



Influence of Type of Culture Media and Light Colors on Biomass and Harvesting Time of Green Algae (*Chlorococcum humicola*)

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Abstract

The current study aimed to determine the effect of exposure to different colors of light and culture media with different concentrations of nutrients on growth curves, growth rates, doubling times of *Chlorococcum humicola* and different harvest times. The studied algae were grown in two different media, Chu13 and BG11 for comparison purpose with the effect of three colors of white, blue, and red lights. White light was adopted as a control factor. The harvest was carried out after the days (7, 14, 21, 28, 35) days of development to determine the highest growth rate in the shortest time during five weeks and therefore to determine the best harvest time in producing the highest growth rates and growth curves and the lowest doubling time during the experiment period of 35 days by the effect of different treatments and at a temperature of $(2\pm 25)^{\circ}\text{C}$ and a light intensity of 3000 lux and a light system of 8/16 hours of light / darkness by conducting daily measurements by reading the absorption values and calculating the number of cells for each culture medium in each light color. The results of the study showed that the highest growth rate and the least doubling time depending on the values of absorption and the number of cells was recorded in the medium BG11 when exposed to blue light at harvest on the seventh day of the experiment. The results recorded the highest growth rate based on absorbance values and cell numbers on the seventh day of the experiment in BG11 medium with the effect of blue light.

Keywords: Microalgae, Green algae, *Chlorococcum humicola*, BG11 media, Chu13 media, Growth rate, Harvesting time, Light colors.

1. Introduction

Microalgae have been studied for more than a century, yet modern biotechnological applications have fostered new research on their use as renewable energy sources as well as in the production of many important industrial and food products (1). Several studies have confirmed the impact of environmental factors on increasing algae production, abundance, distribution and diversity (2). Microalgae have a simple cellular structure that enables them to grow in the most difficult conditions (3). Microalgae use carbon from the air or organic carbon to produce energy (4).

Microalgae convert light energy during photosynthesis into chemical energy by fixing CO_2 (5). Algae characterized by containing many bioactive compounds (6). Microalgae produce



proteins, fats, carbohydrates, pigments, vitamins and minerals (7, 8). When algae cultivation is used in processed culture media, harvest time is a key point for the chemical content of the studied algae because of the algae harvesting refers to the separation of algae from their growing medium (9).

Through the daily measurement of the number of cells and the values of absorption, it is possible to deduce the growth rates, growth curves and doubling times of algae, so we see researchers in the field of algae developing and culture always seeking to increase cell numbers, biomass and productivity using different culture media containing the largest amount of major essential nutrients such as nitrates, silicates and phosphates, and nutrient deficiencies lead to different responses within the cell depending on the species of algae and the culture medium (10-13).

It is important in the field of algae development and culture to obtain a good and rich composition of its components for the culture medium and to be suitable for the nature of the studied algae and low economic cost and give wide growth curves because of the successful culture medium is the medium that shows the largest growth rate and gives the highest biomass and achieves a good biochemical and physiological condition for the studied algae that has been grown in an ecosystem that contains limited amounts of necessary elements (14).

The culture media BG11 and Chu13 were chosen in this study because they contain important and necessary elements in the growth of algae such as ferric of all types, which affects the process of photosynthesis and plays an important role in the chemical content of chlorophyll within the cell (15).

Magnesium (Mg) plays an important role in increasing cell numbers with increasing algal cell size (16) because it is one of the main components of photosynthetic pigments and influences the activity of the photosynthetic enzymes, deficiency leads to a decrease in the amount of chlorophyll followed by a decrease in the growth rates (17-19), as well as affecting cell division and enzyme activity (20). The term Growth curve is a graph of the increase in biomass over time; it is characterized by five phases or stages that the cell passes through in pure algae cultures, which are: Lag, Exponential, Stationary, Declining relative growth and Death phases respectively (21). One of the most important ways to test the environmental success of strains or genera isolated in vitro or in vivo cultures is the growth rate (22, 23).

