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Study of Light Wavelength Dependency in Red-Orange Spectrum on Continuous Culture of *Synechocystis sp. PCC6803*

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ABSTRACT

In this study, the effect of light wavelength on growth rate and lipid production of Synechocystis was investigated. Continuous cultivation system was used to have uniform cell density and avoid self-shading in order to obtain more precise results. Based on previous studies, red light is more efficient than other colors in the visible spectrum for cultivation of Synechocystis; however, the optimum wavelength in red light spectrum remains still unknown. In order to determine the most efficient wavelength of red light, five different wavelengths including 600, 635, 660, 670, and 730 nm were used for growing Synechocystis in a chemostat setup. The results revealed that 635 nm was the most efficient wavelength for cultivation of Synechocystis in terms of both biomass production yield and growth rate. These findings can be attributed to the existence of phycocyanin, the principal light-harvesting supercomplex in Synechocystis, which absorbs maximally at around 620 nm. The results also indicated that cell size and fatty acid profile of Synechocystis were almost the same for different light wavelengths; however, the maximum light was absorbed at 635 nm.

1. Introduction

Photosynthesis is the main process of converting light energy to chemical energy that allows microorganisms to produce biomass and lipids [1]. Light, the driving force of photosynthesis, is a major abiotic parameter that influences cellular metabolism. In addition to temperature, CO_2, pH, and nutrients, light conditions should be optimized in photobioreactor to maximize certain production [2]. Since the microalgae represent a photosynthetic microorganism, quantity and quality of light have a huge influence on its growth rate. Generally, microalgae can harvest light at wavelengths between 400 and 700 nm [3]. However, their photosynthetic apparatus cannot utilize all the wavelength in this region and the optimal wavelength to achieve maximum photosynthetic efficiency is highly dependent on the chemical nature of algal constitutive pigments and is highly different from species to species [4]. Further, the energy consumption of artificial light is higher for shorter wavelengths [5]. Thus, illumination should be done at a specific wavelength in order to ensure energetica efficiency [6].

There are some examples of using monochromatic lighting for optimal growth of microalgae and cyanobacteria [7-14]. For

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batch culture of microalgae, Mutaf et al. [7] showed that *Stichococcus bacillaris* had a similar growth rate under red light and blue light illumination; however, the fatty acid content was higher for red light. Hwang and Maier investigated the effect of red and white lighting on growth and fatty acid contents of *Neochloris oleoabundans* in a 1-L batch reactor [8]. They showed that red light yielded the maximum specific growth rate and the greatest proportions of total lipids as well as Monounsaturated Fatty Acids (MUFAs). For light-activated heterotrophic growth of *Synechocystis* sp. PCC6803 *(Synechocystis)*, it was shown that a daily pulse of blue (450 nm) light was necessary to keep growing [9]. Even though growth on continuous orange-red (636 nm) light does not require blue light (pulsed or continuous), a broad-spectrum white LED was shown to be increasing the growth rate of *Synechocystis* beyond that possible with only blue and red light [10].

In most of the previous studies, batch cultivation has been used for investigating the effect of light, which suffers from self-shading of culture by increasing the biomass density. One solution to this problem is to use continuous culture, which attains steady state and provides well-controlled and constant cell density [11]. It should be noted that this self-shading phenomena sometimes may produce incorrect or contradictory results. For example, Toe et al. [12] and Das et al. [13] who used batch culture for their experiments showed that *Nannochloropsis* sp. had a better growth rate under blue wavelength. In contrast to these results, Kim et al. [14] showed that red light exhibited a higher growth rate than the blue light in continuous culture of *Nannochloropsis* sp. These findings indicated the importance of using continuous cultivation system to get more precise results about the effect of light quality and quantity on growth rate.

It was previously shown that red light would be more efficient than blue light for growing *Synechocystis*. The aim of this study is to improve the previous researches in this field by finding the optimum wavelength in the orange-red spectrum for growing *Synechocystis*. A continuous chemostat system was illuminated at five different wavelengths between 600 and 730 nm. The efficiency of these five wavelengths was compared by determining the growth rate, fatty acid content, and absorbed photons.

