

## **EFFECT OF DIFFERENT LIGHT WAVE LENGTHS ON THE GROWTH AND OIL CONTENT OF *CHLORELLA VULGARIS***

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### **Abstract:**

Cultures of *Chlorella vulgaris* were grown in fully transparent polyethylene bottles 20L and exposed to different light colors (*i.e.*; yellow, green, blue, red and white) by covering the growth vessel with fully transparency colored Nylon sheets with fixed light quantity (120 $\mu$ e), growth containers provide the desired light. Growth was performed with fully recommended media of BG-11. By the end of growth period (17 days), oils were also determined. Growth parameters including dry weight, total chlorophyll and carotenes were daily assessed. Growth parameters were varied due to the used containers color type. Yellow and white colors enhanced dry weight accumulation, despite inhibited chlorophyll accumulation. Maximum chlorophyll content was obtained by green light grown cultures followed by blue and red lights. Carotenoids were increased with all used colored light, but green light represented the lowest increase as compared with control culture at zero time. Concerning dry weight growth evaluation, maximum growth rate ( $\mu_{max}$ ) was found to be the highest with cultures exposed to yellow color followed by white color. On the contrast, a negative relationship was observed with cultures that exposed to red light. As for total chlorophyll, slight differences were observed. Regarding carotenes, green and red colors represented the lowest, while white, yellow and blue colors had a little difference. The oil content was found to be slightly associated with carotenoids. White color container represented the maximum oil content following by the blue and yellow color. Oil content was closely related to carotenes content and maximum oil content was observed with yellow light grown alga (12.7%), followed by 10.02% of blue light comparing with 9.4% of white light (control) and 9.34% of red light. The lowest oil content was observed with green light that gave 8.22%.

**Key words:** *Chlorella vulgaris* – Light color – Dry weight – Total chlorophyll – Carotenoids – Oil content

### **Introduction**

Most of photosynthetic organisms including algae can acclimate to the ambient environmental light by changing their pigmentation system. Changes in pigmentation in photosynthetic organisms in response to light quality have been termed chromatic adaptation (**Grossman *et al.*, 1993**). Rhythms of photosynthetic

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capacity was found to be algae dependent, where it was maximized in white light in unicellular algae such as *Lingulodinium polyedrum* (Hastings *et al.*, 1961) or *Euglena* sp. (Walther and Edmunds, 1973) or in red light in the filamentous brown alga *Ectocarpus* sp. (Schmid *et al.*, 1992). *Chlorella* has much higher utilization rate (10-20%) of light energy for the photosynthesis when compared to common plants (Zhang *et al.*, 2000).

Light supply within the water column is a critical factor that also affects phytoplankton production and species composition in lakes. Although the abundance of many species is greatest in the epilimnion, where irradiance is greatest, other species including several algal flagellates are adapted to deeper waters (Lund and Reynolds, 1982). Red light increases carotenoid synthesis and chlorophyll fluorescence of *Dunaliella bardawil* (Sánchez-Saavedra *et al.*, 1996).

Planktonic algae absorb blue light most strongly, and green light most weakly. The blue light absorption by algae strengthens the combined absorption effects of water and planktonic algae of that region. Light qualities affect the ultrastructure of *Chlorella*

Compared to red light cells, blue light cells exhibited higher photosynthetic rates per chlorophyll molecule and contained less chlorophyll per dry weight. Blue light stimulated the content of soluble protein as well as that of soluble carbohydrates. The combined absorption effect shows a sharp peak, in the red light spectral region (600- 700 nm) when chlorophyll-a concentration is greater than 2.0mg. m<sup>-3</sup> and when the chlorophyll-a concentration is less than 2.0mg.m<sup>-3</sup>, the combined absorption effect is not selective. The main cause of water color shift in the ocean is the absorption of blue light by planktonic algae (Shengguang *et al.*, 1991). The absorbance properties of pigments facilitate their qualitative and quantitative analyses (Nusch, 1980; Sartory, 1982) and it was determined that the pigment level was influenced by limiting factors such as highlight, lack of nitrogen and limited nutrient (Greene *et al.*, 1991 ; Collier and Grossman, 1992).

