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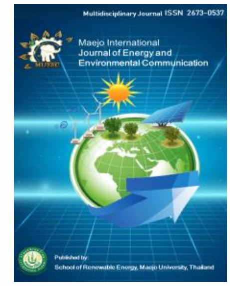

ARTICLE

Impact of biomass density on growth rates of *Spirulina platensis* under distinct light spectra

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ABSTRACT

Light is the core environmental factor that affects the growth and biomass production of microalgae. However, the high density of microalgae will lead to reduction of the growth rate of microalgae culture due to availability of light decreases. Therefore, this experiment was conducted with aim of determining the influence of biomass density on growth of *Spirulina platensis* under different density of culture and LED lights. The result found that, the growth rate *Spirulina platensis* was reduced under high biomass density (9:1) due to lacking light penetration into the culture. However, white LED helps maintain the light acclimation process in the cells. Light spectra enhance the growth biomass. However white light contains all the light spectra highly contribute to the biomass production. The maximum light was penetrated into the culture due to minimal density of culture. More light was observed by the cells. Photosynthetic microalage may frequently experience irradiance fluctuations of one to two orders of magnitude in the natural environment. Microalgae have created several acclimation mechanisms to deal with such shifts.

1. Introduction

The optimizing of microalgal biomass cultivation is receiving a great deal of focus because of its potential value in a variety of applications. The main force behind this is the utilization of microalgae as a sustainable supply of basic foods and biofuels to feed the rapidly growing global economy and population growth (Vadiveloo et al., 2015). The growth of microalgae highly influences by the environmental factors such as temperature, pH, nutrient intake and light intensity, colour, and photoperiod (Palanisamy et al., 2021a; Palanisamy et al., 2022).

A light source, which can be either natural light from the sun or artificial light from lamps, is necessary for autotrophic microalgal growth. Sunlight frequently has a negative impact on process control, resulting in inconsistent production in terms of quantity and biomass composition (Borella et al., 2021). Sunlight is the least expensive option, but it also depends on the location, the weather, and the time of year. Besides that, light depends on the finished product of microalgae

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and its commercial value, but artificial illumination has been advocated as a solution to these issues (Palanisamy et al., 2022). Therefore, light emitting diodes (LEDs) are very efficient lighting technology which generate low heat energy and high conversion efficiency. LED has more advantages than conventional light, in applications of microalgae cultivation. It provides high intensity between the range of 2 to 2.55 µmol photons s-1w-1 of photosynthetically active radiation (Blanken et al., 2013).

Algal growth highly influences by the light spectrum. During the photosynthesis process, microalgae cells were transformed the light energy into chemical energy to accumulate the biomass. Based on photosynthesis-irradiance curve, the rate of photosynthesis to changes according to the light intensity (Palanisamy et al., 2021b). Photo limitation takes place when the cells absorb low intensities (Palanisamy et al., 2022). Photo acclimation takes place when the light intensities proportional until it achieves saturation point where the maximum growth rate of cells. If the intensities go beyond the saturation point, cells undergo photoinhibition where the cells get damages and loss of photosynthesis.

The amount of light intensity received by the cells are based on the volume and density of culture. At lower density, maximum amount of light can be absorbed whereas high density causes limited amount of light can be penetrated the culture and absorbed by the cells. In this situation, different light spectra can be applied to the culture (Palanisamy et al., 2020)

Different microalgae species using different spectrum of lights to synthesis their own cellular metabolite compounds (Kanagesan et al., 2021). Among light spectra, white light can work for all species, whereas red and blue can be highly absorbed by all microalgae and tend to produce rapid growth rates. Izadpanah et al. (2018), reported that, red light maximizes the cell density fir *Chlorella vulgaris* IG-R-96. Under blue light, *Nannochloropsis* sp. grew at its maximum rate under both phototrophic and mixotrophic cultivation conditions. The amount of synthetic pigment produced by microalgae is also influenced by the light's quality. Additionally, it has been noted that *Chlamydomonas reinhardtii grown* under various light wavelengths exhibits light-quality-dependent cell division. Due to a delay in cellular division procedures, the microalgae grown under blue light had larger cells than those grown under red light (Izadpanah et al., 2018; Oldenhof et al., 2004).