2. Materials and methods

2.1. Algal strain and culture media

Synechocystis sp. PCC6803 was first inoculated in 100 ml flasks containing 25 ml autoclaved modified BG-11 medium [15] supplemented with 5 mM $NaHCO_3$ and was then incubated in a shaking incubator under continuous fluorescence illumination (50–70 $\mu E/m^2/s$) and at 120 rpm and 30 ^oC. Cells were transferred to the photobioreactor when they reached the logarithmic phase as determined by optical density (OD) at 730 nm using spectrophotometer Novaspec II, Pharmacia.

2.2. Chemostat cultivation system

A flat-plate glass vessel with volume of 225 ml was used as a photobioreactor for continuous culture of *Synechocystis* in chemostat system. The system was aerated by Nitrogen flow containing 3 % carbon dioxide with a flow rate of 10 ml/min. To regulate the temperature of the photobioreactor, it was kept in water bath at a temperature of 30±0.2 °C. Culture medium, modified BG-11, was injected to the photobioreactor using a

peristaltic pump. The pump flow rate can be changed to adjust the dilution rate. The OD_{730} and pH of the system were manually measured by taking out a sample from the culture. Schematic diagram of this chemostat system is shown in Figure 1.

The front side of the photobioreactor which sticks to the water bath wall was illuminated by a LED panel with five different wavelengths of 600, 635, 660, 670, and 730 nm. The spectral irradiance of the LED lamps is shown in Figure 2.

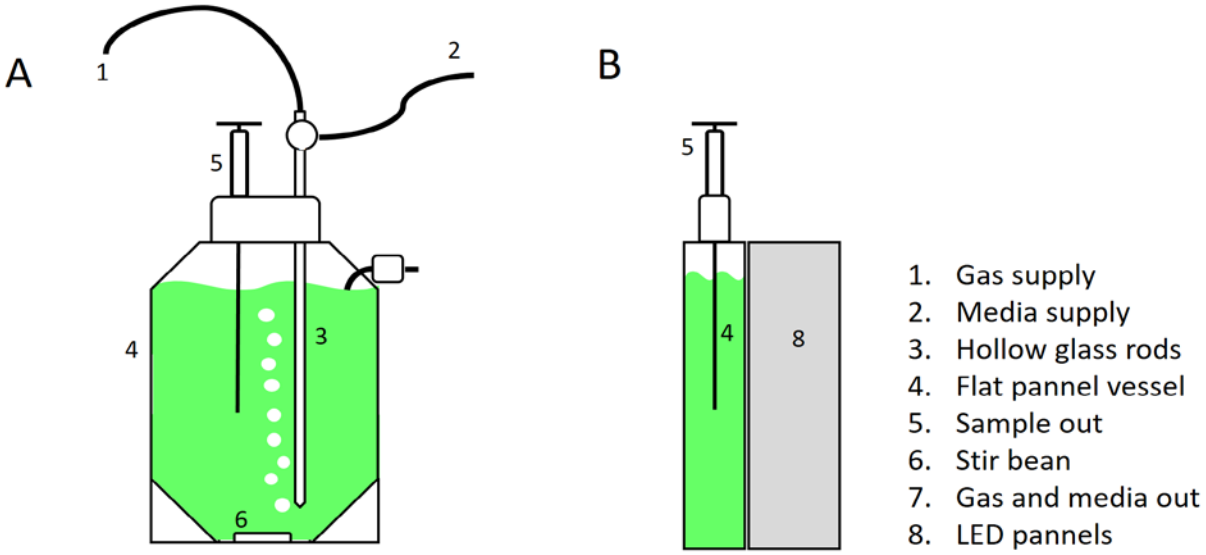

Figure 1. Schematic diagram of the photobioreactor used for chemostat experiments: a) front view, b) side view.

