000 lux F) 40°C, 17000 lux G) 33°C, 4000 lux H) 33°C, 30000 lux I) 33°C, 17000 lux J) 33°C, 17000 lux at (I) OD_{680} and (II) OD_{750}.

Table 1 shows the combination effects of temperature and light intensity on lipid productivity. The highest biomass (115 mg/L/d) and lipid productivity produced (27 mg/L/d) was Set D at 36°C and light intensity of 23500 lux thus reflecting the ideal growth condition for *T. obliquus*. The biomass productivity of Set F was only at 45 mg/L/d. Result of this study is in accordance to Han et al. [20] who found that
longer exposure at temperature at 40°C led to the decrease in biomass and lipid content of microalgae. Temperature is an important parameter because it influences the cellular chemical composition, carbon dioxide fixation, and microalgae growth rate. When the temperature increases beyond an optimum level, the growth rate of the microalgae will decrease thus explaining the culture entering stationary phase faster (day 4) at 40°C. Longer exposure of high temperature reduced the viability of cell culture as temperature induced photoacclimation of cell culture which lead to the decrease in biomass as mortality rate is increased [21].

The fatty acid content of the lipid extract is presented in Table 2. Set D (36°C, 23500 lux) was chosen for FAME analysis as it produced the highest lipid content. According to Table 2 only 3 fatty acid was included in this study as they have the highest retention time compared to the others and have the highest confidence measure which is closer to 100 when compared to library search. The highest fatty acid is octadecatetraenoic acid (61.75%) with lipid number C18:3, followed by hexadecanoic acid (20.89%) and stearic acid (17.36%). Higher percentage of polyunsaturated fatty acid (PUFA) was discovered in T. obliquus compared to saturated fatty acid. PUFA is suitable for cold weather due to its outflow properties; however, the substance has low oxidative stability, which can be overcome with antioxidants [22]. Meanwhile, saturated fatty acid has been shown to have excellent combustion properties because it is more stable compared to PUFA, although the former can be problematic with cold outflow [23].

Table 1: Combination Effects of Temperature and Light Intensity on Biomass and Lipid Productivity of T. obliquus

| Set | Temperature (°C) | Light Intensity (lux) | Biomass Productivity (mg/L/d) | Lipid Productivity (mg/L/d) |
|-----|------------------|----------------------|------------------------------|-----------------------------|
| A   | 29               | 10500                | 72                           | 16                          |
| B   | 36               | 10500                | 91                           | 22                          |
| C   | 29               | 23500                | 88                           | 17                          |
| D   | 36               | 23500                | 115                          | 27                          |
| E   | 25               | 17000                | 53                           | 10                          |
| F   | 40               | 17000                | 45                           | 8                           |
| G   | 33               | 40000                | 52                           | 9                           |
| H   | 33               | 30000                | 89                           | 20                          |
| I   | 33               | 17000                | 49                           | 9                           |
| J   | 33               | 17000                | 50                           | 10                          |

Table 2: Fatty Acid Profile of T. obliquus by GCMS

| Index Name          | IUPAC Name                  | Lipid Number | FAME (%) |
|---------------------|-----------------------------|--------------|----------|
| Hexadecanoic acid   | Methyl palmitate            | C15:0        | Saturated | 20.89    |
| Octadecatetraenoic | 5,,9,12-Octadecatrienoic    | C18:3        | PUFA     | 61.75    |
| acid                | acid                        |              |          |          |
| Octadecanoic acid   | Stearic acid                | C18:0        | Saturated | 17.36    |

Tetradesmus sp. previously known as Scenedesmus sp. [24] is commonly used as feedstock for biofuel productivity for its capability to adapt to various environmental conditions and produce high biomass and lipid content. Tetradesmus sp. is also well known as potential oil-producing microalgae [25]. A few studies had been conducted to show the ability of Tetradesmus sp. to produce lipid in various
environmental condition, as presented in Table 3. It was found that under a nitrogen-depleted condition, a lipid productivity up to 53.5 mg/L/d can be achieved [26]. The lipid productivity for *T. obliquus* UPSI-JRM02 is comparable with that found in other studies [27, 28].