When *Dunaliella* was exposed to high light intensity of blue light, photo-inhibition of O<sub>2</sub> evolution, photo-destruction of carotenoids in the order 9-cis-β-carotene, all-trans-β-carotene, chlorophyll and finally the destruction of the cell were occurred (Ben-Amotz *et al.*, 1989). Sequency, the initial oil might be quantitatively and qualitatively varied. In commerce, production of β-carotene is controlled by numerous stress factors like high light intensity, high salinity,

temperature and availability of nutrients and an inverse relationship exists between  $\beta$ -carotene content and the specific growth rate of *Dunaliella* (**Raja et al., 2007**).

Exposure to low percentage of far red light did not decrease of growth rate of *Chaetoceros* sp. (**Sánchez -Saavedra and Voltolina, 2006**). It has also been observed that the accumulation of  $\beta$ -carotene and its isomeric ratio are strongly dependent on the light intensity and the quality of light used (**Senger et al., 1993**). In addition to the visible wavelength, ultraviolet (UV) radiation also affects  $\beta$ -carotene production.

Chlorophyll a content relating to the pigment level was almost the same in all algae groups, but chlorophyll b and c changed, Also the carotene level varied depending on the algae species and environmental conditions as there was an increase in the carotene level under some stress conditions (**Martin et al., 1991 and Grung et al., 1992**). Illumination is the most expensive factor of algae production during indoor growth (**El-Sayed, 2007**) and blue light tends to yield the highest oil production by *Chlorella* sp. and *Saccharomyces* (**Shu et al., 2012**). The lipid contents of the three *Nannochloropsis* sp., *N. salina*, *N. oceanica*, and *N. oculata* and bio-mass increased by using LED light than with ordinary fluorescent light using a two-phase culture system and the high lipid production was obtained under green LED light stress in the second phase of the two-phase culture (**Chae et al., 2016**).

The current work was achieved to determine the color container effect on algal growth in terms of dry weight, pigmentation and oil content that can increase outdoor productivity rate through controlling light containers or source during night illumination.

## ***Materials and Methods***

### ***Alga and growth conditions***

The green alga *Chlorella vulgaris* (NRC) was early grown on fully saturated BG-II growth medium containing 17.6mM nitrogen (**Stainer et al., 1971**) to obtain the proper inoculum under white light. Illumination of white color was provided from 40w fluorescent lamps. Aeration was done by dried compressed air. The formed inoculum was harvested by cooling centrifuge and washed three times by glass bi-distilled water and then inoculated within

polyethylene bottles containing five liters of fully saturated BG-II growth medium. In three replicates, polyethylene bottles were covered by 0.1 $\mu$  fully transparent Nylon sheet. Sheet colors used were yellow, blue, green and red. Control cultures were performed within the colorless polyethylene bottles. Each color treatment was carried out in three replicates.

### ***Growth determination***

Daily sampling and determination of dry weight, total chlorophyll and carotenes were employed. Dry weight was measurement by filtering a defined volume of the algal slurry (5-10 ml) over pre-weighted dried membrane filter (0.45  $\mu$ m). Filters were dried at 105°C for 30 minutes, kept over anhydrous calcium chloride till room temperature and then re-weighted. The difference between weights monitored the net dry weight of the grown alga within defined sampling time. Chlorophyll was extracted from the pre-centrifuged algal bulk by 95% DMSO (**Burnison, 1980**). Chlorophyll absorbance was measured at 666 nm and concentration was calculated ( $\text{mg.g}^{-1}$ ) according to **Seely et al.(1972)**. To recover carotenes, saponification was performed by 5% KOH/30% MeOH and the residual was re-extracted by DMSO after the addition of 5 drops of concentrated acetic acid (**Boussiba et al., 1992**). Carotenes absorbance was measured at 468nm and concentration was calculated ( $\text{mg.g}^{-1}$ ) according to **Davis (1976)**. Growth analysis; mainly growth rate ( $\mu$ ) and percentage increase (y %) was performed using the methods adopted by **Wolf and Carson (1973)**. Oil content was determined by n-hexane/isopropanol (3:2) extraction using Soxhelt technique.