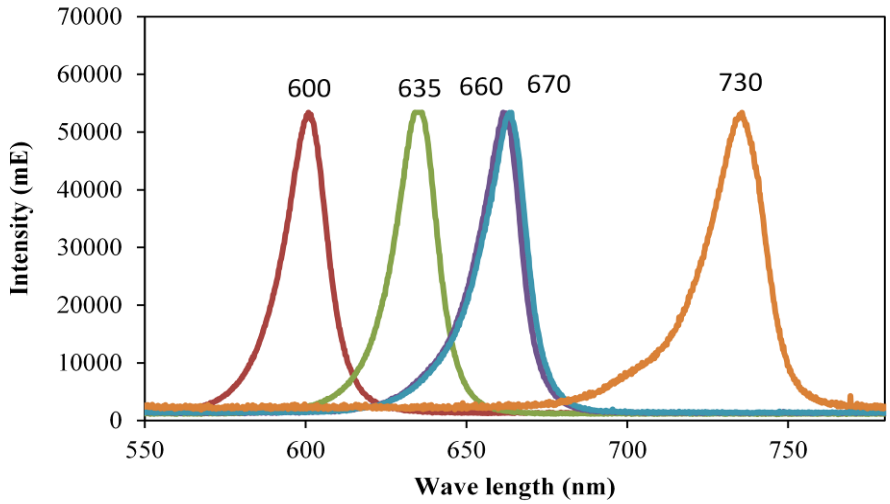

Figure 2. The spectral irradiance of each wavelength used in chemostat setup.

2.3. Growth rate determination

The effectiveness of each wavelength in growing *Synechocystis* was compared in terms of biomass production yield and growth rate. For determining the biomass production yield, the dilution rate was set to 0.081 h^{-1} and the steady state OD_{730} was measured for each wavelength.

The growth rate was measured in washout experiment, in which the dilution rate was set

to 0.205 h^{-1}, supposed to be much higher than the maximum growth rate of *Synechocystis*. The growth rate was calculated by taking into account the dilution rate and slope of the log (OD_{730}) versus time.

2.4. Cell size determination

The algal cell size was measured by using cell counter + analyzer system model TCC. Then, 20 µl of the culture was diluted in 10 ml of the casy liquid and then, analyzed by the equipment.

2.5. Biomass concentration determination

The biomass concentration was measured by Dry Cell Weight (DCW). A filter paper was dried for 2 h at 90 °C, cooled in a desiccator, and weighed. Then, a sample expected to yield around 2 mg of dry weight was collected from the culture and filtered and was dried and cooled before weighing again. Biomass concentration was obtained by the difference between the two weights.

2.6. Fatty acid determination

To determine the fatty acid profile of biomass, an aliquot of the culture corresponding to at least 10 mg of biomass was collected from the culture and harvested by centrifugation at 4000 rpm for 5 min and the biomass was transferred to a bead beater tube which contained around 0.5 g glass beads. 50 µl of 0.2 mg/ml heptadecanoic acid (C17:0) was spiked to the sample as internal standard for fatty acid determination. The tube was bead beated for 6 times at 2500 rpm for 60 seconds with 40 seconds internal between each beating. Then, the solution was transferred to a glass tube and 6 ml of chloroform/methanol (1:2 v/v) was added to the biomass and the mixture and vortexed for 5 min. The solution was centrifuged at 4000 rpm for 5 min and the extraction mixture was collected. The above mentioned extraction process was repeated three times to ensure complete extraction of lipids. Collected solvents were combined and evaporated to dryness under nitrogen flow. The total lipid contents were expressed as a percentage of DCW.

The fatty acids present in the lipid were converted into fatty acid methyl esters (FAMEs) using 2 ml of 1 % KOH in methanol, followed by heating for 15 min at 65 ^{o}C, then adding 2 ml of 5 % methanolic HCl, and heating again for 15 min at 65 ^{o}C. Finally, 1 ml Milli-Q water was added to the sample to stop the reaction. FAMEs were extracted by hexane and 1 µl of it was analyzed by GC–MS, according to the following temperature profile: initial temperature 323.15 K held for 1 min, then raised to 453 K at 15 K/min, 503.15 K at 7 K/min, and finally to 613.15 K at 30 K/min with total analytical run time equal to 20 min.

