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Effect of Light Quality on the Growth of Microalgae *Haematococcus lacustris*

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Authors' contributions

This work was carried out in collaboration between all authors. Author MTD designed and implemented the research, performed the statistical analysis. Author LVKT wrote the first draft of the manuscript, managed the analyses of the study. Authors LVKT and MTD wrote the manuscript with input from all authors. Authors LVKT, NTT, TVTN and NTS performed the experiments, contributed to the interpretation of the results. All authors read and approved the final manuscript.

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ABSTRACT

Haematococcus lacustris (formerly *Haematococcus pluvialis*) has been widely known as a promising natural source of astaxanthin. To produce a higher amount of astaxanthin content in *H. lacustris*, two major aspects are considered including increasing biomass production of green cells or enhancing astaxanthin accumulation in red cysts. The aim of this study was to investigate the effect of light quality of Light emitting diodes (LEDs) on the growth of *H. lacustris.* In the present research, the effect of different light spectrum, light intensity on the growth of *H. lacustris* was simultaneously investigated for 11 days of cultivation period. The experiments were performed in Bold's Basal Medium, illuminated with a circadian light/dark cycle of 16/8 and maintained at 25ºC. A mixture of red and blue at the light intensity of 30 µmol photons m^{-2} s^{-1} was found to be the most effective light quality for growing. The highest cell density obtained with mixed red-blue illumination at the light intensity of 30 µmol photons m^{-2} s^{-1} after 9 days of cultivation was 49.57 (10^5 cells/ml) corresponding to the specific growth rate of 0.509 d^{-1}.

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1. INTRODUCTION

Astaxanthin (3, 3'-dihydroxyl-β, β'-carotene-4, 4'-dione), belongs to xanthophyll class of carotenoids, is a powerful biological antioxidant that occurs naturally in a range of organisms, such as aquatic animals (salmon, trout, krill, shrimp and crayfish), some birds (flamingoes, quails) and various microorganisms [1]. Astaxanthin can act as a stronger antioxidant activity than vitamin C, β-carotene, canthaxantin, lutein or α-tocopherol and has potential biological functions including protection against UV- light effects, anti-cancer, preventing or reducing the risk of many diseases, pigmentation, reproductive behavior and improved reproduction, therefore, it has considerable promising application in food, feed, cosmetic, aquaculture, nutraceutical and pharmaceutical industry [2,3]. However, humans and animals lack of the ability to synthesise astaxanthin and must be provided through food products. Although the synthetic products dominate the astaxanthin commercial market today, it has not been approved for human consumption to date [4]. Therefore, the demand of the natural astaxanthin in human health applications has generated much interest nowadays. One of the best sources of natural astaxanthin is *Haematococcus lacustris* which can accumulate higher astaxanthin content comparing to yeast or bacteria [5]. Natural astaxanthin from *H. lacustris* has been considered as a preferred choice for high – end markets [6] and already approved as a color additive in salmon feeds with as a dietary – supplement ingredient for human consumption [7]. It is available in the market as a dietary supplement in dosages from 3.8 to 7.5 mg per day due to potential benefits [8].

The green microalgae *H. lacustris* is also known as *Haematococcus pluvialis or Sphaerella lacustris* [3]. It belongs to the class Chlorophyceae, order Volvocales and family Haematococcasea. There are both motile and non-motile forms in its growth stages. The former stage is also called "green vegetative phase" consisting of three types of cellular morphologies: zoospores, micro-zooids, palmella. Hematocysts (aplanospores) are referred as "red non-motile encysted phase" [9,3]. Astaxanthin is accumulated during the process of transformation of the green motile vegetative cells to the aplanospore stage, so, this microalga strain requires different culture conditions for vegetative growth and astaxanthin production. The factors inducing the astaxanthin synthesis have been extensively studied such as light, temperature, salt concentration, nutritional stress [10,11,12,13]. However, *H. pluvialis* has slow growth rate and the vegetative cells being easy to transform to cyst cells in the adverse environmental conditions, therefore, the achievement of high yield of *H. lacustris* vegetative cells are one of the main problems to be solved. Several attempts have been carried out to establish the nutritional requirements of *H. lacustris* and optimise media formulation [14]; only a few culture conditions were considered. One of the most relevant environmental factors affecting the growth of this microalga is light exposure. Light quality influences the growth and biochemistry of microalgae, so artificial lighting can be used to manipulate the final biomass for special applications [15].

