

Optimization Of Light Intensity and Color Temperature in The Cultivation of *Chlorella Vulgaris* Culture Using the Surface Response Method

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Abstract. Microalgae have an important role as a source of biomass in producing energy. One type of microalgae that has the potential to be developed is *Chlorella vulgaris*. Several factors that affect the growth and biomass production of *Chlorella vulgaris* microalgae are color temperature and light intensity because they play an important role in the photosynthesis process. This study aims to influence the effect of light and color temperature and optimize these parameters using Response Surface Methodology (RSM). Two independent variables were varied: light intensity 200, 400, 600, 800, 1000 lux and color temperature 3000, 4000, 5000, and 6000 K. The results showed that the average value of *Chlorella vulgaris* growth was higher along with higher light intensity. At a color temperature of 4000 K, the highest biomass yield and the most negligible biomass production were found at 6000 K. At a color temperature of 4000 K, it is feasible to apply it as an alternative lighting source in the production of *Chlorella vulgaris*. The combination of light intensity and color temperature shows that the specific growth rate and doubling time have opposite trends where high values produce low values and vice versa. Growth in dark conditions, the specific growth rate was 0.0026 day⁻¹, and the optimal light intensity at 600 lux treatment. ANOVA evaluation showed that color temperature greatly affected growth. Based on the optimization, the optimal specific growth rate of 0.0751 day⁻¹ with the conditions of light intensity and color temperature of 556 lux and 4152 K, respectively.

Keywords: *Chlorella vulgaris*; microalgae; light intensity; color temperature; lipid production

1. Introduction

Microalgae are known as a potential source of biomass for energy production. The biomass could be utilized by simple firing or converted to other products such as bioethanol and biodiesel (Razzak et al., 2017; Shuba & Kifle, 2018). The fast growth of the microorganism could be a potential source of biomass among other conventional sources such as wood and other higher plants. Therefore, microalgae are considered the third generation of biomass which is not competing with food sources (Wang et al., 2017).

One of the microalgae with the potential to be developed is *Chlorella vulgaris*. *Chlorella vulgaris* is a microalgae size 2 to 10 μm and with a structure similar to higher plants because it contains; cell walls, mitochondria, and chloroplasts to carry out photosynthesis (Krienitz et al., 2015; Safi et al., 2014). In addition, these microalgae contain large amounts of intracellular proteins, carbohydrates, lipids, vitamin C, -carotene, and vitamins B (B1, B2, B6, and B12) (Panahi et al., 2012). *Chlorella vulgaris* can be applied in several fields, such as biofuel products, supplements or food coloring additives, wastewater treatment, and agrochemicals (Safi et al., 2014). Thus, the cultivation of the microalgae *Chlorella vulgaris* in terms of increasing production continues to be developed considering the many potentials of the microalgae. Based on current microalgae technology, wastewater can be used as a source of nutrients, which also shows great potential for microalgae growth (Chiu et al., 2015). Based on various studies that have been reported, the biomass produced by microalgae can increase when environmental conditions such as light intensity, pH, salinity, and temperature are met; while the nutritional needs such as the ratio of nitrogen, and phosphorus, are reached (Luangpipat & Chisti, 2017; Metsoviti et al., 2020; Qiu et al., 2017; Ras et al., 2013). Light is an important factor because microalgae generally

grow in autotrophic conditions by utilizing light intensity as an energy source through photosynthetic reactions (Zachleder et al., 2021). The conditions are essential for large-scale production; therefore, it is necessary to find the optimum condition for the growth.

Light intensity is commonly known as the main factor for the growth in a large-scale area. However, at a very high light intensity, the efficiency of photosynthesis becomes low (Malkin & Fork, 1996). To increase the efficiency, previous research has successfully demonstrated that the color temperature, specified by warm white (3200K), cold white (6500K), and very cold white (8500K), and light intensity contributed to the growth of *Chlorella vulgaris* (Kondzior et al., 2019). However, in the previous research, color temperature and light intensity were not well optimized and studied for the interactions between the factors. We hypothesized that light intensity and color temperature influenced growth and biomass production.

This paper study about cultivation of *C. vulgaris* with different light intensities and color temperatures. This research aimed to study the effect of light and color temperature and optimize the parameters by employing Response Surface Methodology (RSM). The microalgae biomasses would be weighted every two days for eight days to determine the specific growth rate.

