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Effect of Different Light Wavelength on the Growth of Marine Microalgae

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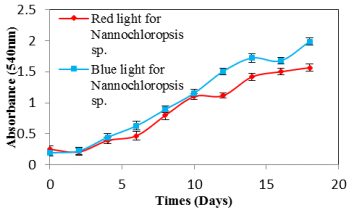
Graphical abstract



Abstract

Tetraselmis sp. and *Nannochloropsis sp.* are common marine microalgae used as substrates for production of biodiesel which is the third generation alternative energy. Recently, wavelength of light is believed to influence the growth of marine microalgae. In this investigation, two different types of light wavelength; red and blue which can influence the production of microalgae were investigated. The marine microalgae growths were observed in terms of biomass which was then measured using the UV-vis spectrophotometer. The results showed that both *Tetraselmis sp.* and *Nannochloropsis sp.* grow better in the blue light compared to the red light indicated by the higher absorbance readings.

Keywords: *Tetraselmis sp.*; *Nannochloropsis sp.*; light wavelength; biomass

Abstrak

Tetraselmis sp. dan Nannochloropsis sp. ialah mikroalga marin biasa digunakan sebagai substrat untuk pengeluaran biodiesel yang merupakan generasi ketiga tenaga alternatif. Baru-baru ini, jenis panjang gelombang cahaya yang dipercayai mempengaruhi pertumbuhan alga marin. Dalam penyiasatan ini, dua jenis panjang gelombang cahaya, merah dan biru yang akan mempengaruhi pengeluaran mikroalga telah disiasat. Pertumbuhan mikroalga marin diperhatikan dari segi biomas yang kemudiannya diukur dengan menggunakan spektrofotometer UV-vis. Hasil kajian menunjukkan bahawa kedua-dua Tetraselmis sp. dan Nannochloropsis sp. berkembang lebih baik dalam cahaya biru berbanding dengan lampu merah yang ditunjukkan melalui bacaan keserapan yang lebih tinggi.

Keywords: Tetraselmis sp.; Nannochloropsis sp.; cahaya gelombang, biomas

1.0 INTRODUCTION

The world has been plagued in the last decade with energy problems, associated with irreversible reduction of traditional material of fossil fuels, unsustainable use of energy, accumulation of greenhouse gases in the atmosphere and global warming problem. With the necessary need to decrease carbon production and the decreasing reserves of crude oil, liquid fuels derived from plant material also called biofuels appear to be an attractive substitute source of energy. Compared with other forms of renewable energy in the world such as wind, tidal and solar, biofuels allow energy to be chemically stored, and can also be used in existing engines and transportation infrastructures after combining with various degrees with petroleum diesel [1].

At present, the most popular available form of biodiesel comes from such oil crops such as sugar cane, oil palm, oilseed rape and soybean. However, there are concerns about sustainability of this mode of production: to produce 2,500 billion liters of biodiesel from oilseed rape. In order to meet the current demand of petroleum diesel in the whole United Kingdom, 17.5 Mha would be required for the plantation of such crops which is more than half the land area of United Kingdom. Furthermore, the production of biodiesel process is energy saving and less greenhouse gas emissions effect. Example material of biodiesel production such as oilseed rape or soya [2]

Microalgae are photosynthetic microorganisms that convert sunlight, water and carbon dioxide to algal biomass. Many microalgae are exceedingly rich in oil [3], which can be converted to biodiesel using existing technology. Recently, significant research efforts have focused on the production of biofuel from microalgae. Compared with other available feedstocks, microalgae possess several advantages for biodiesel production including: fast growth, high photosynthetic efficiency, high oil content, and the fact that microalgae can obtain nutrients from water unsuitable for human consumption. In addition, microalgae require a smaller land area compared to other feedstocks [4, 5].