The growth rates differ significantly between the different genera of algae as they depend on the quality and quantity of macro and micronutrients and their effect on the speed of algal cell division, where they increase and decrease according to the type of the culture medium (24, 25). For example, a study of *Pediastrum boryanum* saw the highest growth rate in low concentrations of Ca, Mg, and SiO₂ while diatoms recorded the highest growth rate in high concentrations of Ca, Mg, and SiO₂ (26). In another study on 8 types of algae under the influence of different concentrations of N and P, the results recorded the highest growth rate and highest biomass in high concentrations of N and P (27). The growth curve is an important factor for determining the appropriate time to harvest a farm, so growth curves must be measured at the beginning of any algae cultured (28, 29). In the present study, two different nutrient concentration media were used on the same genus and this explains why growth rates increase and decrease (30-33). In addition to the effect of different colors of light, light and its intensity is one of the main factors that directly affect the stimulation of microalgae and is necessary for photosynthesis (34-37). The doubling time can be expressed as the time required obtaining twice the cellular number or twice the absorbance values that represent the criteria for measuring productivity (38).

The current study aimed to record the highest growth rate and the poorest doubling time of green alga *Chlorococcum humicola* after growing in two culture media chu13 and bg11, by effect of three light colors white, blue and red during 35 days.

2. Materials and Methods

The experiment was conducted on *Chlorococcum humicola* alga, where the modified culture media used in the present study were prepared as Chu13 modified by (39) and BG11 modified by (40) in the form of stock solutions. Distilled water was used to prepare the media and sterilization by ultraviolet (UV) rays for 15 minutes, and then the solutions were left to cool at room temperature and then kept in the refrigerator at 4°C until use. A sterile agricultural room was prepared to save algae samples, and three sterile incubators were equipped, and each incubator was equipped with a different light color with temperature control, and the necessary devices, equipment and materials were provided to carry out the experiment. The isolation of each incubator grew in two mediums and by three repeaters with daily counting of cells and measuring the absorption values at the wavelength of 650 nm using an optical spectrometer to draw the growth curves of the studied algae under standard conditions (25±2)°C and a light system of 8/16 hours of light/ darkness and a light intensity of 3000 lux while maintaining daily shaking and once a day throughout the duration of the experiment.

Table 1. Chu-13 medium components by Yamaguchi *et al.* (39).

No		Nutrient solution ml	Stock solution g/L
1	KNO ₃	10	40
2	K ₂ PO ₄	10	8
3	CaCl ₂	10	10.7
4	MgSO ₄ .7H ₂ O	10	20
5	Citric acid	10	10
6	Ferric citrate	10	2
7	Micro element	1	
	a. H ₃ BO ₃		5.720
	b. CoCl ₂		0.02
	c. ZnSO ₄ .7H ₂ O		0.44
	d. CuSO ₄ .5H ₂ O		0.16
	e. Na ₂ MoO ₄		0.362
	f. MnCl ₂		

Table 2. BG11 medium components by Rippka and Herdman (40).

No		Nutrient solution ml	Stock solution g/L
1	NaNO ₃	10	150
2	K ₂ HPO ₄ .3H ₂ O	10	4.0
3	MgSO ₄ .7H ₂ O	10	7.5
4	CaCl ₂ .2H ₂ O	10	3.6
5	Citric acid	10	0.6
6	Ferric citrate	10	0.6
7	EDTA-Na	10	0.1
8	Na ₂ CO ₃	10	2.0
	Micronutrient Solutions		
	a. H ₃ BO ₃		61.0
	b. MnSO ₄ .H ₂ O		169.0
9	c. ZnSO ₄ .7H ₂ O	1	287.0
	d. CuSO ₄ .5H ₂ O		2.5
	e.(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂		12.5

The experiment was conducted in the form of five stages, where the number of cells and the absorption values of the culture were measured daily to determine the highest growth rate and the lowest doubling time at harvest for days 7, 14, 21, 28, 35 respectively. Growth rates and doubling times were calculated based on the following equations (41):

$$\text{Growth rate (K)} = \frac{\text{Log } N_t - \text{Log } N_0}{t} \times 3.322 \quad (1)$$

$$\text{Doubling time (G)} = \frac{K}{\text{Log } 2}$$

Whereas:

N_t = optical density in time t or number of cells in time t .

3. Results

The results showed the effect and difference in the number of cells and the values of absorption in the media used when developing in each of the light colors used in the experiment. The increase in the growth curve of each medium appeared on the second day for each color of light.