2. Method

2.1. Microalgae culture

Stations Commercial *Chlorella vulgaris* was cultured in a 1000 mL Erlenmeyer flask containing 200 mL of modified Bangladesh medium no. 3 (Hadiyanto & Azimatun Nur, 2014) consisted of 1 g·L⁻¹ NaHCO₃, 0.06 g·L⁻¹ urea, and 0.01 g·L⁻¹ Triple Super Phosphate, and an aeration system used for agitation purposes. The experiment was conducted at a temperature of 25 °C for eight days. Bench LED lamp (DU-PLEX/DP-510LS) was chosen because it has four different kinds of color temperatures (3000, 4000, 5000, and 6000 K), and every color temperature has five different light intensities, i.e., 200, 400, 600, 800, and 1000 lux. The Red, Green, Blue (RGB) composition and Hex color of every color temperature can be shown in Table 1 and Figure 1 and their color appearance. Sterile cotton was used to cover the top of Erlenmeyer to prevent microalgae from contamination.



Figure 1. The visual color temperature is from 3000 to 6000 K (left to right)

Table 1. The RGB and hex color code of color temperature from 3000 to 6000 K

Colour Temperature	RGB	Hex Colour
3000	255, 177, 110	#FFB16E
4000	255, 206, 166	#FFCEA6
5000	255, 228, 206	#FFE4CE
6000	255, 246, 237	#FFF6ED

RGB = Red Green Blue composition

2.2. Microalgae biomass analysis

The biomass concentration of the microalgal cultures was determined daily according to a previously reported method (Chiu et al., 2009) using a UV-Vis spectrophotometer (Spectroquant prove 100) at a wavelength of 682 nm, following:

$$W = 0.3101 \times OD_{682} - 0.0065 \quad (R^2 = 0.9981) \quad (1)$$

Where W is biomass ($\text{g}\cdot\text{L}^{-1}$), and OD_{682} is the absorbance of microalgae culture at 682 nm. Moreover, doubling time (t_d) was calculated by using

$$t_d = \frac{\ln 2}{\mu} \quad (2)$$

where t_d is the doubling time (day) and μ is the specific growth rate (day^{-1}).

2.3. Optimization of microalgae biomass production

The two input factors were varied, i.e., light intensity 200, 400, 600, 800, 1000 lux and color temperature 3000, 4000, 5000, and 6000 K, selected as independent variables, while the microalgae growth rate was used as response variables to determine the combined effects of the inputs. One of the objectives of this study is to investigate the correlation between light intensity and light temperature in the trend of growth rate by using the Response Surface Method (RSM) using the statistical software Minitab 17 (Pennsylvania, USA). That statistical software was used to model the empirical equations and plot the response surface. The One-way Analysis of Variance (ANOVA) was also used to deal with the statistical parameters. Therefore, 20 designs with single replication were produced. A control experiment with the optimized conditions determined in the analysis was performed to verify the validity of this approach, and the precision between model and experimental results was projected.

3. Result and Discussion

3.1. Growth profile of microalgae *Chlorella Vulgaris*

The range Four different color temperatures with various light intensities (i.e., 3000, 4000, 5000, and 6000 K) with the conditions of total darkness reference were applied to the cultivation of *C. vulgaris* (Figs. 2). It is shown that *C. vulgaris* produced small amounts of biomass when cultured in the dark. Conversely, the higher light intensity increased the growth. In this research, high light intensity was not applied to avoid photoinhibition. At 4000 K color temperature, the highest biomass was obtained in all light intensity ranges tested. 4000K color temperature is considered the most efficient color temperature required for algae photosynthesis. The color temperature of 4000 K tends to be neither too warm nor too cold. Based on the RGB composition, the color temperature is closer to the red color; thus, the chlorophyll antenna from *C. vulgaris* quickly absorbs red color from the light (Shin et al., 2016). At temperature 3000 K, the composition of the color is mixed between reddish and orange. We expected that the biomass could be boosted. However, the color temperature did not affect growth based on the result. In this experiment, the lower biomass production was found at 6000 K, which has a cooler temperature compared to the others. White light is a combination or mixture of several light spectrums, including those from inefficient light spectrums, to not significantly increase the yield of biomass (Table 2).