3. Results and discussion

3.1. Effect of different wavelengths on growth rate

The OD_{730} versus time for each of the studied wavelength is shown in Figure 3. The dilution rate was 0.081 h^{-1} and light intensity was 70 $mE/m^2/s$ for all the light sources. For 730 nm, a very low rate of growth was observed even in the batch culture; therefore, the steady state OD_{730} for this wavelength was not reported in this figure. According to these results, the steady state OD_{730} for 635 nm wavelength was higher than the other wavelengths, which indicated better biomass production yield of this wavelength. This behavior can be attributed to the existence of *phycocyanin*, the principal *phycobilisome* in *Synechocystis*, which was absorbed maximally at around 620 nm. *Synechocystis* also contained chlorophyll

a and cph1 photoreceptor, with maximum absorptions in 680 and 670 nm, respectively. The results of this experiment indicated that existence of phycobilisome was more effective than chlorophyll a and cph1 in growing *Synechocystis*.

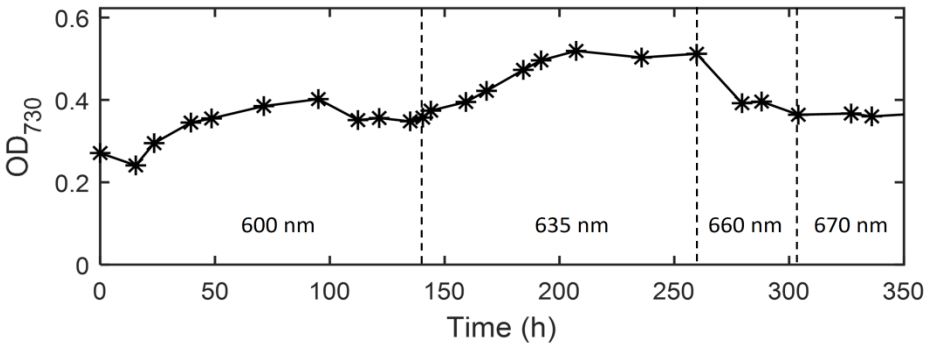

Figure 3. OD_{730} versus time for the studied wavelength in chemostat setup.

To compare the maximum growth rate of *Synechocystis* for each of the studied wavelengths, washout experiments were performed in the chemostat setup. In these experiments, the dilution rate was set to 0.205 and the optical density of the culture was measured accordingly. The log (OD_{730}) versus time for this experiment under illumination of 600 nm wavelength with 75 $mE/m^2/s$ light intensity is shown in Figure 4. The slope of this diagram was -0.0694. Thus, by considering the dilution rate of 0.205, the maximum growth rate of *Synechocystis* in this wavelength was 0.1356. The reported value of the maximum growth rate of *Synechocystis* exposed to the red light was 0.11 h^{-1} [16]. Since we used washout experiments for the measurement of the maximum growth rate, we obtained a slightly higher value.