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Figure (1), which shows the growth curve of *C. humicola* algae, which was grown in two media and the effect of three colors of light, indicated close absorption values on the first day and then began to vary from the first day to the seventh day, and the highest value was recorded in white light 0.434 in the Chu13 medium and 0.397 in the BG11 medium, and the differences were significant on the seventh day, while in blue light, the highest value was recorded 0.538 in Chu13 medium and 0.374 in BG11 medium, and the differences were significant in the first and second day in the color of red light recorded the highest value of 0.471 in Chu13 medium and 0.374 in BG11 medium, and the differences were significant on the second day during seven days respectively. Then it was found that there was a slight increase in the absorbance values in the colors of light, where white light recorded the highest value of 0.627 in Chu13 medium and 0.649 in BG11 medium and the differences were not significant, either in the blue light, Chu13 medium recorded a value of 0.699 and BG11 medium recorded a value of 0.685 and the differences were significant in days 8, 13, 14. In the color of red light, Chu13 medium recorded a value of 0.647, while BG11 medium recorded a value of 0.639 and the differences were non-significant on day 14 during fourteen days respectively.

Upon completion of the twenty-eight days of the experiment, Chu13 medium showed a clear increase in the absorbance values of all light colors, and in white light a value of 0.832 was recorded in Chu13 medium and 0.789 in BG11 medium, and the differences were non-significant in 23 and 28 days and in the blue light, the Chu13 medium recorded a value of 1.033, while BG11 medium recorded 0.936, and the differences were non-significant in 22, 23 days. In the red light, the Chu13 medium recorded a value of 0.998 and BG11 medium recorded a value of 0.746 and the differences were significant on the day 23 for twenty-eight days respectively.

Chu13 medium continued to rise and at white light recorded a value of 0.939, while BG11 medium recorded 0.897, and the differences were not significant in days 29, 32, 33. In the blue light, Chu13 medium recorded a value of 1.090, and BG11 medium recorded a value of 0.855, and the differences were significant, while in the red light, Chu13 medium recorded a value of 1.042, and BG11 medium recorded a value of 0.816, and the differences were

significant during thirty-five days respectively.