Based on Table 2. The concentration of *Chorella vulgaris* on different light intensity and color temperatures was then varied to the growth kinetics using the general formula of growth rate analysis. It showed that the specific growth rate (μ) and doubling time (t_d) have opposite trends where high μ values produce low t_d and vice versa. This is consistent with previous research, which demonstrated that the growth rate is inversely proportional to the doubling time of a microorganism (Vidyashankar et al., 2015). Furthermore, the culture of *Chorella vulgaris* in all operating conditions did not result in a lag time/phase of adaptation in all cultures. The measurements verified this in Figure 2a-f, where no adaptation phase was found in the culture of *Chorella vulgaris* during the cultivation process. This result indicated that *Chorella vulgaris* is a potential candidate to be used in large-scale cultivation.

The interaction of light intensity and the specific growth rate can be seen in Fig 3. It showed that the growth under dark conditions resulted in a low growth rate, while the growth rate increased when light intensity was switched to high for all color temperatures. In this experiment, the optimum light intensity was 600 lux). At above 600 lux, the growth seems to decrease due to photoinhibition (Béchet et al., 2013; Kim et al., 2015)

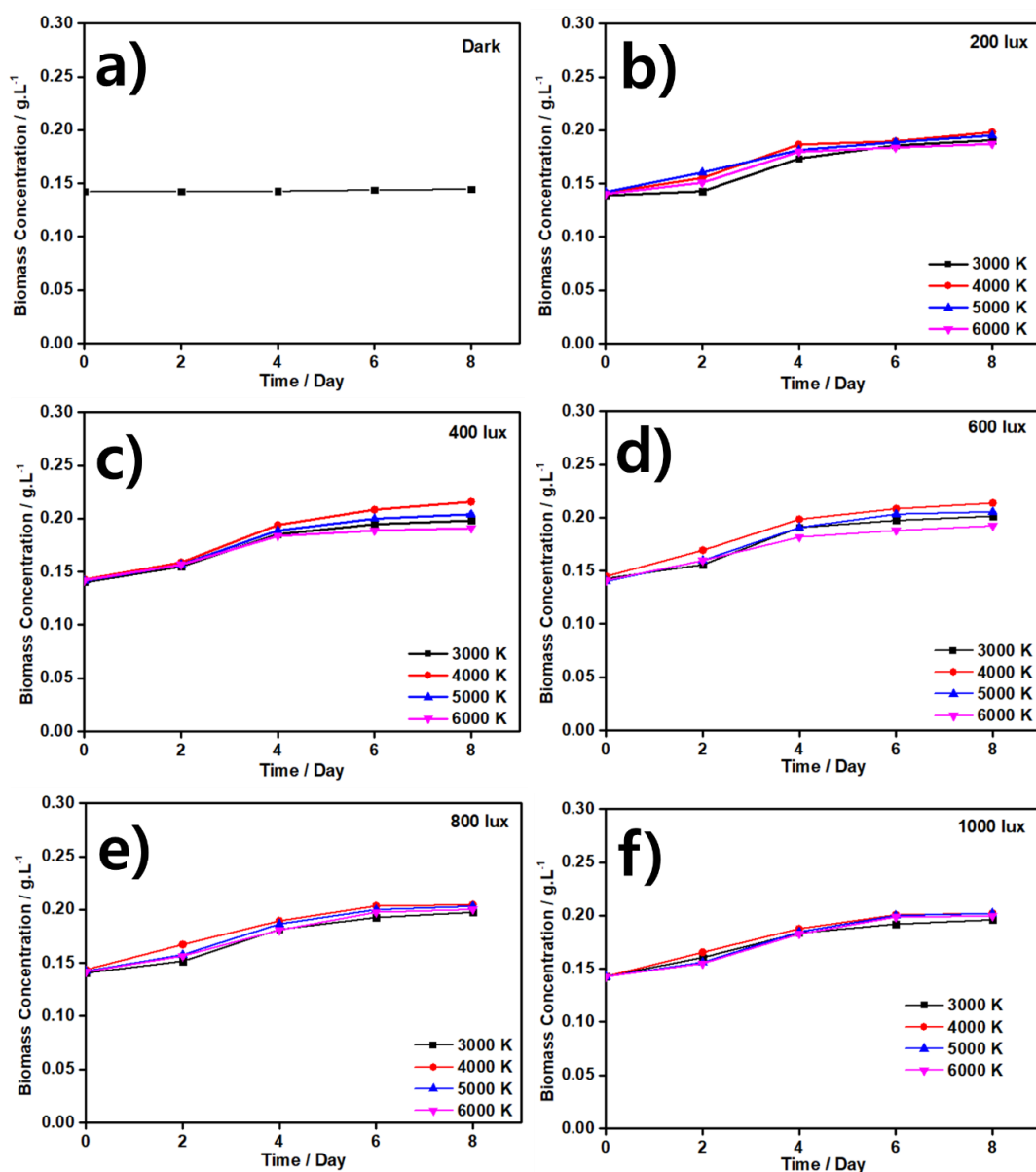


Figure 2. Microalgae biomass dry weight under the conditions of various color temperatures (3000, 4000, 5000, and 6000 K) with the light intensity of a) 0, b) 200, c) 400, d) 600, e) 800, and f) 1000 lux

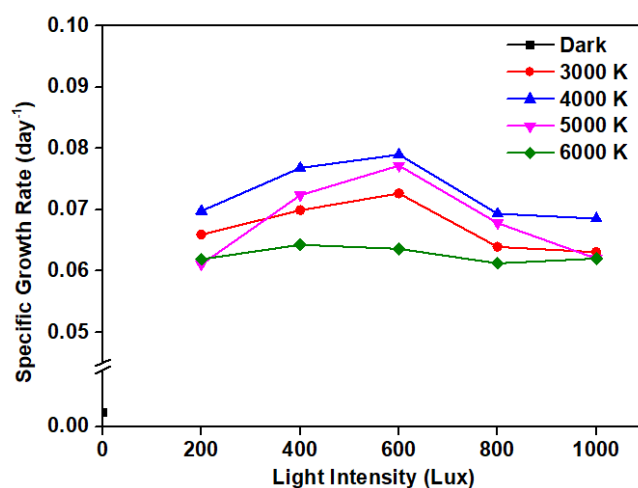