Microalgae are discovered to have higher photosynthetic efficiencies than most photoautotrophic living things and have ten to fifty times higher carbon elimination efficiencies than other terrestrial floras [6]. Light is an essential parameter in photosynthesis and is important for microalgae photoautotrophic growth. When there is insufficient light supply, the growth of microalgae is under photo limitation condition. It can be concluded that the light intensity increase will improve the microalgae growth until a saturation stage. The growth of microalgae becomes negative when the light intensity is more than optimum or saturation condition of light intensity. The microalgae growth is then said to be undergoing photo inhibition condition [7, 8]. Biomass cultivation of microalgae is in general carried out under regular light intensity. However, at the beginning of the lag stage, the light intensity used may be so high when the number of microalgae cells are low and can be considered under a state of photo inhibition. On the other hand, the growth of microalgae to a moderately dense broth can cause strict light attenuation due to mutual shading. In this condition, the state of photo limitation could be considered for the microalgae cells in the zone which is far away from the light source. A lumostatic process is thus developed to improve the microalgae growth by optimizing the light energy supply. In a lumostatic procedure, light is supplied in a continuous increasing method in order to avoid photo inhibition and photo limitation [9-19] Chlorophyll is an important constituent of the chloroplast that is responsible for photosynthesis. In the different forms of chlorophyll, chlorophyll 'a' is the main pigment for the conversion of light energy to chemical energy. The presence of chlorophyll 'a' is found to be helpful for the photosynthesis reaction in microalgae [20].

A majority of the microalgae cultivation are using open ponds which use solar energy [21]. In outdoor cultivation of marine microalgae, light source from sunlight is the main source, while the artificial light source such optical fiber or LED are suitable for indoor cultivation of marine microalgae. Some studies reported [21] that conventional artificial light source produce higher biomass due to large illumination area, high stability of light source and low construction cost. In LED the light source consumes lower energy and has longer life-expectancy, lower heat generation, low construction cost and ability to tolerate higher frequency of no-off switching [21].

In order to cultivate marine microalgae commercially, a durable, reliable, low cost and high efficiency light source is required. If the light source has a narrow spectral output that overlaps the absorption spectrum of photosynthesis, the emission of light in the unusable frequencies would be eliminated, directly advancing the whole energy conversion. Nowadays, among the variety of light sources, light-emitting diodes (LEDs) are the only ones that can achieve this criteria. LEDs are light and small enough to fit into almost any type of photobioreactor and their advantages such as lower heat generation, longer life-expectancy, higher conversion efficiency and others [21] has made them commercially viable.

2.0 EXPERIMENTAL

2.1 Microalgal Cultures

The marine microalgae strain was bought originally from BioMac Ltd now known as Algae Tech (Ltd). *Tetraselmis sp.* and *Nannochloropsis sp.* was maintained in Walne's medium agar which contains 15% agar (1 L Walne's medium was mixed with 15 g agar powder).

2.2 Inoculation of Microalgae to Culture Medium

The marine microalgae strains *Tetraselmis sp.* and *Nannochloropsis sp.* which were maintained in dormant condition (in agar plate) were inoculated into Walne medium which is shown in Table 1:

Table 1 Walne medium formulation

Walne medium (per litre)
Nitrogen Sources:
100.0 g $NaNO_3$
20.0 g $NaH_2PO_4.2H_2O$
1.3 g $FeCl_3.6H_2O$
33.6 g H_3BO_3
45.0 g $Na_2EDTA.2H_2O$
Trace elements:
2.1g $ZnCl_2$
0.36g $MnCl_2.4\ H_2O$
0.9g $(NH_4)_6MO_7O_{24}4H_2O$
2.0 g $CoCl_2.\ 6H_2O$
2.0 g $CuSO_4.5H_2O$
Vitamin Solution (per 100 ml):
0.01 g Thiamine
0.01 g Vitamin B_{12}
0.0002 g Biotin

The *Tetraselmis sp.* and *Nannochloropsis sp.* were inoculated in different 150 ml shake flasks which contain 10 ml microalgae and 40 ml medium. The cultivation was performed at 21°C ±0.5°C, pH 7.8±0.2 and under a light intensity of 100 µmol $m^{-2}s^{-1}$ with a 24:0 light-dark cycle for 16 days so as to give enough time to activate the *Tetraselmis sp.* and *Nannochloropsis sp.* which were inoculated from agar plate. The cultures were then placed onto orbital shakers to avoid sedimentation.