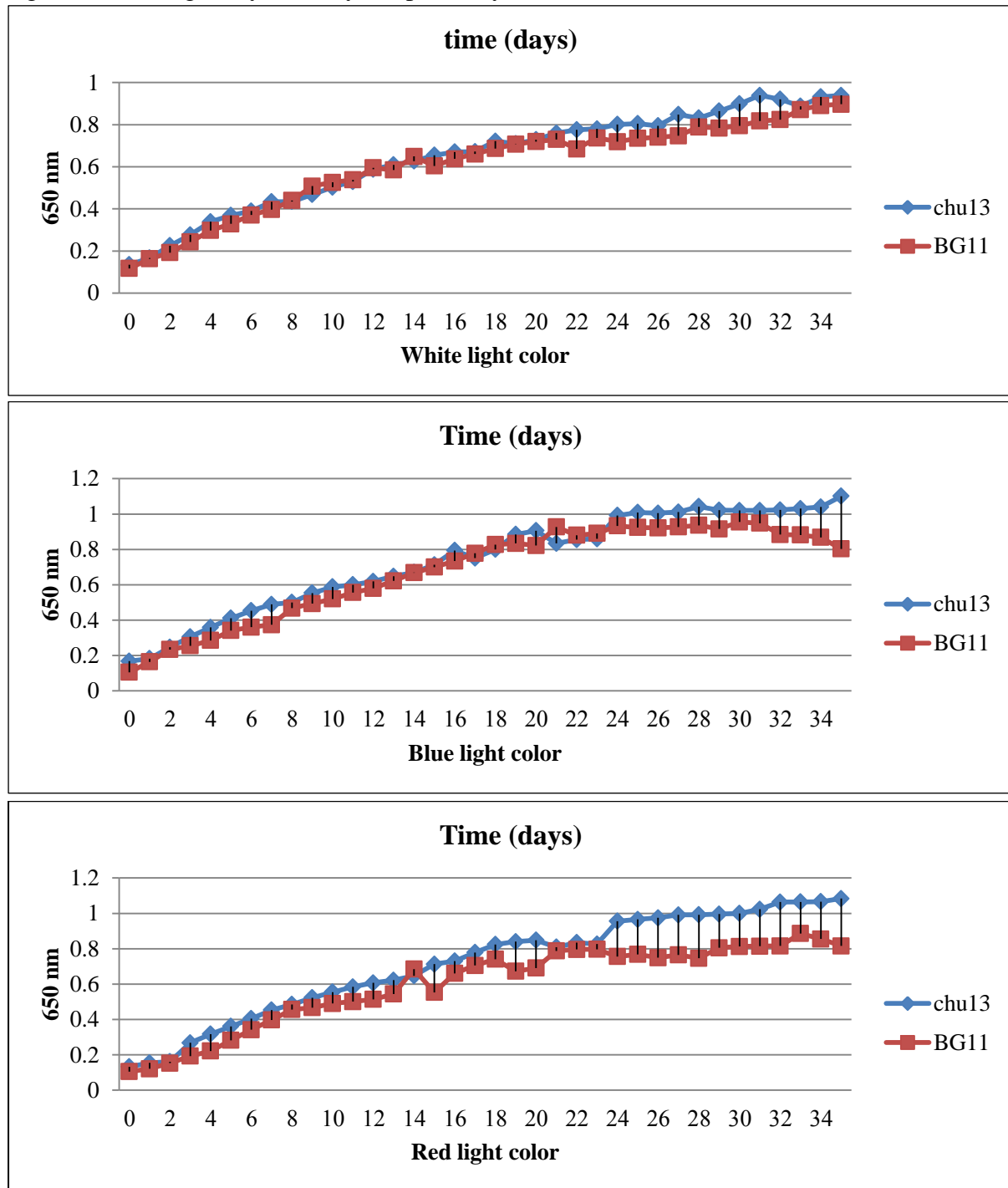


Figure 1. Growth curve of *Chlorococcum humicola* in two media for 35 days with the effect of three colors of light based on absorbency values (650nm).

When relying on the number of cells to draw the growth curve of the studied algae, Chu13 medium recorded a number of cells 1.2×10^4 cells.ml⁻¹ compared to the BG11 medium, which recorded a number of cells of 1.1×10^4 cells.ml⁻¹ in white light and the differences were significant on the second day, while in blue light, Chu13 medium recorded a number of cells 1.7×10^4 cells.ml⁻¹ and BG11 medium recorded a number of cells 1.5×10^4 cells.ml⁻¹ and the differences were significant on the second day too. In the red light, Chu13 medium recorded a number of cells 1.5×10^4 cells.ml⁻¹ while BG11 cells.ml⁻¹ recorded 1.3×10^4 cells.ml⁻¹ and the differences were significant on the first and second days and during seven days respectively. Then the cells of the studied algae continued to doubled and showed larger numbers from day 7 until day 14 in different treatments. Chu13 medium recorded a number

of cells ranging between $2.1-2.3 \times 10^4$ cells.ml⁻¹. While BG11 medium recorded a number of cells 2.6×10^4 cells.ml⁻¹ in white light and the differences were not significant.

In blue light, Chu13 medium recorded a number of cells ranging from $3-3.2 \times 10^4$ cells.ml⁻¹ and BG11 medium recorded a number of cells 1.9×10^4 cells.ml⁻¹ and the differences were significant on day 14. In the red light, Chu13 medium recorded a number of cells 3.7×10^4 cells.ml⁻¹ and BG11 medium recorded a number of cells ranging between $2.6-3 \times 10^4$ cells.ml⁻¹ and the differences were non-significant during fourteen days respectively. When the experiment continued, Chu13 medium recorded a number of cells 2.1×10^4 cells.ml⁻¹ and BG11 medium recorded a number of cells 2.4×10^4 cells.ml⁻¹ in white light and the differences were non-significant, while in blue light, Chu13 medium recorded a number of cells ranging from $5.4-5.7 \times 10^4$ cells.ml⁻¹ and BG11 medium recorded a number of cells ranging from $2.7-3 \times 10^4$ cells.ml⁻¹ and the differences were significant. In the color of red light, Chu13 medium recorded the number of cells ranging from $3-3.2 \times 10^4$ cells.ml⁻¹ and the BG11 medium recorded 2.6×10^4 cells.ml⁻¹ and the differences were not significant during twenty-one days respectively.

