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INFLUENCE OF PHOTOBIOREACTOR ON CULTIVATION OF *TETRASELMIS* SP. UNDER VARIOUS COLORS AND LIGHT INTENSITIES FOR BIODIESEL PRODUCTION

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Abstract— In microalgae cultivation, artificial light sources have impactful influences on their growth pattern. The biomass production rate, lipid and fatty acid profile is observed in microalgae under various color of lights. Therefore, microalgae *Tetraselmis sp.* was cultivated in 20 L photobioreactor under different color (Blue= 490 nm, Green= 560 nm, Red= 635 nm) with different light intensities (120, 240 and 480 µmol photon $m^{-2}s^{-1}$) at 24±2℃ for 15 days to determine the specific light absorption rate. The highest biomass 42.34 mg/L and lipid 35±2% was obtained under blue light with 240 µ mol photon $m^{-2}s^{-1}$ light intensity as compared to other lights.

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Palmitic acid and Stearic acids were dominant fatty acids which found from the lipid analysis. The observation proves that there is a compelling association between light colors, intensity, and the growth of microalgae.

I. Introduction

Phototrophic microalgae cultivation has been identified as a promising platform for the generation of renewable biofuels. In order to enhance the biomass production at maximum level with low cost, photobioreactor is highly efficient in cultivation of the microalgae [1]. The photobioreactor allows for the separation of the biomass growth and lipid generation phases with efficient stress induction strategies as light. The growth is highly influence by artificial light rather than sunlight due to fluctuation [2]. The total number of photons received per square metre over the course of a specific period is referred to as light intensity, which is a wavelength-weighted energy measurement. Because of the total amount of photons received rather than the energy obtained by each photon drives photosynthetic photochemical reactions, its usage has been widely spread in research. The vertical migration of diatoms can be impacted by the composition of the light as well as locomotion speed. The fraction of photons proportional to each wavelength that are available per unit area at a given time is influenced by spectral differences.

The photobioreactor can be constructed and optimized according to the strain of choice. This closed system takes up little space while boosting light availability and considerably reducing contamination risks. In natural waterways, water molecules, organic and inorganic chemicals, and microalgae themselves alter the underwater light field through scattering at specific wavelengths and polarizations as well as through wavelength-dependent absorption [3]. These optical

contact mechanisms significantly modify the polarisation characteristics of propagating light. When the light travels through water, it partially polarises and loses strength mostly in wavelengths of red, yellow, and purple [3].

Light spectra shift as reactor depth increases due to absorption of blue and red by green microalgae. Different colors and light intensity highly affect the biomass production at optimum condition and reduce the cultivation cost [2, 4]. Therefore, this study aims to investigate the response of light spectral composition and light intensity on microalgae *Tetraselmis* sp. using 20 L photobioreactor based on physiological and behavioural photo regulation mechanisms for biomass and lipid accumulation for biofuel production.

II. Method & Materials

Tetraselmis sp. was obtained from Algae Biotechnology Laboratory, Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang and it was maintained in BG-11 medium. Microalgae *Tetraselmis* sp. was cultivated in 20 L photobioreactor under different color (blue= 490 nm, green= 560 nm, red= 635 nm) and white light as constant with different light intensities (120, 240 and 480 µmol photon m^{-2} s^{-1}) at 24±2°C for 15 days. The growth of biomass measured by filtered the suspension (50 ml) onto pre-weighed whatman GF/f filters and algae rinsed to determine the biomass specific light absorption rate. One way of avoiding oversaturation is to use light with a spectral composition that minimizes light absorption. The biomass productivity and lipid extraction were measured following the protocol mentioned in [5].

III. Results and Discussion

A. Effects of Lights Color

The effects of colour and light intensities were obviously noticed in the growth of *Tetraselmis* sp. as shown in Figure 1. The absorbance reading value for red light were higher than other blue and green light which reflect the growth of culture. The cell grows rapidly

under blue (490 nm), and the highest biomass (42.34 mg/L) obtained as compared to red (34.32 mg/L) and green (25.55 mg /L). The growth and biomass accumulation rapidly increase until day 13 than it reached to stationary phases where the rate of cells multiplication and deaths are almost same.