Figure 3. Correlation between light intensity vs. specific growth rate during *Chlorella vulgaris* cultivation under a various color temperature

Table 2. The specific growth rate of microalgae *Chlorella vulgaris* under the conditions of various color temperatures (3000, 4000, 5000, and 6000 K) and various light intensities (0, 200, 400, 600, 800, and 1000 lux)

Light Temperature	μ (Day ⁻¹)	t _a (Day)
Dark	0.0026	266.5951
200 Lux		
3000 K	0.0659	10.5182
4000 K	0.0698	9.9305
5000 K	0.0611	11.3445
6000 K	0.0619	11.1979
400 Lux		
3000 K	0.0699	9.9163
4000 K	0.0768	9.0254
5000 K	0.0724	9.5739
6000 K	0.0643	10.7799
600 Lux		
3000 K	0.0727	9.5343
4000 K	0.0790	8.7740
5000 K	0.0772	8.9786
6000 K	0.0636	10.8985
800 Lux		
3000 K	0.0639	10.8474
4000 K	0.0694	9.9877
5000 K	0.0678	10.2234
6000 K	0.0613	11.3075
1000 Lux		
3000 K	0.0631	10.9849
4000 K	0.0685	10.1189
5000 K	0.0620	11.1798
6000 K	0.0620	11.1798

3.2. Light intensity and color temperature optimization using response surface method

Based on to optimize the effect of light intensity and color temperature, we employed RSM and central composite design (CCD). Linear regression from the RSM was generated as follows (Eq 3).

$$\mu = 0.0169 + 4.4 \times 10^{-5}X_1 + 2.2 \times 10^{-5}X_2 - 0X_1^2 - 0X_2^2 - 0X_1X_2 \quad (3)$$

Where X_1 and X_2 are light intensity and color temperature, respectively, since X_1 , X_2 , and $X_1.X_2$ are almost zero. Thus, the regression can be simplified as

$$\mu = 0.0169 + 4.4 \times 10^{-5}X_1 + 2.2 \times 10^{-5}X_2 \quad (4)$$

Groups Zero coefficient showed that the interaction between light intensity and color temperature is shallow. It demonstrated that the growth rate of *Chlorella vulgaris* was influenced by the variables independently.