| Table 3: Lipid Production of *Tetradesmus* sp./*Scenedesmus* sp. under Various Conditions |
| Species | Factors | Lipid Content (%) | Lipid Productivity (mg/L/d) | References |
|---|---|---|---|---|
| *Scenedesmus* sp. | Nitrogen stress condition (70 mg/L/N) | - | 53.5 | [26] |
| *Scenedesmus quadricauda* | Carbon dioxide concentration | - | 35.1 | [27] |
| *T. obliquus* UPSI-JRM02 | Combination of temperature and light intensity | 23 | 27 | This study |
| *Scenedesmus* sp. LX1 | Cultured in secondary effluent | 31 | 8 | [28] |
| *Scenedesmus obtusiusculus* | One stage cultivation process | 19.9 | - | [29] |

4. Conclusion

The combination effects of temperature and light intensity influenced the growth of microalgae and lipid productivity. In this study, the combination of temperature at 36°C and light intensity of 23000 lux produced 27 mg/L/day lipid with a total lipid content of 23%. The composition of the fatty acid was 61% (PUFA) and 39% (saturated), respectively. Further study on the effect of nutrient and pH stress on lipid production in *T. obliquus* is still being conducted.

References

[1] Lee, O., Seong, D., Lee, C., & Lee, E. 2015 Sustainable production of liquid biofuels from renewable microalgal biomass. *Journal of Industrial and Engineering Chemistry*, 29, 24-31.

[2] Zhu, S., Wang, Y., Xu, J., Shang, C., Wang, Z., Xu, J., & Yuan, Z. 2015 Luxury uptake of phosphorus changes the accumulation of starch and lipid in Chlorella sp. under nitrogen depletion. *Bioresource Technology*, 198, 165-171.

[3] McGinn, P., Dickinson, K., Bhatti, S., Frigon, J., Guiot, S., & O’Leary, S. 2011 Erratum to: Integration of microalgal cultivation with industrial waste remediation for biofuel and bioenergy production: opportunities and limitations. *Photosynthesis Research*, 109 (1-3), 249-249.

[4] Sun, X., Cao, Y., Xu, H., Liu, Y., Sun, J., Qiao, D., & Cao, Y. 2014 Effect of nitrogen-starvation, light intensity and iron on triacylglyceride/carbohydrate production and fatty acid profile of *Neochloris oleoabundans* HK-129 by a two-stage process. *Bioresource Technology*, 155, 204-212.

[5] Breuer, G., Lamers, P., Martens, D., Draaisma, R., & Wijffels, R. 2013 Effect of light intensity, pH, and temperature on triacylglycerol (TAG) accumulation induced by nitrogen starvation in *Scenedesmus obliquus*. *Bioresource Technology*, 143, 1-9.
[6] Ruiz-Marin, A., Mendoza-Espinosa, L., & Stephenson, T. 2010 Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource Technology*, 101(1), 58-64.

[7] Gris, B., Morosinotto, T., Giacometti, G., Bertucco, A., & Sforza, E. 2013 Cultivation of *Scenedesmus obliquus* in Photobioreactors: Effects of Light Intensities and Light–Dark Cycles on Growth, Productivity, and Biochemical Composition. *Applied Biochemistry and Biotechnology*, 172(5), 2377-2389.

[8] Gonçalves, A., Pires, J., & Simões, M. 2016 The effects of light and temperature on microalgal growth and nutrient removal: an experimental and mathematical approach. RSC Advances, 6(27), 22896-22907.

[9] Wu, Y., Yu, Y., & Hu, H. 2014 Effects of Initial Phosphorus Concentration and Light Intensity on Biomass Yield per Phosphorus and Lipid Accumulation of *Scenedesmus* sp. LX1. *Bioenergy Research*, 7(3), 927-934.

[10] Chen, G., Jiang, Y., & Chen, F. 2008 Variation of lipid class composition in *Nitzschia laevis* as a response to growth temperature change. *Food Chemistry*, 109(1), 88-94. doi: 10.1016/j.foodchem.2007.12.022

[11] Fernando, A. 2014 Effect of temperature, dissolved inorganic carbon and light intensity on the growth rates of two microalgal species in monocultures and co-cultures. The University of Texas at Austin.

[12] Karmee, S., Linardi, D., Lee, J., & Lin, C. 2015 Conversion of lipid from food waste to biodiesel. *Waste Management*, 41, 169-173.