Blue light significantly increase the development of microalgae *Tetraselmis* sp. Beyond that, it increased the amounts of carbohydrates, lipid and protein. However, red light, as opposed to blue and white light, inhibited the growth of algae and the production of lipids.

Blue has higher penetration capacity into the culture and enhance the growth and cell density of *Tetraselmis* sp. blue light has higher energy content as compared to other lights. Therefore, it enhances the cell density and biomass productivity. Green light has greater influences in cells growth. But compared to blue light it slows in biomass production and lower lipid content. The growth of culture under red light environment was lower than blue because wavelength of red inhibits the growth and damage the cell at one point. The cultures were easily reached saturation point where the maximum growth level achieved.

B. Effect of Light Intensity

The higher growth found under light intensity 240 µmol photon $m^{-2}s^{-1}$ environment. Lower growth found under 120 and 480 µmol photon $m^{-2}s^{-1}$ as shown in Figure 2 and Figure 3. 120 µmol photon $m^{-2}s^{-1}$ light intensity was insufficient to culture. Therefore, it could not performed high rate of photosynthesis to multiply the number of cells. However, over exposure of light intensities to cells causes photoinhibitions where the light harvesting pigments were stop absorbing light wavelength which destroy the pigments and cells mechanisms. Therefore, lower growth found under too low and too higher light intensities.

C. Lipid Content

Bligh and dyer method used and extracted maximum lipid content of cells were extracted.

This method helps to separate lipids from the biomass with more efficient. From the experiment, total lipid content 35±2% was extracted from biomass which grown under blue environment. Under red and green light biomass contain lower lipid content were 30±2% and 28±4% respectively. The blue wavelength can penetrate much deeper than other light wavelength. Therefore, more culture cells especially in the deeper area was exposed blue light in photobioreactor than other light.

D. Fatty Acid Profile

Table 1 shows the fatty acid profile of *Tetraselmis sp*. The extracted lipids were trans esterified to breakdown the macromolecules into micro molecule where separated the fatty acids methyl ester (FAME) from the triacyl glycerides using Methanol KOH as catalyst. The individual FAME's separated cleanly and identified via gas chromatography mass spectroscopy (Agilent 7890A gas chromatograph; mega wax MS capillary column with size of 30 m × 00.32 mm internal diameter × 0.5 μm film). From the Table 1 also it can be found that the high mount of saturated fatty acids were present in the lipid following with polyunsaturated fatty acid (23.57%) and monosaturated fatty acid (12.31%). Palmitic acid and Stearic acids present in higher quantity as compared to other fatty acids which can noticed from the result of gas chromatography. The observed changes are caused by a biological component that is closely related to the metabolic activity of specific microalgal strains. It may be crucial to analyse FAs profiles in connection to growth stage and time biomass collection to the most advantageous time for the best fatty acid composition. The percentage of saturated, monosaturated and polyunsaturated fatty acid were varied under different light spectra. However, this experiment has found that, high percentage of saturated fatty acids were obtained under optimum conditions.

Beyond the optimum conditions the cells gone through the physical and chemical properties which can detect through the growth and lipid production of cells. Fettah et al. [7] reported that, each wavelength has a particular minimum intensity threshold for growth. Variable growth results under various light/dark cycles indicate the need for factor tuning. Therefore, *Tetraselmis* sp. highly influential to the light spectra and intensities to grow and formation of internal composition. The lipid composition highly suitable for the biodiesel production since it contains fatty acid methyl ester which suitable for combustion.