## ***Results and Discussion***

### **Light color affecting dry weight**

Among the different light color containers used, yellow color was found to be the most effective color on growth dry weight accumulation, however white color was early considered as the common used color especially for inoculum preparation or indoor cultivation The obtained result was found in harmony with **Sebastián et al., 2013** which showed that *C. vulgaris* does not adapt production of

their photosynthetic pigments to absorb light from a wavelength spectrum different from one that they would normally be exposed to. Other colors (blue, white and red) resulted in somewhat less biomass production. Motile cells released in continuous green or blue lights, but not in red one were really observed (Cepak *et al.*, 2006).

On the other hand, Barghbani *et al.* (2012) reported that light color indicated the least significant effect on *Chlorella vulgaris* production among different examined factors including salinity, temperature, iron and light color. They also added that yellow and blue lights increased the algal growth; however salinity (NaCl concentration) had the highest impact on the biomass production.

Regardless yellow color, white color surpasses blue, green, and red light. In addition, red light seems to be having no effect as compared with zero point dry weight (Fig. 1). The effect of light; as a whole; on algal growth composition is referred to the given wavelength that varied on their energy content. This hypothesis could be confirmed by other earl investigations. Total power of emission of the white lamps is higher than those of the other light sources, because of the differences in the respective light spectra.

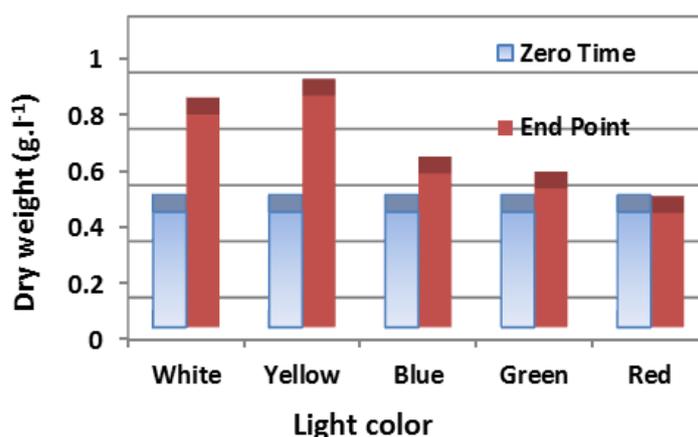


Fig. 1. Dry weight of *Chlorella vulgaris* as grown under different light color containers.

On the contrary, red LED light was found to be the most effective in cell division and dry weight accumulation, while blue LED light was the most effective in cell enlargement (Koc *et al.*, 2013).

The rate of percentage increase was listed in Table (1); showing the slight decline that observed with red light. Maximum growth dry weight was obtained with yellow light, while negative relationship was observed with red light. Thus, it could be concluded that yellow light seems to be the ambient and universal color of sun light that naturally enhances the most surface grown algae in their habitats. Such phenomenon is much desired for biomass production that aims to produce the maximum dry weight within define area or growth volume. To meet that, sun energy must be used all over the day and store to use by night illumination by electric yellow white sources. Moreover, the differences in growth rates and in biomass production should be considered as the effect of the differences in light absorption caused by those of spectral emission (Sánchez-Saavedra and Voltolina, 2006).

**Table 1. Percentage increase and average growth rate ( $\mu_{avr}$ ) of some growth metabolites of *Chlorella vulgaris* as affected by light color.**

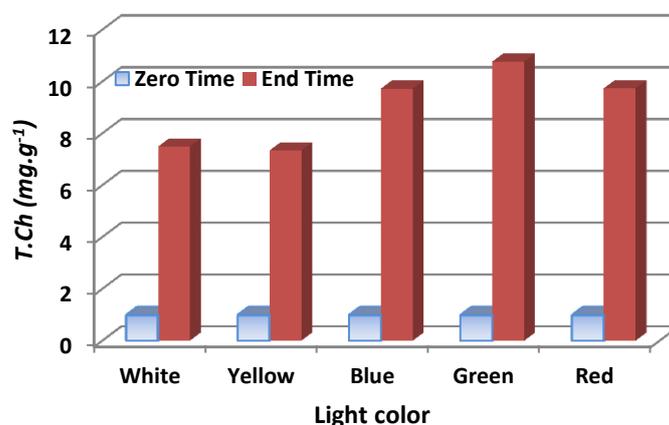
Light color	Dry weight		Total chlorophyll		Total carotene	
	y%	$\mu_{avr}$ .	y%	$\mu_{avr}$ .	y%	$\mu_{avr}$ .
White	84.6	0.61	657	0.12	801	0.13
Yellow	100.8	0.70	642	0.12	682	0.12
Blue	33.3	0.29	883	0.13	774	0.13
Green	20.3	0.19	989	0.14	84	0.04
Red	-0.8	-0.01	885	0.13	462	0.10