Light emitting diodes (LEDs) have recently been recognised as effective light sources and low-cost energy sources for microalgae cultivation. Two properties of light conditions being important for improving biomass production of *H. lacustris* are light intensity and spectral quality [15]. Therefore, it is important to develop the emphasis on the effect of LEDs. In this research, *H. lacustris* growth was observed as a potential source for next step astaxanthin production. Herein, the growth of *H. lacustris* was examined for various light spectra with different light intensity in order to find the most effective light quality of LEDs for growing this strain.

2. MATERIALS AND METHODS

2.1 Microalgae Culture

The microalga *Haematococcus lacustris* (formerly *Haematococcus pluvialis*) was provided from Faculty of Biotechnology – Vietnam National University of Agriculture. The stock culture was maintained photoautotrophically on agar at 25ºC under light/dark cycle of 16/8 illumination with the light intensity of 30 µmol photons m^{-2} s^{-1} and sub-cultured every month. The medium for maintenance of the microalga was Bold's Basal medium (BBM medium) [16]. For activating the microalgae, the cells were inoculated into a 250 ml Erlenmeyer flask containing 50 ml BBM medium (sterilised by autoclaving at 121ºC, 1 atm for 20 minutes) at

25ºC, illuminated with a circadian light/dark cycle of 16/8 (the light intensity of 30 µmol photons m^{-2} s^{-1}) and supplied sterilised air.

2.2 Experimental Designs

The effect of different light conditions on the growth of *H. lacustris* was simultaneously investigated for 11 days of cultivation period. All experiments were performed under the same culture media, temperature and the photoperiods. *Haematococcus lacustris* was cultivated in 50 ml BBM medium contained 10% volume/volume (v/v) inoculum (4.10^4 cells/ml), illuminated with a circadian light/dark cycle of 16/8 and supplied sterilised air. Light emitting diodes (LEDs) were used as a light source for the seed culture and the cultivation was maintained at 25ºC.

Table 1. Setting up different light conditions

Light spectrum	Light intensity (µmol photons m^{-2} s^{-1})		
White plasma	30	45	60
Blue LED	30	45	60
Red LED	30	45	60
Blue-red LED	30	45	60

2.3 Analytical Methods

The growth of *H. lacustris s* was determined through cells/ml density using improved Neubauer Hemocytometer: $D = A \times 10^4/(X \times Y)$, whereas D is cells/ml density, A is the total number of cells in all blocks, X is the dilution factor and Y is the number of blocks in the large central square.

The specific growth rate (µ): $\mu = \ln(X_2/X_a)/(t_2 - t_1)$, where X_2 and X_1 are the cell density (cells/ml) at t_2 to t_1 (sample day) respectively at the exponential phase.

2.4 Statistical Analysis

All experiments were performed in triplicate. The obtained results were shown as the average and standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyse data (IBM SPSS 16.0 software) and post – hoc Turkey's honestly significantly difference test. The significant test was set at $p \leq .05$.

3. RESULTS AND DISCUSSION

3.1 Effect of Light Intensity on the Growth of *H. lacustris*

The effect of light intensity on the growth of *H. pluvialis* is displayed in Table 2. Overall, there were significant differences on the growth of cells at different light intensities. Maximum cells/ml density was observed on the light intensity of 30 µmol photons m^{-2} s^{-1} under most of different light spectrum. As shown in Figs. 1; 2; 3; 4, the cells/ml density reached the maximum value after 9 days with the light intensity of 30 µmol photons m^{-2} s^{-1} including 31.40, 37.93 and 49.57 (10^5 cells/ml) at blue, red and blue – red LED light, respectively. At white plasma, the light intensity of 30 µmol photons m^{-2} $s^{-1}$and 45 µmol photons m^{-2} s^{-1}also resulted in the highest cells/ml density of above 37.10^5 cells/ml (Fig. 1).