Therefore, this study aims to investigate the impact of culture density on growth and biomass productivity of *Spirulina platensis* under different light spectra. The influence of light spectra was investigated to determine the changes on photo acclimation of cell. This study to enhance the growth of cells by altering the lights spectra on culture environments to optimize the cultivation conditions.

2. Material and methods

2.1 Material preparation

Mother culture of *Spirulina platensis* was obtained from the Sustainable Resources and Sustainable Engineering Research laboratory, Maejo University, Chiangmai Thailand. This culture was selected due to chosen for its good spiral pitch and lengthier trichome, which have a significant impact on the efficiency of harvesting biomass The culture was cultured and maintained in modified fertiliser-based media which contain Nitrogen phosphorus, sodium chloride (NaCl), magnesium sulphate ($MgSO_4{\cdot}7H2O$), ammonium nitrate (NH_4NO_3) and sodium bicarbonate ($NaHCO_3$). This composition was derived from Zarrouk media (Zarrouk, 1966). The media prepared by dissolving the ingredients one by one and adjust the pH to 8.5±0.3 using 1M NaOH buffer solution. The media was autoclaved at 121 ℃ for 21 min for sterilization. Culture was maintained in 2 L of Erlenmeyer flask by providing light environment using white LEDs between under 2000-3000 Lux, temperature at 22±1 ℃ and pH at 9.0±0.2. For every 7 days, the media changed by inoculating the grown culture into new flask of media for maintaining under aseptic conditions.

2.2 Experimental set up

To investigate the growth of culture in different biomass density and light spectra, the experiment was conducted in two phases. In first phase the culture was grown in different biomass density where the young culture was mixed in sterilised media in different ratio (1:9, 3:7, 1:1, v/v). From the best growth of biomass density, a ratio was selected and grown in different LED spectra (red, blue and blue-red). These LED lights were selected due to help of enabling the creation of illumination with a specific wavelength, monochromatic lighting with a particular selected spectral composition. The cultivation was conducted for 7 days for each parameter. The growth and safety of culture was monitored daily.

2.3 Growth measurement

The cleanliness and healthiness of culture monitored daily by absorbing the culture under light microscope. The shape and cell size were examined regularly. The growth of *Spirulina platensis* was estimated with optical density at 620 nm using UV-Vis spectrophotometer. the biomass dry weight was quantified, after centrifuged 50ml of Spirulina platensis culture at 7000 rpm for 5 min. the collected wet biomass was rinsed with distilled water, centrifuged again and dried at 80°C for 6 h. all the measurement was taken in triplicate to minimise the error of data.

The weight of dry biomass calculated using:

Dry biomass (g/L) = (FFW-IFW)/ (Amount of sample taken filtration) ×100

Where,
FFW = Final filter weight (g) and
IFW=initial filter weight

3. Result and Discussion

3.*1 Effect of biomass density on cell growth and biomass yield*

The experiment was conducted for 7 days continuously and monitored the growth and biomass production of *Spirulina platensis*. The growth profile of spirulina corresponding to different biomass density are shown in Figure 1. The initial concentration of culture in all flasks were 1.43×10-4. The growth was gradually increased and reached the maximum growth at each biomass density. However, growth at biomass density ratio 7:3 was the highest growth compared to other culture density. The maximum light was penetrated into the culture due to minimal density of culture. More light was observed by the cells. Photosynthetic microalage may frequently experience irradiance fluctuations of one to two orders of magnitude in the natural environment. Microalgae have created several acclimation mechanisms to deal with such shifts. As result less photosynthesis and cell division process might happen in the culture which can reflect in the optical density value. To achieve balance between the light and dark photosynthetic responses, acclimation procedures are used. Lowest growth found in the biomass density 1:9 ratio. The cells in the flask were received only minimal amounts of lights was observed. High amount of culture cells resists the light penetration which becomes light limitation to the cells. Less energy been absorbed by the cellular pigments. If the biomass density low, it causes photoinhibition due to over exposure of light in the culture. The cells temperature rising when receiving overexposure of lights. As a result, chlorophyll resist light absorption to prevent organelle damage due to over exposure. Meanwhile photolimitation takes place when the biomass density is high. It prevents the light from penetration. As result cells doesn't receive enough light for photosynthesis process. Therefore, optimum biomass density with sufficient media as in good ratio are preferable for cultivation of *Spirulina platensis* with maximum biomass production. The yield of biomass which was harvested in 2 days interval time was shown on Figure 2. The highest biomass yield was obtained at 7:3 ratio and followed by 9:1 with biomass of 0.507 mg/L and 0.422 mg/L. The lowest biomass yield was harvested in 1:9 ratio of biomass density.