At the completion of 28 days, Chu13 medium recorded a number of cells 3.4×10^4 cells.ml⁻¹ and BG11 medium recorded a number of cells 3.5×10^4 cells.ml⁻¹ in white light and the differences were significant in days 25, 26. In blue light, Chu13 medium recorded a number of cells ranging from $7.3-7.5 \times 10^4$ cells.ml⁻¹ and BG11 medium recorded a number of cells 5×10^4 cells.ml⁻¹ and the differences were significant. In the color of red light, Chu13 medium recorded a number of cells of 5.7×10^4 cells.ml⁻¹ and BG11 medium recorded the number of cells ranges from $3.2-3.5 \times 10^4$ cells.ml⁻¹ and the differences were non-significant on day 24 during twenty-eight days respectively.

At the end of the experiment, Chu13 medium recorded a number of cells 4.5×10^4 cells.ml⁻¹ and BG11 medium recorded a number of cells 4.6×10^4 cells.ml⁻¹ in white light and the differences were significant in days 31, 32, 33, while in blue light and red light, the number of cells continued to decrease during the last week of the experiment for both media during thirty-five days respectively, as shown in **Figure 2**.

The highest growth rate depending on the absorbance values, the results recorded values of 0.060 and 0.079 in white light color and the differences were non-significant, while recorded 0.066 and 0.104 in blue light color and the differences were significant in days 2 and 3 only, and recorded 0.055 and 0.081 in red light color and the differences were non-significant in days 4 and 5 only, in Chu13 and BG11 media respectively on the seventh day of the experiment.

The results also recorded the lowest doubling time depending on the absorption values 5.02, 4.02 in white light and the differences were non-significant and recorded 4.50, 3.30 in blue light and the differences were significant on the third day only and recorded 4.57, 3.95 in red light and the differences were significant in days 3, 6 and 7 and the differences were significant in Chu13 and BG11 media respectively on the seventh day of the experiment as shown in **Figure 3**.

As for the highest growth rate depending on the number of cells, the results recorded values of 4.76 and 4.81 cells.day⁻¹ in white light and the differences were significant on the second day only and recorded 4.87, 4.97 cells.day⁻¹ in blue light and the differences were significant on day 2 and 4 and recorded 4.85, 4.97 cells.day⁻¹ in red light and the differences were significant on day 1, 2, 5 in the Chu13 and BG11 media respectively on the seventh day of the experiment.

Depending on the number of cells, the results recorded the lowest doubling time in white light with values of 3.11 and 3.07 cells.day⁻¹ and the differences were significant on the second day only and recorded 3.04, 2.99 cells.day⁻¹ in blue light and the differences were significant on day 2 and 4, while recorded 3.04, 3.03 cells.day⁻¹ in red light and the differences were significant on the first day only in Chu13 and BG11 media respectively on the seventh day of the experiment. The highest growth rates and the lowest doubling times were recorded depending on the values of absorption and the number of cells in BG11 medium and this is consistent with (49) study which it was in blue light followed by red light and then white light as shown in **Figure 4**.