Light energy plays a vital role in the life cycle of *H. lacustris* for converting simple organic nutrients into macromolecules such as carbohydrate, protein and lipid. Microalgae contain chlorophyll which is essential for photosynthesis to generate energy from light. Under optimal growth conditions, light is absorbed by pigments in chloroplast and then converted to chemical energy forming ATP and NADPH through a photosynthesis electron chain. Finally, chemical energy is stored in starch by fixing CO_2 through the Calvin cycle [17].

In this study, the highest specific growth rates obtained at the light intensity of 30 µmol photons m^{-2} s^{-1} under all light spectra, increasing the light intensity to 60 µmol photons m^{-2} s^{-1} led to a poor cell growth (Table 2). This finding is agreement in Esra et al. [18] reported that decrease in cell concentration of *H. lacustris* was observed with increasing light intensity from 40 to 60 µmol photons m^{-2} s^{-1} under the white fluorescent lamps (18W). This phenomenon could be explained that the light intensity of 30 µmol photons m^{-2} s^{-1} supplied effective energy for the growth of *H. lacustris*. Insufficient light intensity resulted in slow growth because the microalgae consumed carbohydrates and oxygen during photorespiration, though they were unlike to cause fatal damage [19]. On the other hand, excessive light intensity could lead photoinhibition and damaged microalgae due to overloaded photosystems [9,19].

Table 2. Effect of light conditions on the growth of *H. lacustris*

Light spectrum	Specific growth rate day^{-1}		
	30 µmol photons m^{-2} s^{-1}	45 µmol photons m^{-2} s^{-1}	60 µmol photons m^{-2} s^{-1}
WHITE	0.480 ± 0.091^{a}	0.478 ± 0.015^{a}	0.468 ± 0.033^{a}
BLUE	0.458 ± 0.021^{a}	0.397 ± 0.028^{b}	0.375 ± 0.014^{b}
RED	0.480 ± 0.019^{a}	0.471 ± 0.028^{a}	0.465 ± 0.015^{a}
BLUE -RED	0.509 ± 0.027^{a}	0.464 ± 0.050^{ab}	0.376 ± 0.068^{b}

**Means ± SD = Means ± Standard Deviation of three independent experiments. The means difference is significant at the p ≤ .05. Means followed by the different letters are significantly different on the same row.*