### Total chlorophyll

Chlorophyll content (mg.g-1) was found to be more affected by light color rather than dry weight accumulation as a variation between the define growth period. In addition, an opposite manner was found verses dry weight accumulation. Despite yellow light surpasses all other color used in concern dry weight accumulation, green color was found to be the more effective light color affecting chlorophyll accumulation (Fig. 2).

Values of percentage increase were listed in Table (1). However percentage increases presented a great variation (642–989%); growth rate showed the minor

variation (0.12-0.14 g.d<sup>-1</sup>). The effect of light color on chlorophyll formation might be ascribed to the energy coming from the used color in concern wavelength. Total chlorophyll per volume of *Chlamydomonas* and *Chlorella* was increased 20 % while the chlorophyll a/b ratio was decreased during the adaptation period (10 days) in blue light. In red light no change was observed regarding total amount of chlorophyll or chlorophyll a/b ratio (**Hess and Tolbert, 1967**). Green color showed high chlorophyll content, these results agreed with **Mattos et al., 2015** they concluded that weakly absorbed colors of light such as green results in a higher photosynthetic efficiency for high density cultures of *Scenedesmus bijuga*.

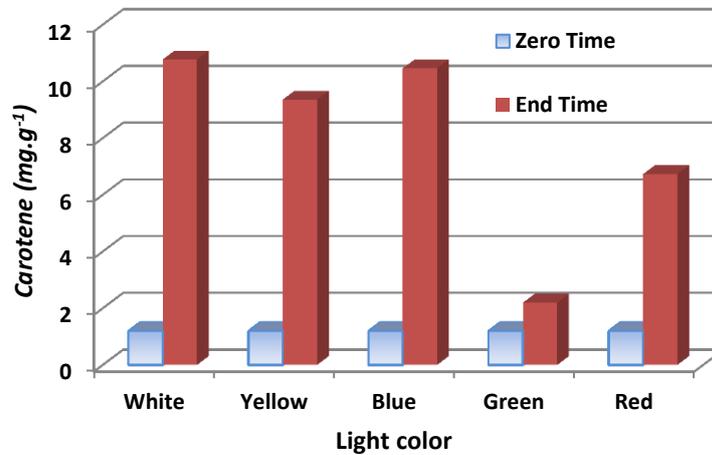


**Fig. 2.**Total chlorophyll of *Chlorella vulgaris* grown under different light color containers.

### Total carotenoids

The role of carotene pigments in algae has been suggested as a passive light protecting filter and has got the role of accessory pigments transferring energy and oxygen (**Yong and Lee, 1991** and **Bidigare et al., 1993**). Carotene content (mg.g<sup>-1</sup>) of *Chlorella vulgaris* represented another different manner as growth response was compared with those of dry weight and total chlorophyll under different light color. White light possesses the most enhancing effect. Despite green light

increases chlorophyll accumulation, it was represented the lowest increase of carotene accumulation (Fig. 3).



**Fig. 3. Total carotenes of *Chlorella vulgaris* grown under different light color containers.**

Numerous factors have been shown to induce massive carotenoid accumulations and these include high photosynthetic photon flux density (**Brown et al., 1997**); far red light and nutrient limitation conditions (**Borowitzka and Borowitzka, 1988**). All of these factors are known to accumulate carotenoid content along with a reduction in the growth rate (**Radmer, 1996**). Thus, an inverse relationship exists between  $\beta$ -carotene content and the specific growth rate (**Ben-Amotz et al. 1982; Raja et al., 2007**). Far red light may affect the growth rates of marine phytoplankton (**Lipps, 1973**) and increases carotenoid synthesis as well as chlorophyll fluorescence of *Dunaliella bardawil* (**Sánchez-Saavedra et al., 1996**).