[13] Nordin, N., Yusof, N., & Samsudin, S. (2014). Microalgae biomass production and nitrate removal from landfill leachate. In *Proceeding of International Conference on Research, Implementation and Education of Mathematics and Sciences 2014*. Yogyakarta.

[14] Teoh, M., Wong, C., & Phang, S. 2013 Effect of Increased CO2 And Temperature on Growth, Photosynthesis and Lipid Content of Tropical Algae. Malaysian Journal of Science, 32(3), 85-94.

[15] Singh, P., Guldhe, A., Kumari, S., Rawat, I., & Bux, F. 2015 Investigation of combined effect of nitrogen, phosphorus and iron on lipid productivity of microalgae *Ankistrodesmus falcatus* KJ671624 using response surface methodology. *Biochemical Engineering Journal*, 94, 22-29.

[16] Li, L., Cui, J., Liu, Q., Ding, Y., & Liu, J. 2015 Screening and phylogenetic analysis of lipid-rich microalgae. *Algal Research*, 11, 381-386.

[17] Zhu, S., Huang, W., Xu, J., Wang, Z., Xu, J., & Yuan, Z. 2014 Metabolic changes of starch and lipid triggered by nitrogen starvation in the microalga *Chlorella zofingiensis*. *Bioresource Technology*, 152, 292-298.

[18] Ishaq, A. G., Matias-Peralta, H. M., & Basri, H. 2016 Bioactive Compounds from Green Microalga *Scenedesmus* and its Potential Applications: A Brief Review. *Pertanika Journal of Tropical Agricultural Science*, 39(1), 1–16.

[19] Martínez, M., Jiménez, J., & El Youssi, F. 1999 Influence of phosphorus concentration and temperature on growth and phosphorus uptake by the microalga *Scenedesmus obliquus*. *Bioresource Technology*, 67 (3), 233-240.

[20] Han, F., Pei, H., Hu, W., Han, L., Zhang, S., & Ma, G. 2016 Effect of high-temperature stress on microalgae at the end of the logarithmic phase for the efficient production of lipid. *Environmental Technology*, 37(20), 2649-2657.

[21] Serra-Maia, R., Bernard, O., Gonçalves, A., Bensalem, S., & Lopes, F. 2016 Influence of temperature on *Chlorella vulgaris* growth and mortality rates in a photobioreactor. *Algal Research*, 18, 352-359.
[22] Knothe G. Improving biodiesel fuel properties by modifying fatty ester composition. Energy & Environmental Science. 2009; 2(7): 759.

[23] D’Alessandro, E., & Antoniosi Filho, N. 2016 Concepts and studies on lipid and pigments of microalgae: A review. Renewable and Sustainable Energy Reviews, 58, 832-841.

[24] Carreres, B., de Jaeger, L., Springer, J., Barbosa, M., Breuer, G., & van den End, E. et al. 2017 Draft Genome Sequence of the Oleaginous Green Alga Tetradesmus obliquus UTEX 393. Genome Announcements, 5(3).

[25] Sharma, T., Gour, R., Kant, A., & Chauhan, R. 2015 Lipid content in Scenedesmus species correlates with multiple genes of fatty acid and triacylglycerol biosynthetic pathways. Algal Research, 12, 341-349.

[26] Ördög, V., Stirk, W., Bálint, P., Lovász, C., Pulz, O., & van Staden, J. 2012 Lipid productivity and fatty acid composition in Chlorella and Scenedesmus strains grown in nitrogen-stressed conditions. Journal of Applied Phycology, 25(1), 233-243.

[27] Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G., & Tredici, M. 2009 Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnology and Bioengineering, 102(1), 100-112.

[28] Xin, L., Hong-ying, H., & Jia, Y. 2010 Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, Scenedesmus sp. LX1, growing in secondary effluent. New Biotechnology, 27(1), 59-63.

[29] Schulze, C., Reinhardt, J., Wurster, M., Ortiz-Tena, J., Sieber, V., & Mundt, S. 2016 A one-stage cultivation process for lipid- and carbohydrate-rich biomass of Scenedesmus obtusiusculus based on artificial and natural water sources. Bioresource Technology, 218, 498-504.

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