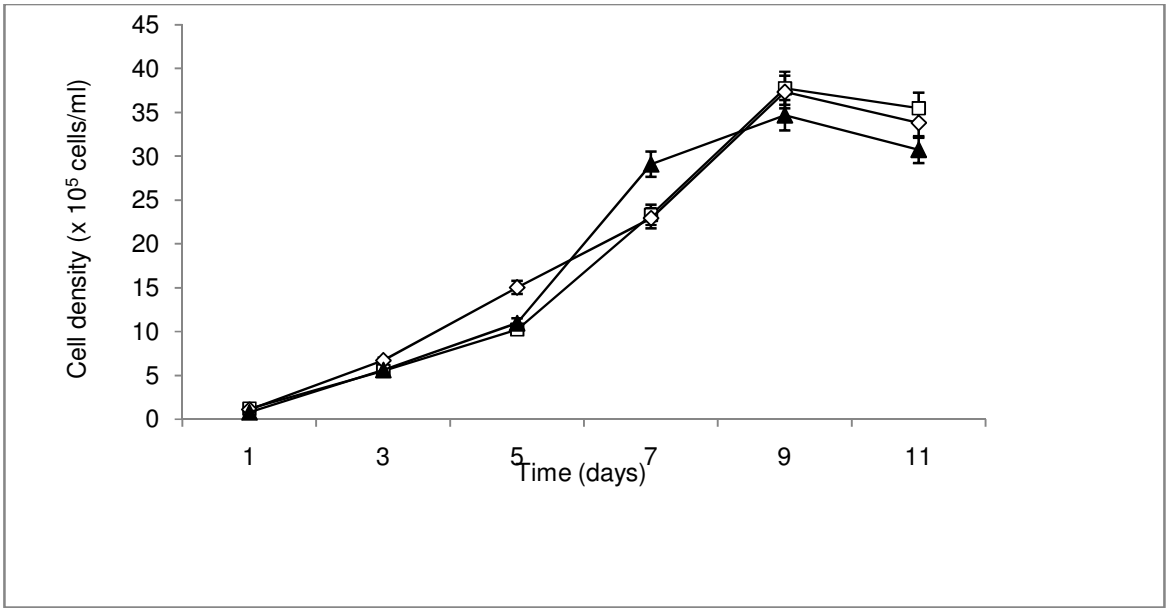

Fig. 1. Effect of light intensity on the growth of *H. lacustris* at white plasma light. Symbols for different light intesity: (□) 30 µmol.m^{-2}.s^{-1}, (◇) 45 µmol.m^{-2}.s^{-1}, (▲) 60 µmol.m^{-2}.s^{-1}. The error bars in the figure indicated the standard derivations.

3.2 Effect of Light Spectrum on the Growth of *H. lacustris*

The importance of selecting an optimised light condition for the cell growth depends on some factors such as photoperiods, intensity and light sources. This research carried out varying the light spectrum from white plasma light, blue LED light, red LED light and the combination of blue-red LED light. The growth of *H. lacustris* was significantly varied in different light spectrum under the same light intensity of 30 µmol photons m^{-2} s^{-1} (Figure 5). The highest specific growth rate of 0.509 d^{-1} was observed at blue-red LED light and the lowest at the blue LED light (0.458 d^{-1}).

To maximise photosynthesis efficiency, all photons released from a light spectrum should be captured by the photosynthetic apparatus of microalgae. The number of photons at blue or red wavelength that can be absorbed by the molecular chlorophyll in algae depends on the pigment composition. The research of Keeling (2013) reported that microalgae can grow under either blue (λ = 420 – 470 nm) or red (λ = 660 – 680 nm) light [20]. White plasma is a visible light consisting of a broad spectrum from 380 (violet) – 750 (red) nm, therefore it covers both the absorption peaks at 430 nm and 662 nm of chlorophyll α. On the other hand, red LED light which operated at a relatively low light intensity was more suitable for cell growth than blue LED light [21]. Red light provides a high level of activation in the chlorophyll electrons producing water hydrolysis leading to the synthesis of ATP which is used for synthesis of carbohydrates that advance the growth of *H. lacustris* [22,23,24]. Therefore, white plasma light and red LED light were more effective for cell growth comparing to blue LED light (Fig. 5).