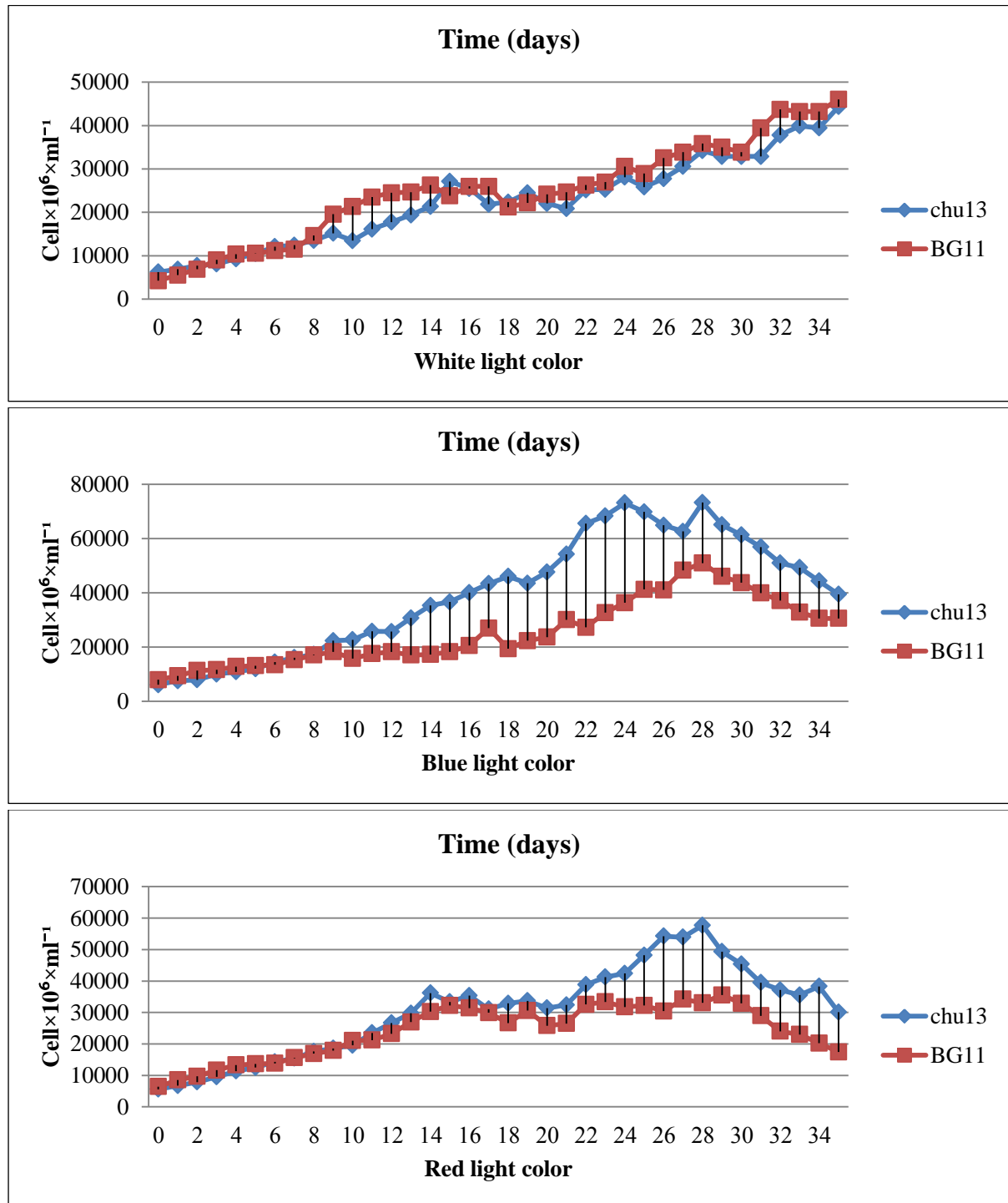


Figure 2. Growth curve of *Chlorococcum humicola* in two media for 35 days with the effect of three colors of light depending on the number of cells (Cells × 10⁶ × ml⁻¹).