2.3 Cultivation of Marine Microalgae Under Wavelength of Light

After 16 days, *Tetraselmis sp.* and *Nannochloropsis sp.* were cultivated in Walne medium with 10% starting inoculum taken from stock culture. The marine microalgae, *Tetraselmis sp.* was cultivated at 21°C ±0.5°C and pH 7.8±0.2 under red and blue L.E.D illumination (LH-R30T50X-DF, Sun Led Company, China) of 100 µmol $m^{-2}s^{-1}$ with a 24:0 light-dark cycle with aeration condition as the control growth environment. The whole set up was placed in an enclosed box. The marine microalgae growths were measured using the optical density (OD) method. Samples were taken every two days for 18 days where OD readings were taken using a UV-vis spectrophotometer at 540 nm. Each experiment was performed in duplicates so as to ensure reproducibility of results.

2.4 Observation of Marine Microalgae Under Microscope

The cultures were sampled at every 24 hr interval and microalgal growth was observed by a light microscope (Olympus CX21, Japan).

3.0 RESULTS AND DISCUSSION

3.1 Effect of the Growth Rate in Different Wavelength of Light

Figure 1 shows *Tetraselmis sp.* grows better under blue light compared to red light. This can be seen from the higher growth rate observed for *Tetraselmis sp.* under Walne medium. Figure 1 clearly shows that the blue illumination condition results in higher biomass starting from the 2nd day when compared with red light condition. The biomass from all the species steadily increased starting from day 1 until around day 16. After day 16 the growth tends to slow down and remain stationary with time due to nutrient depletion and wastes start to accumulate in the culture medium.

Similar results are shown in Figure 2, *Nannochloropsis sp.* also grows better under blue light condition compared to red light condition. This could be seen from the higher growth rate observed for *Nannochloropsis sp.* grown under blue illumination condition. Figure 2 clearly shows that blue light condition has higher biomass starting from day 4 when compared with red light condition. The amount of biomass increased steadily from day 1 until around day 16. Similarly after day 16 biomass growth slowed down and remained almost stationary with time in blue light condition.

The results show that there is a significant difference between the growth of *Tetraselmis sp.* and *Nannochloropsis sp.* culture under different wavelength of light (Figure 1 and 2). This indicated that change in the wavelength of light influenced the growth rate of *Tetraselmis sp.* and *Nannochloropsis sp.* during 18 days of cultivation due to chlorophyll 'a' which is at a maximum at two points (430 and 662 nm) which influenced the grow of biomass. The blue light wavelength was around 420-450 nm and the red light wavelength was around 660-700 nm. Thus the blue light wavelength tends to be narrower and thus closer to the chlorophyll 'a' wavelength requirement. The author suggested that blue light condition was a better medium than red light in cultivation of *Tetraselmis sp.* and *Nannochloropsis sp.* Ruyters [22] stated that blue light enhanced in the regulation of activation of enzymes and gene transcription. When cell was damaged by red light, the blue light allows the cell to repair by exposure to low intensity of blue light. The growth rate and biomass production of marine microalgae were related to the type of light wavelength [23]. In addition, Madiha [24] reported the blue LED allows the light intensity to penetration deep into the batch culture thus enhanced the doubling of microalgae and cell density increased faster.