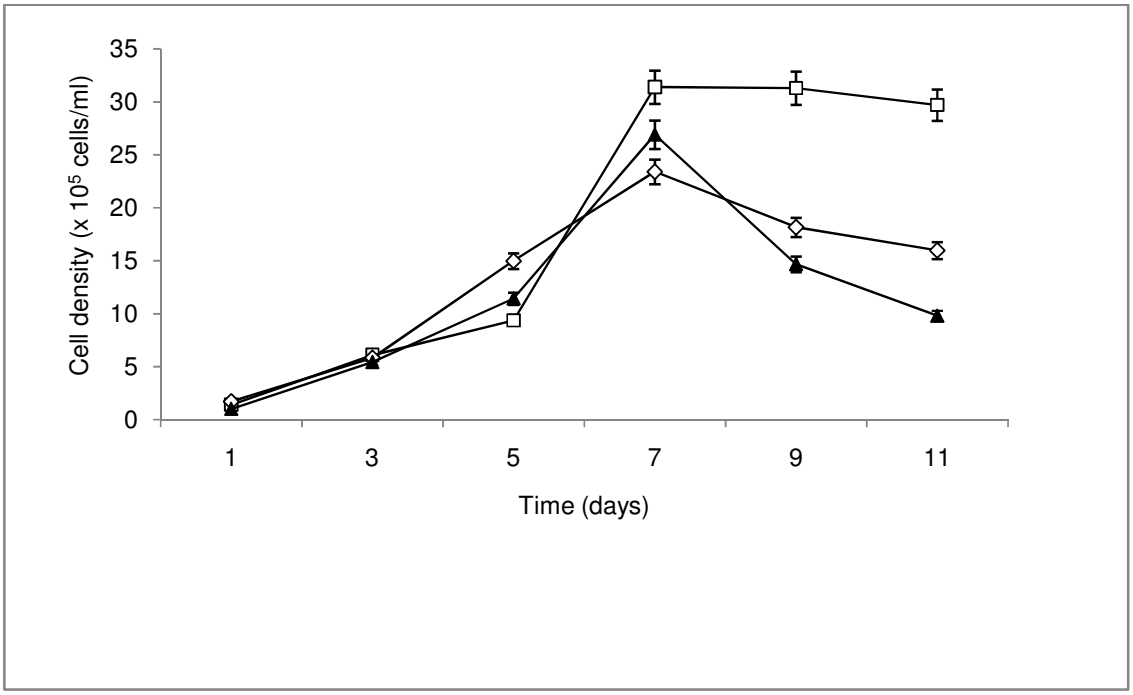

Fig. 2. Effect of light intensity on the growth of *H. lacustris* at blue LED light. Symbols for different light intesity: (□) 30 µmol.m^{-2}.s^{-1}, (◊) 45 µmol.m^{-2}.s^{-1}, (▲) 60 µmol.m^{-2}.s^{-1}. The error bars in the figure indicated the standard derivations.