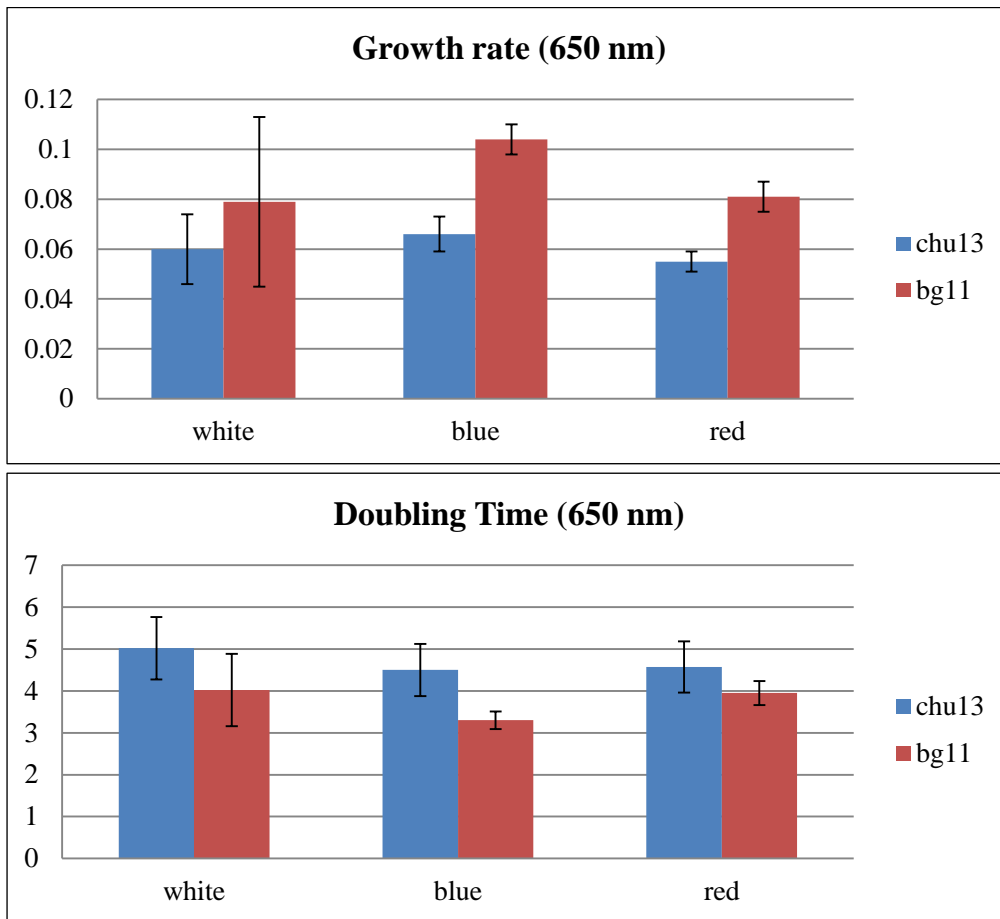


Figure 3. Growth rates and doubling times of *Chlorococcum humicola* with the effect of two media in three colors of light on the seventh day depending on the absorption values.

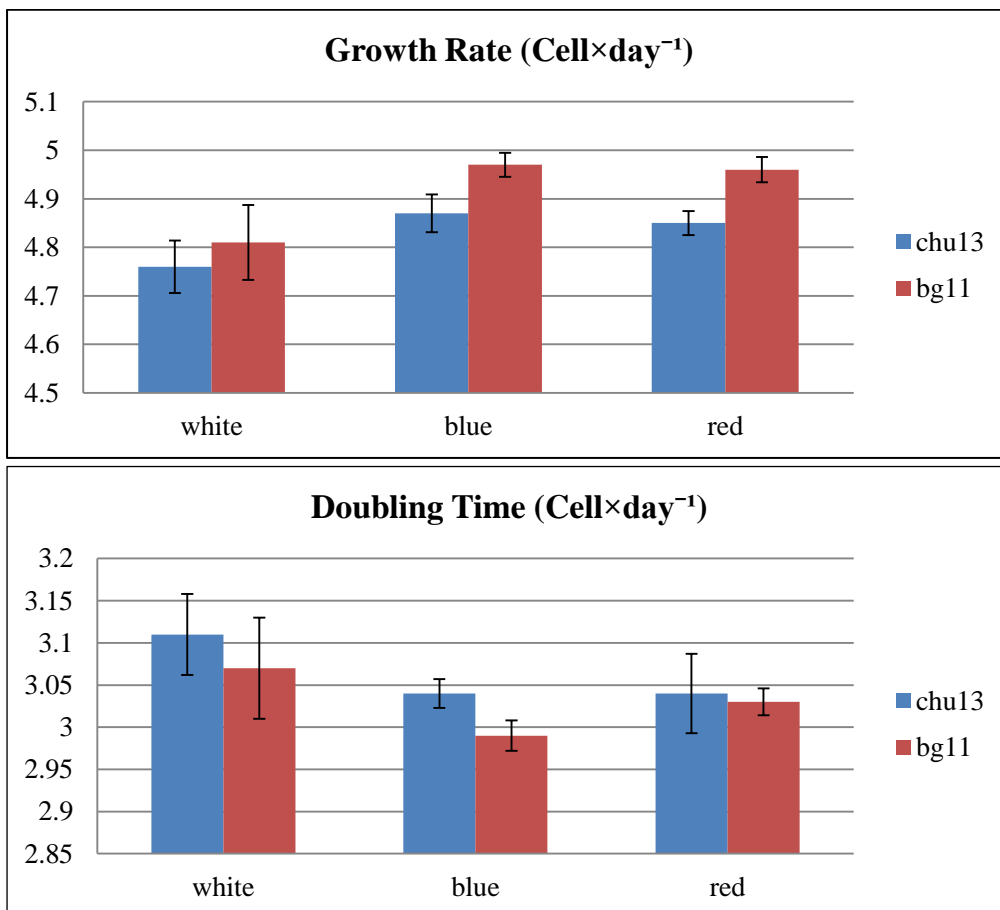


Figure 4. Growth rates and doubling times of *Chlorococcum humicola* with the effect of two media in three colors of light on the seventh day depending on the number of cells.