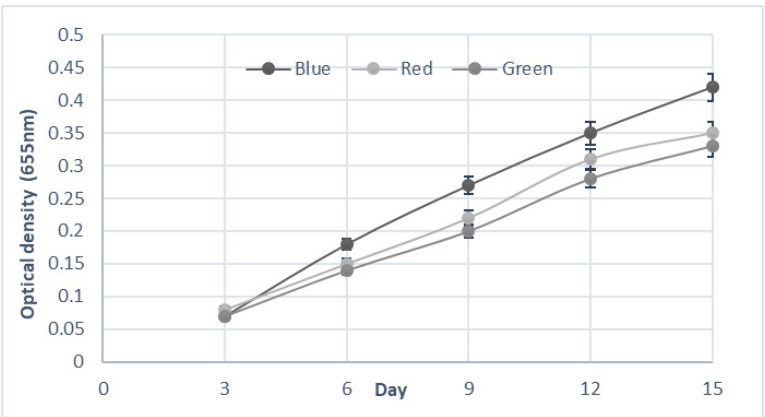

Figure 1: Growth curves of *Tetraselmis* sp. under different color

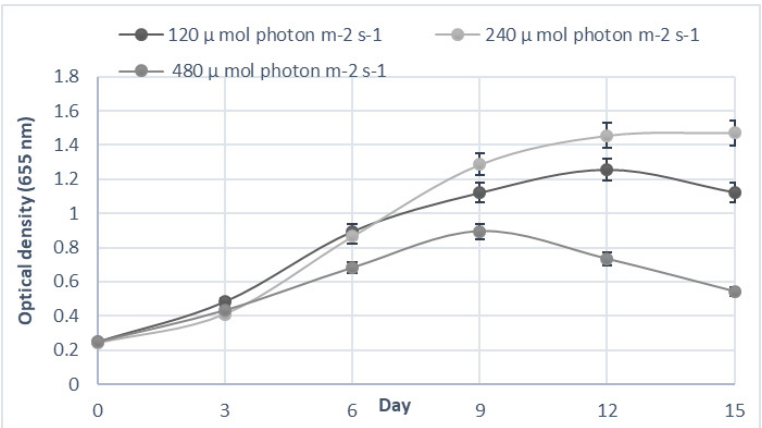

Figure 2: Growth curve of *Tetraselmis* sp. under different light intensities

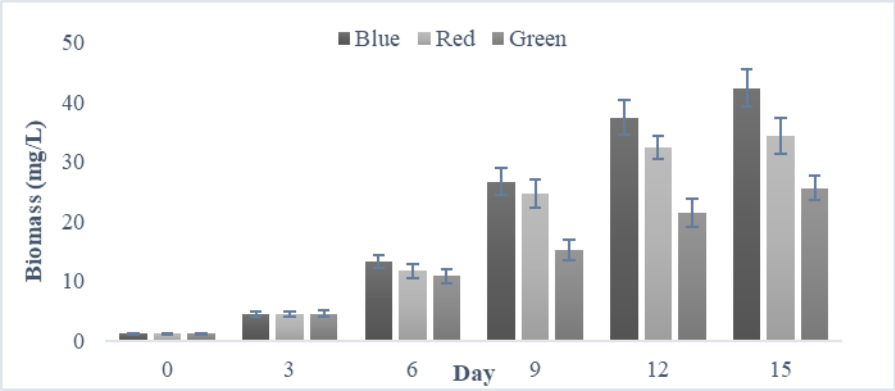

Figure 3: The biomass production of *Tetraselmis sp.* for the 15 days.

Table 1: The fatty acid profile of *Tetraselmis* sp.

Fatty acid	Carbon number	Percentage (%)
Myristic acid	C14:0	3.77 ± 0.2
Pentadecanoic Acid	C15:0	2.59 ± 0.2
Palmitic	C16:0	25.28 ± 0.1
Stearic acid	C18:0	32.46 ± 0.1
Palmitoleic acid	C16:1	7.75 ± 0.2
Oleic acid	C18:1	4.56 ± 0.1
Linoleic acid	C18:2	18.82 ± 0.1
Eicosatrienoic acid	C20:3	4.75 ± 0.2
Saturated		64.1
Monosaturated		12.31
Polysaturated		23.57

VI. Conclusion

The photosynthesis capacity of *Tetraslmis* sp. has changed marginally based on the external environment. The volume of photobioreactor significantly contribute to biomass growth by preventing from biological contamination. The result showing highest growth, biomass and lipid production under blue light with 240 µmol photon $m^{-2}s^{-1}$. Fatty acid profile of *Tetraselmis* sp. has confirmed that it has great potential for biodiesel production.

VII. Acknowledgements

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