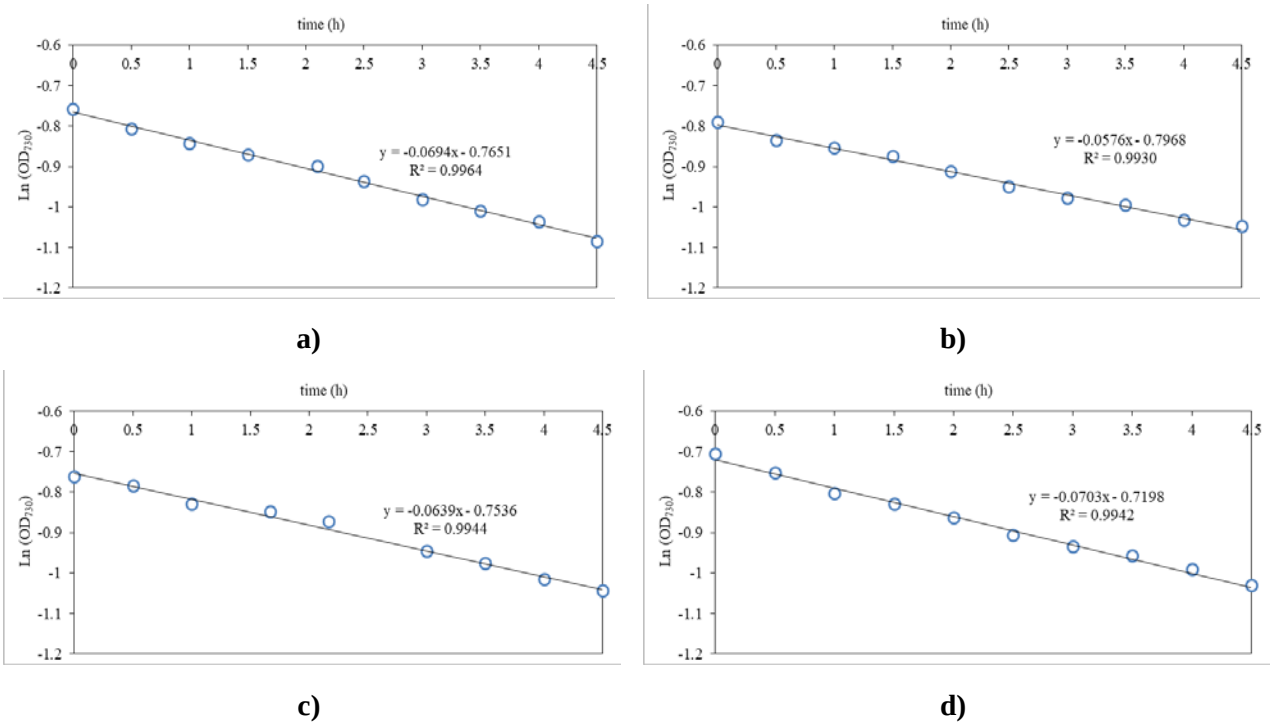

Figure 4. Washout experiment results for lighting with: a) 600 nm, b) 635 nm, c) 660 nm, and d) 670 nm.

The same washout experiment was repeated for other wavelengths and maximum growth rate was determined using the same calculation; the results are shown in Figure 5. These findings demonstrate that the growth rate by illumination at 635 nm is higher than other wavelengths, which is in agreement with previous results on biomass production yield.

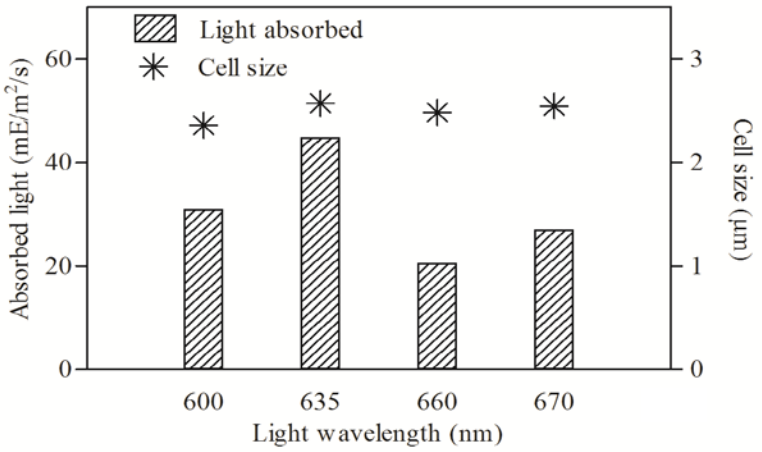

Figure 5. Maximum growth rate for different light wavelengths obtained from washout experiments.

3.2. Effect of different wavelengths on cell size and photon absorption

The adsorbed photon and average cell size for the steady state culture of each wavelength are shown in Figure 6. The results indicated that the light absorption in 635 nm was more than other wavelengths. This can be due to the fact that the steady state OD_{730} for 635 nm wavelength was higher than the other wavelengths. The cell size for each of the studied wavelengths was quite the same, indicating that light wavelength and growth rate had no significant effect on cell size. It should be noted that previous studies showed that increasing the light wavelength reduced the microalgal cell size [17, 18]. However, since we only changed the light wavelength in a narrow range, no significant influence on cell size was observed.