4. Discussion

The difference in growth curves is mainly due to the different chemical components of the nutrient media, where the cultural media are key factor for growth. The researchers confirmed their effect on growth curves, growth rates and doubling times (42). The selection of the appropriate cultural medium is a key factor in the success of algae cultured (43). In addition to the difference in the source, color and intensity of light affects the algal culture and the way it grows (44, 45).

After that, a very slight increase in the absorbency values appeared in Chu13 medium compared to BG11 medium, and the white light was recorded 0.760 for Chu13 medium, while BG11 medium recorded 0.730, and the differences were not significant. In the color of the blue light, Chu13 medium recorded 0.850, and BG11 medium recorded 0.897, and the differences were significant in the days 15, 19, 20, while in the red light, Chu13 medium recorded 0.809, and BG11 medium recorded 0.793, and the differences were non-significant in the days 16, 17, 21 during twenty-one days respectively. Many studies have indicated that the differences are significant on some days and non-significant on other days depending on the type of algal studied, the color and intensity of light and the nutrient medium (46).

From the results, we can see that Chu13 medium recorded the highest value of absorbency in the color of light blue, red and white respectively. Other studies have confirmed that among the different colors of light, the color of red and blue light plays an important role in the process of photosynthesis and leads to a fluctuation in the growth curve (47).

The results showed that the studied algae recorded different growth curves of absorbency values and cell numbers in different colors of light and media, it was found that the highest growth curves of *C. humicola* were in the Chu13 medium then BG11 medium respectively and this is consistent with (48, 49, 25) who emphasized that different media and nutrient concentration affect growth curves, growth rates and doubling times.

The best light color is blue, red, then white, respectively and this is consistent with (45). Blue and red light colors play a key role in the photosynthesis of algae (47) because they provide ATP in addition to important chemicals that play an essential role in the growth of algae cells (50, 51).

This is because growth rates also vary depending on the quality and quantity of nutrients that make up the development medium and their effect on the speed of algal cell division and on the type of algal studied (25), (24).

Studies have also shown that light is one of the most important environmental factors affecting the growth and reproduction of algae, From these studies, a group of algae was grown under the influence of five different colors: red, blue, green, white, and yellow, for a period of twenty-one days and studied Its effect on productivity and chlorophyll, the results recorded the algae grown under red light showed a higher growth rate, followed by white light. The poorest performance was observed under green and yellow colors, while the highest level of chlorophyll was under the influence of green light color (53). There is a direct relationship between the growth of microalgae and the type, intensity and duration of light, one of these studies checked the effects of photoperiod and radiation on the formation of fatty acids (FA) and biomass of the alga *Chlorella vulgaris*, the results indicated significant differences in biomass and FA at different photoperiods and light intensities (54). The quality of light must be determined in addition to periods of light and darkness, which play an important role in photosynthesis in algae, and that light is necessary for the synthesis of ATP and NADPH, which lead to dark reactions to produce great structures of carbon (55). Another important factor that plays an important role is temperature, which is the most

important determinant of algae development, as the presence of algae at an optimal temperature leads to an enhanced rate of photosynthesis, leading to high growth rates (56).

The optimum temperature for most algae species is 20-30°C (57). Low temperatures can also affect photosynthesis by reducing carbon absorption activity, while temperatures above normal reduce photosynthesis by disrupting photoproteins and an imbalance occurs in the energy balance in the cell in addition to reducing cell size and respiration and this the decrease in photosynthesis leads to a decrease in the growth rate (58).

This explains the differences in the curves and growth rates and doubling times in each color of light, in addition to the impact of environmental conditions on photosynthesis levels, which plays an important role in the growth of algae, as well as the presence of oscillation due to the different type of nutrient medium and the different color of light (47). Many studies have also confirmed the importance of nutrients in culture media and their important role in increasing the growth rate (59).

5. Conclusion

The study aimed to record the highest growth rate and the poorest doubling time of green alga *Chlorococcum humicola* after growing in two culture media Chu-13 and BG-11, by effect of three light colors white, blue and red during 35 days, exposure to different light colors and different culture media recorded significant changes for some days depending on the number of cells and absorbance values, the results recorded the highest growth rate and poorest doubling time on the seventh day of harvesting in BG-11, Chu-13 culture media respectively with the effect of blue light color.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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