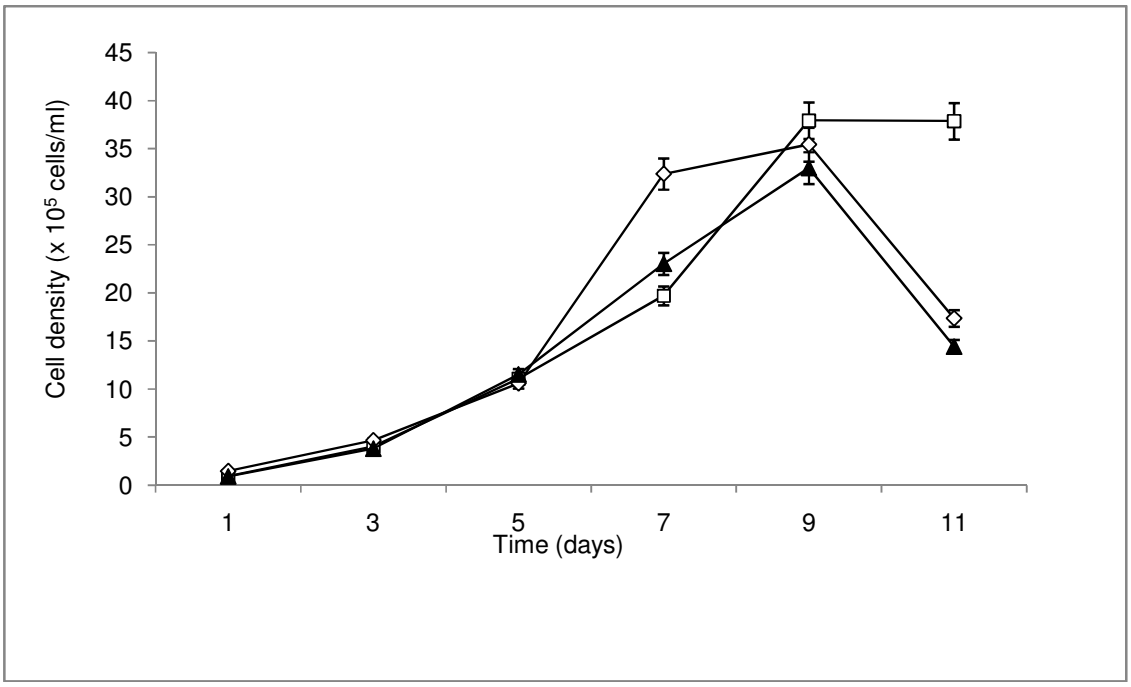

Fig. 3. Effect of light intensity on the growth of *H. lacustris* at red LED light. Symbols for different light intesity: (□) 30 µmol.m^{-2}.s^{-1}, (◊) 45 µmol.m^{-2}.s^{-1}, (▲) 60 µmol.m^{-2}.s^{-1}. The error bars in the figure indicated the standard derivations.

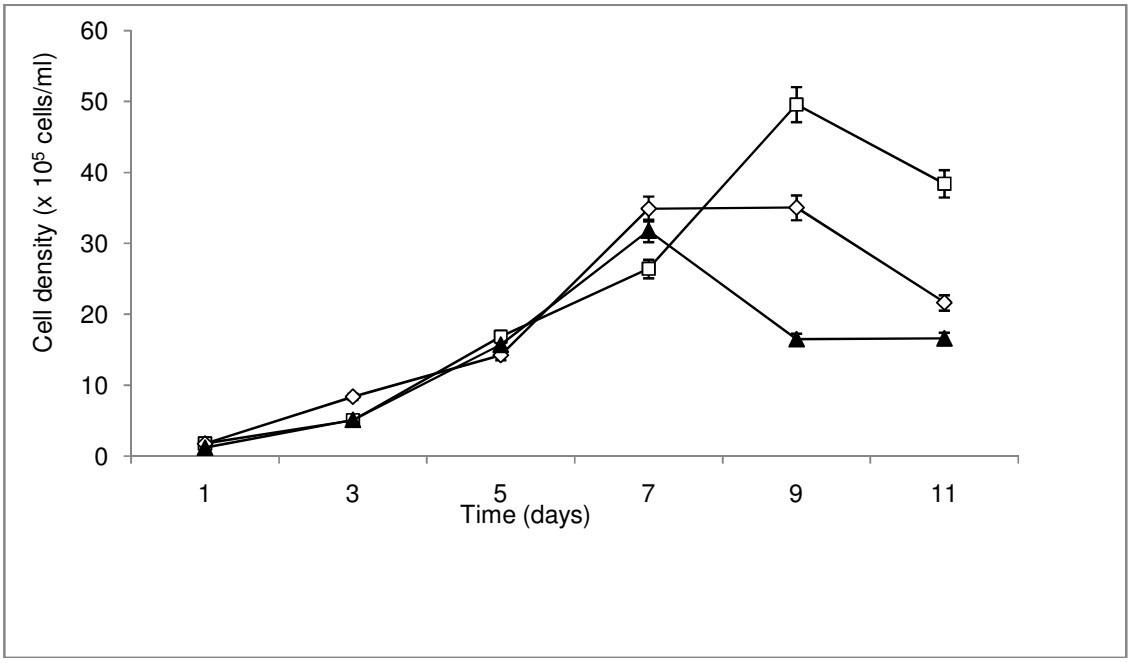

Fig. 4. Effect of light intensity on the growth of *H. lacustris* at blue-red LED light. Symbols for different light intesity: (□) 30 µmol.m^{-2}.s^{-1}, (◊) 45 µmol.m^{-2}.s^{-1}, (▲) 60 µmol.m^{-2}.s^{-1}. The error bars in the figure indicated the standard derivations.