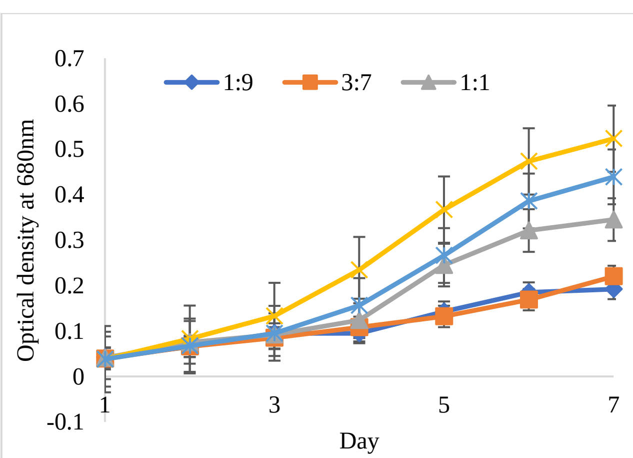

Figure 1 The optical density value of *Spirulina platensis* culture under different biomass density

The photosynthesis of microalgae is influenced by light intensity, which in turn impacts their growth rate. Microalgae need light to create ATP and NADPH and other necessary pigments for growth (Palanisamy et al., 2022). Metsoviti (2020) reported that., increased light intensity accelerates microalgae growth. Up to a certain extent, depending on the microalgae species However, excessive light intensity up to the point of saturation may cause photoinhibition. In this experiment. High density of culture does not support growth due to light blockage. Over density causes decline the of growth and biomass yield. When cell density decreases from high concentration to optimum condition causes increase in the growth of species and biomass yield (Straka and Rittmann, 2018).

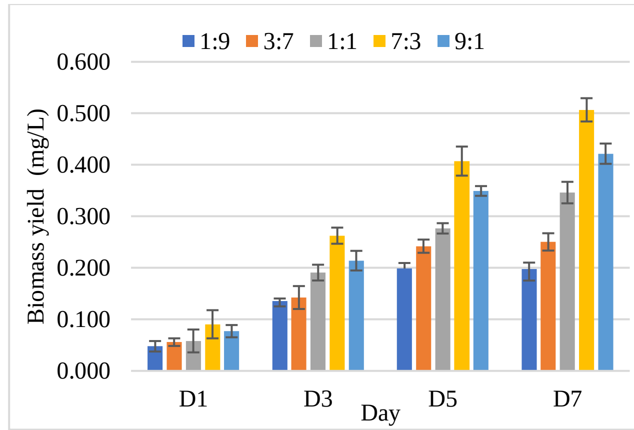

Figure 2 The accumulation of biomass yield from different biomass density

3.2 Effect of light spectra on cell growth and biomass yield

The Figure 3 shown below described the growth of *Spirulina platensis* under different light spectra. The light provided with same irradiation. The optimum cell density was selected from the experiment above to conduct this experiment with different light spectra. From the result can determine the light spectra highly influence the growth. White wavelength highly contributes to growth of *Spirulina platensis*. All the light spectra gradually increase the growth of cells from day two after the lag phase. Among the LED light, White wavelength has highly triggered the growth rapidly and followed by blue-red light. Mono light of red and blue not highly influence the growth as white light but it has minimal influence in the growth of *Spirulina platensis*. There was a opposite result found in a study that light spectrum has an impact on the growth of microalgae cultivated in a biofilm-based system. Red and blue light were more effective at promoting cell growth in *Chlorella* sp. than white and green light.