Figure 4a showed the correlation between predicted and experimental values, which resulted in R^2 of 0.9982. This indicated that the regression could predict the growth rate very well. This relatively high R^2 value is deemed accurate enough to distinguish effects from independent factors. Considering fluctuations in μ experienced in a biological system, limited experiments have implied a satisfying level of confidence between the model (regression) and real experiments.

Table 3. ANOVA results of light intensity and color temperature effect on the specific growth rate of *C. vulgaris*

Specific Growth Rate					
Source	DF	SS	MS	F-value	P-value
Model	5	0.000445	0.000089	7.54	0.001
Linear	2	0.000100	0.000050	4.23	0.037
Light intensity (Lux)	1	0.000018	0.000018	1.57	0.231
Color temperature (K)	1	0.000081	0.000081	6.89	0.020
Quadratic	2	0.000340	0.000170	14.39	0.000
Light intensity (Lux)* Light intensity (Lux)	1	0.000187	0.000187	15.79	0.001
Color temperature (K)* Color temperature (K)	1	0.000153	0.000153	12.99	0.003
2-way interaction	1	0.000006	0.000006	0.48	0.501
Light intensity (Lux)* Color temperature (K)	1	0.000006	0.000006	0.48	0.501
Error	14	0.000165	0.000012		
Total	19	0.000611			
R ²	0.7292				

ANOVA analysis was carried out to elaborate this dataset further, and the effects of light intensity and color temperature on the specific growth rate were investigated, as reported in Table 3. The ANOVA result (Table 3) showed that light intensity was not significantly affected the growth ($P > 0.05$), while color temperature strongly influenced the growth ($P < 0.05$). The interaction between light intensity and color temperature was not affecting the growth ($P > 0.05$). The right combination of light intensity with color temperature can produce a different growth rate, but the initial biomass concentration is also affected. The initial concentration of microalgae biomass that is too small can cause microalgae to experience stress quickly because of the photo-inhibition process.

Atta et al. (Atta et al., 2013) investigated the effect of light intensity. The results showed that the growth of *Chlorella vulgaris* at the blue LED light intensity (12:12 L:D) had a higher density; the maximum growth rate was 1.26 day⁻¹ in cultivation time. They were reduced by eight days. In this study, by testing two types of light sources, namely Blue LED and White fluorescent light, the resulting growth rate range is around 1.15-1.26 day⁻¹. Blair et al. (Blair et al., 2014) studied the effect of light wavelengths (clear LED lights, blue, red) on the specific growth rate on day 3. Their study found that specific growth rates were highest when using clear light ($\mu = 0.369$ d⁻¹), followed by blue light ($\mu = 0.235$ day⁻¹) and red light ($\mu = 0.14$ day⁻¹). In the research of Asuthkar et al. (2016), the specific growth of *Chlorella* was in the range of 0.22-0.51 d⁻¹. Some of this literature report the value of the specific growth rate produced is relatively higher than this study.

Two- and three-dimensional surfaces representing the combined interactions of independent variables in cultivating *Chlorella vulgaris* with low initial biomass concentrations are presented in Figure. 4b and 4c. In these figures, exciting findings are related to the formation of response surfaces that describe specific growth rate behavior in *Chlorella vulgaris*. First, when the light intensity is low, and the color temperature is low, the specific growth rate is relatively low at around 0.067 day⁻¹. However, it rapidly increases when the light intensity and color temperature increase. However, the increase did not occur linearly, where certain conditions caused the growth rate to decline slowly. In this case, the light intensity is around 600 lux, and the color temperature of 4300 K is a turning point. The high light intensity can cause photo-inhibition, while too high color temperature causes *Chlorella vulgaris* cannot optimally absorb the color spectrum for photosynthesis. The turning point is believed to be the optimal operating conditions in this study to get the optimal growth rate of *Chlorella vulgaris*. Optimal conditions were obtained using the 'Response Optimizer' mode found in Minitab 17. The optimum specific growth rate obtained from the optimization process was 0.0751 day⁻¹, with the conditions of light intensity and color temperature being 556 lux and 4152 K, respectively. This result is almost close to the turning point that was predicted before.