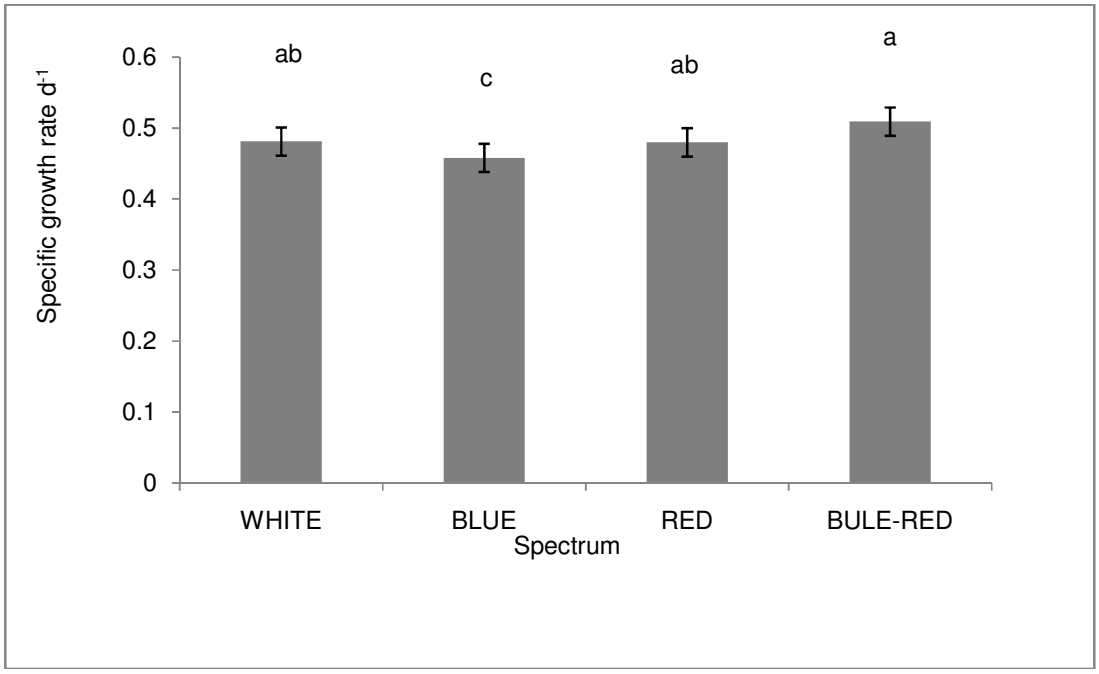

Fig. 5. Effect of different light spectrum on the growth of *H. lacustris* at light intensity of 30 µmol photons m^{-2} s^{-1}. The error bar in the figure indicated the standard derivations.

The combination of red and blue LED light at the light intensity of 30 µmol photons m^{-2} s^{-1} was found to be the most effective for the growth of *H. lacustris.* This result suggests that blue-red

light provided the adequate photosynthesis of this microalga; thereby, the growth rate of *H. pluvialis* under illumination of the mixed blue-red LEDs was higher comparing to monochromatic light.

In addition, blue light plays a vital role for inducing astaxanthin accumulation in *H. pluvialis* [21]. Tran et al. reported that the highest biomass and astaxanthin production was observed at a ratio 1:3 ratio of blue-red LEDs light [25]. It was confirmed that mixed red – blue LEDs light is not only favourable and effective for biomass, but also for astaxanthin production. Therefore, the further research will investigate the optimal ratio of mixed red-blue light for biomass and astaxanthin production from *H. lacustris.*

4. CONCLUSION

Light emitting diodes (LEDs) have been proved to be effective light sources for microalgae cultivation. In the present study, the growth of *H. lacustris* was evaluated for various light spectra at different light intensities. A mixture of red and blue at the light intensity of 30 µmol photons m^{-2} s^{-1} was found to be the most effective light quality for growing. The highest cells/ml density obtained with mixed red-blue illumination at the light intensity of 30 µmol photons m^{-2} s^{-1} after 9 days of cultivation was 49.57 (10^5 cells/ml) corresponding to the specific growth rate of 0.509 d^{-1}. The further research will investigate the optimal ratio of mixed red-blue light for biomass and astaxanthin production from *H. lacustris*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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