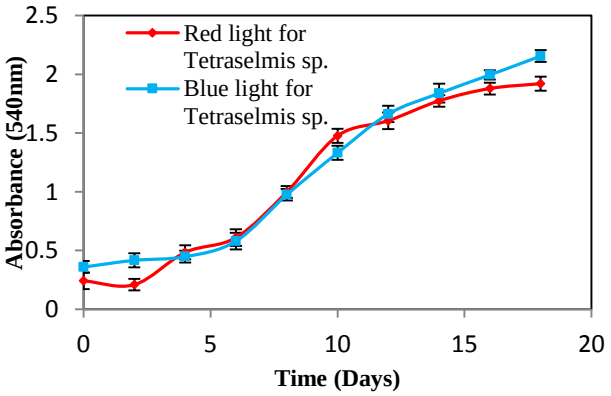


Figure 1 *Tetraselmis sp.* growth curve in different wavelength of light

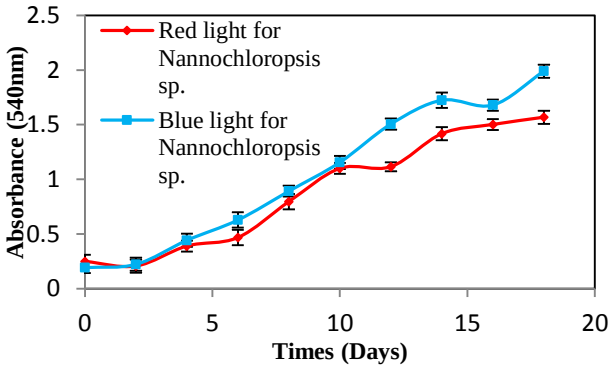


Figure 2 *Nannochloropsis sp.* growth curve in different wavelength of light

3.2 Observation of Marine Microalgae Under Microscope

Tetraselmis sp. is a green marine microalga [25]. This type of microalgae is one of the motile phytoplankton which has 4 flagella. *Tetraselmis sp*. is very important as the main supplier of energy and organic substances in aquatic ecosystems, an important food and fatty acids for sea animals. *Tetraselmis sp*. contains high concentration of natural lipid and also produces amino acids that arouse feeding of other aquaculture [25]. Figure 3 shows that *Tetraselmis sp*. can grow aggressively under both blue and red light separately but results from Figure 1 show that *Tetraselmis sp*. prefer the blue light.

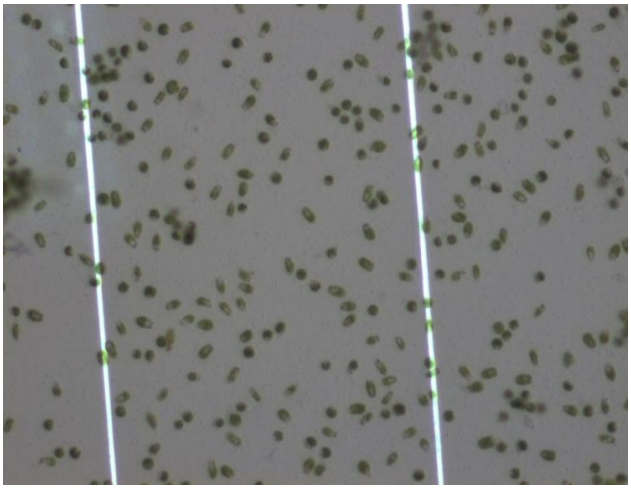


Figure 3 *Tetraselmis sp*. under magnification of 100x

Nannochloropsis sp. (Monodopsidaceae and Eustigmatophyceae) live in waters with high level of nutrients for example estuaries and coastal waters which have very important supply of 5-3 LC-PUFA eicosapentaenoic acid (EPA; 20:5 5-3) and famously used in marine organisms nutrition [26]. *Nannochloropsis sp.* is also a perfect choice for biomass cultivation for biodiesel production [27] because it produces high levels of triacylglcerol under nutrient starvation. Figure 4 shows that *Nannochloropsis sp.* can grow actively under both the blue light and red light. However results in Figure 2 exhibits better growth rate under blue light.

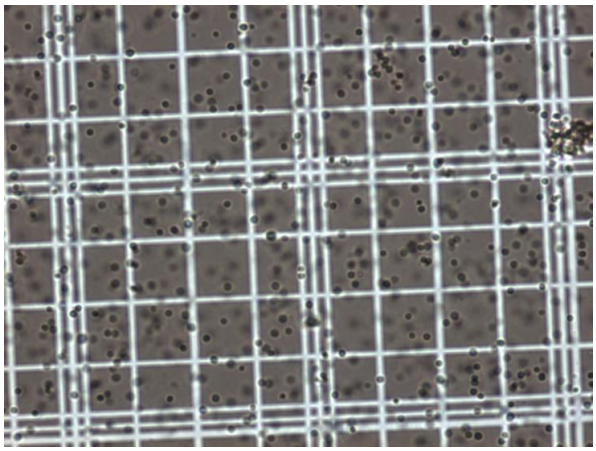


Figure 4 *Nannochloropsis sp.* under magnification of 100x

■4.0 CONCLUSION

Tetraselmis sp. and *Nannochloropsis sp.* were successfully cultivated in Walne's medium within 18 days. According to the initial results, the blue light seems to be more suitable than the red light for the marine microalgae.

Acknowledgement

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