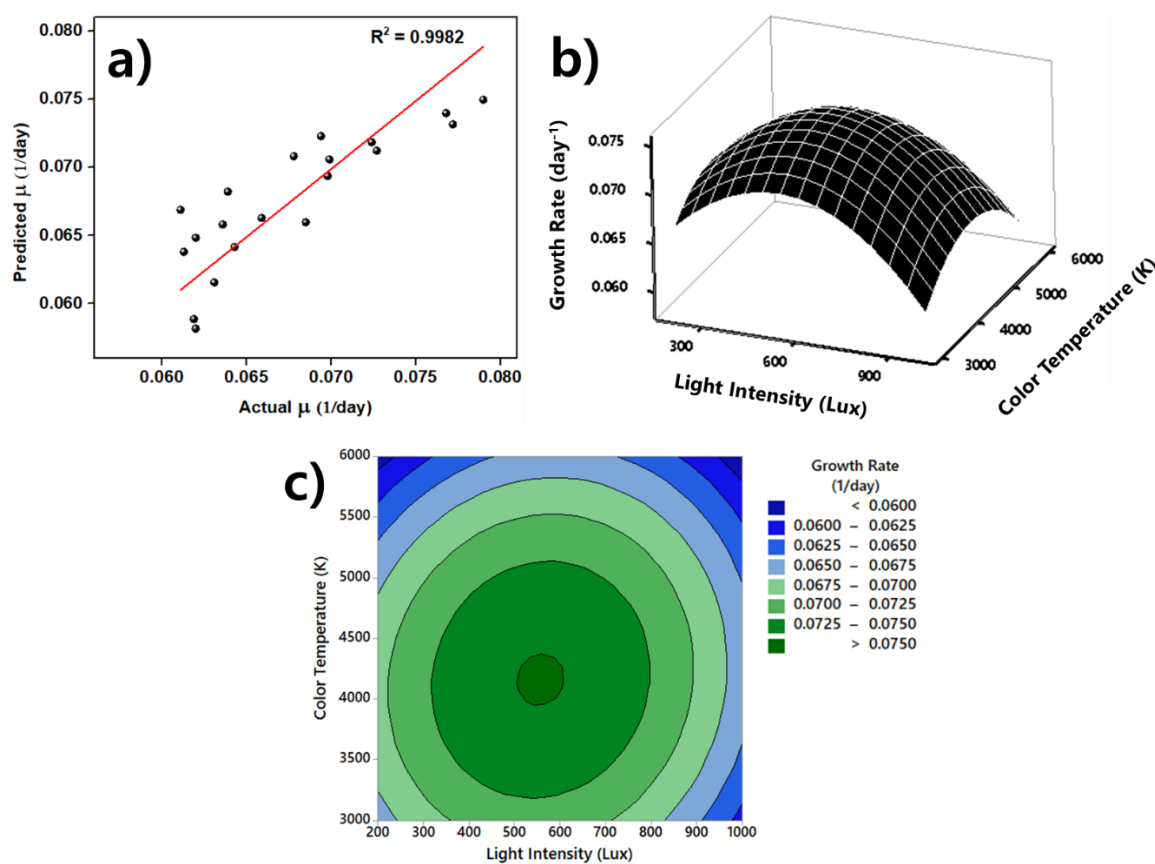


Figure 4. a) Actual vs. Predicted values for the specific growth rate of *Chlorella vulgaris*, b) 3D and c) 2D contour shows the response surfaces of light intensity and color temperature effect on the specific growth rate of *Chlorella vulgaris*.

4. Conclusion

Different color temperatures and light intensity affect the growth of *Chlorella vulgaris* and also its biomass production. The results showed that the average value of *Chlorella vulgaris* growth was getting higher along with the higher light intensity. When tested in dark conditions, the specific growth rate was low, namely 0.0026 day⁻¹, and the optimum light intensity was 600 lux. This study optimized the effect of light and color temperature using RSM. The results showed that the optimal specific growth rate obtained from the optimization process was 0.075 day⁻¹, under light intensity conditions and temperature of 556 lux and 4152 K, respectively.

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