The optimum colour to encourage *Nannochloropsis oculata* cell development was blue light. The outcomes were consistent with those of a suspended microalgae cultivation system (Yuan et al., 2020). Besides that, Palanisamy et al (2022) reported that, radiation with wavelengths between 400 and 500 nm as blue light. This visible spectrum waveband, which is very energetic and significantly affects plant growth, blue light is just as good as green or red light for promoting photosynthesis. It has a great deal of energy, which quickens plant growth. Blue photons, which are responsible for driving the photosynthetic reaction, are less energy-efficient than green or red photons because their high energy is not fully utilized (Runkle, 2017). Beyond the optical density, growth was measured through biomass yield as shown in Figure 4. The highest biomass yield obtained under white (0.42 mg/L) light spectra and followed by mix of blue-red light (0.38 mg/L).

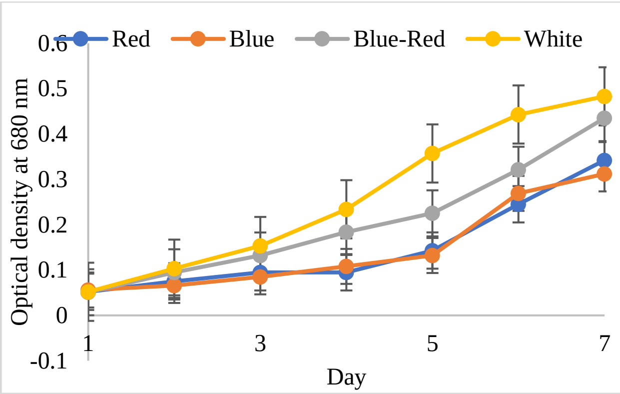

Figure 3 The optical density value of *Spirulina platensis* culture under different light spectra

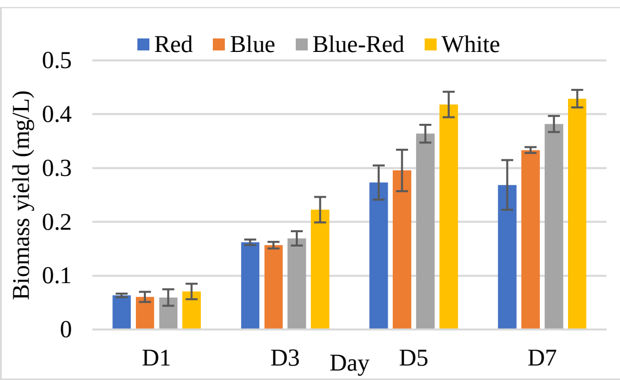

Figure 4 The accumulation of biomass yield from different light spectra.

Light is the most crucial component for photosynthetic activity and the primary source of microalgal autotrophic development. It aids in photosynthesis, respiration, and cell division (Metsoviti et al., 2019). To create ATP and NADPH and other crucial chemicals for growth, microalgae need light. Varied microalgae species have different optimal light levels for growth and biomass production, which also depend on other elements like temperature and the presence of nutrients in the culture medium (Li et al., 2012). Light not only influence the growth, but it also highly contributes to the cells size and composition of cell. Nzayisenga (2020), reported that, increasing the light intensity increase the lipid and fatty acid content. Therefore, suitable light required for microalgae strain to cultivate to obtain desirable product.

4. Conclusion

This investigation determined the influence of biomass density and light spectra on growth of *Spirulina platensis*. biomass density highly contributing to rate photosynthesis by allowing light to penetrate the culture at optimum cell density. Light spectra enhance the growth biomass. However white light contains all the light spectra highly contribute to the biomass production. therefore, biomass production of *Spirulina platensis* can be enhanced by biomass density and light spectra for optimum biomass production.

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