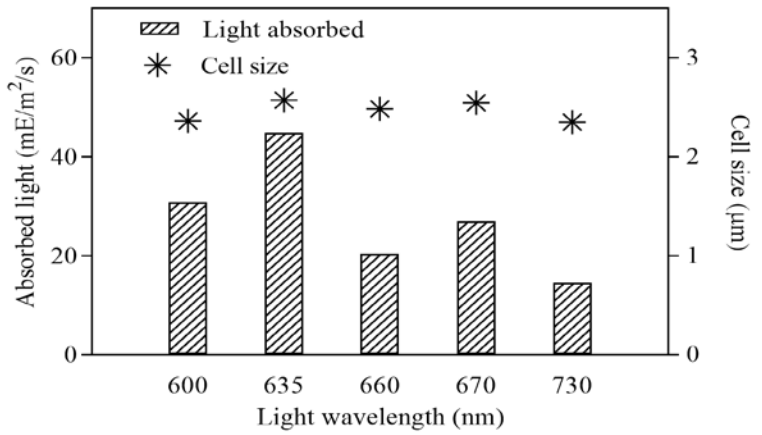

Figure 6. Adsorbed photon as well as cell size for the steady state culture for each wavelength.

3.3. Effect of different wavelengths on fatty acid accumulation

The fatty acid profile for each of the studied wavelengths is reported in Figure 7. Like the turbidostat experiment for *Synechocystis*, the light wavelength had no significant effect on fatty acid profile. The fatty acid profile is in accordance with the ones reported in literature [19, 20].

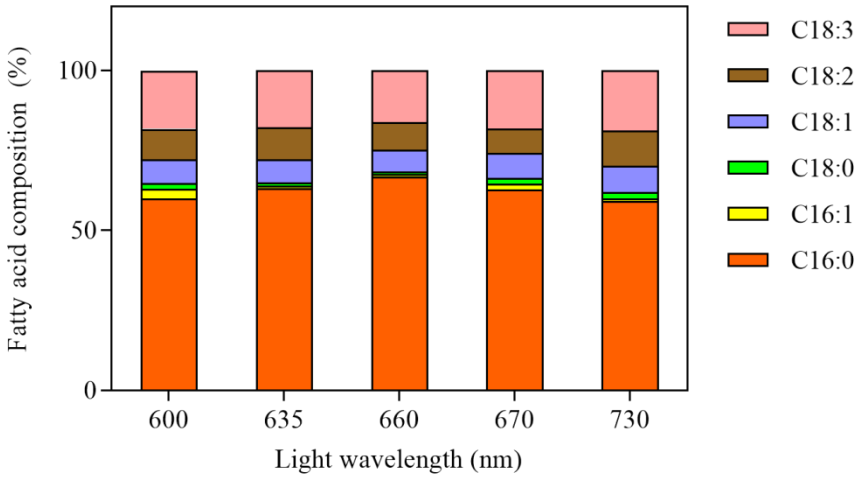

Figure 7. Fatty acid profile of steady state culture for each wavelength.

4. Conclusions

Synechocystis is one of the most well-known cyanobacteria due to its interesting biochemical characteristics. It was already known that *Synechocystis* did not need the whole spectrum of visible light and it could grow very fast by orange-red light illumination; however, the optimum wavelength in this spectrum is still unknown. The aim of this study is to experimentally determine the best light wavelength in the orange-red region for optimum growth of *Synechocystis*. A flat plate photobioreactor was used for continuous culture of *Synechocystis*. The lighting of the photobioreactor was done by using an LED panel with capability of illumination at five different wavelengths including 600, 635, 660, 670, and 730 nm. The results revealed that the highest biomass production yield and growth rate were achieved at 635 nm. This behavior was attributed to the existence of *phycocyanin*, the principal *phycobilisome* in *Synechocystis*, which had maximum absorbance at around 620 nm. The results also indicated that cell size and fatty acid profile of *Synechocystis* were almost the same for different light wavelengths; however, the maximum light was absorbed at 635 nm.

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