In general, protein and chlorophyll content are the most influenced components that sharply decreased, while others like carbohydrates, lipids and carotenoids tended to increase. In all cases, dry weight failure was found to be closely related to both chlorophyll and protein decomposition. Container light might affect algal growth by interprets the penetrated light wave length to energy. Consequently, cell component could be fixed under different container color rather than the exposure light color. As the verse relationship between dry weight

and chlorophyll with the opposite between dry weight-chlorophyll and carotenes, such results were listed in Table (2).

### Growth metabolites ratios

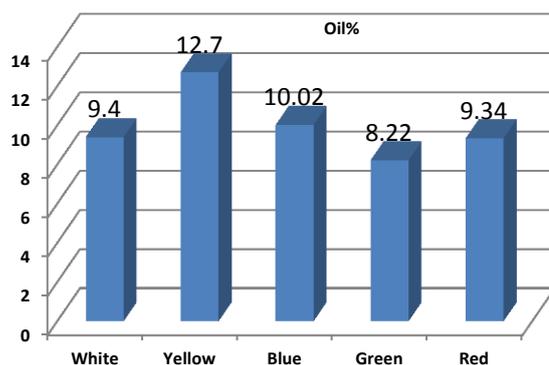
The relationships of growth metabolites (dry weight, chlorophyll and carotenes) were early suggested. Increasing of dry weight was usually accompanied by cell protein content increases. Under non-favorable conditions, growth profile severely changed in relation to stressor factor and stress degree. The composition of microalgae may be modified using different light sources (Mercado *et al.*, 2004), In addition, Red light is also responsible for high carbon fixation with high respiratory rate the wavelength may influence cell composition relative to protein, polysaccharides, and lipids (Riviken, 1989).

**Table 2. Dry weight: pigments ratios (mg/mg) of *Chlorella vulgaris* as affected by light color.**

		D.w/Ch.	D.w/Car.	Ch./Car.
Zero time		<b>39.08</b>	<b>685.6</b>	<b>17.54</b>
End timewith different Light color	<b>White</b>	23.04	140.2	6.04
	<b>Yellow</b>	25.70	175.3	6.82
	<b>Blue</b>	12.89	104.2	8.09
	<b>Green</b>	10.57	451.0	42.67
	<b>Red</b>	9.57	120.4	12.59

### Oil content

Oil content in relation to growth container color was severely acute and mostly associated with carotenoids vis chlorophyll content. Yellow color growth container resulted in the highest oil content (12.7%). Here, the low initial oil content might be ascribed to the enhancing effect of yellow color on vegetative growth and the rising of protein content at the expense of oil level. Colors triggered carotenoids accumulation effect might go back to the initial energy that accelerate chlorophyll decomposition.



**Fig. 4. Oil content of *Chlorella vulgaris* grown under different light color containers.**

Fluorescent light produced more cells than red or natural light with same biomass with *Spirulina platensis* and *Nannochloropsis oculata* referring that light wavelength will impact cell size, reproduction, and composition which determines the value the algae has as a bio-fuel (**Chen and Lee, 2012**).

Several studies reported the growth of microalgae in different light wavelengths. Red (600–700 nm) and blue lights (400–500 nm) stimulate the growth of microalgae, and the growth rates and lipid content of the microalgae differ with light intensity (**Blair et al., 2014**). **Chae et al., 2016** showed that the high lipid production of three *Nannochloropsis* sp. in two-phase culture was obtained under green LED light stress in the second phase of the two-phase culture. The two-phase culture contributed to an increase in lipid productivity by separating the cell growth and lipid production with different LED wavelengths in the microalgae culture. Commercially, factors affecting carotenoids and oils content including light, salinity and nutrient starvation are so hard in implication within open ponds due to insufficient light penetration. Such effect could enhance using photobioreactors. In this case, night illumination of open ponds by desired led light could suggest this result. **Wilhelm et al., 1985** showed that under electron microscopy, blue light cultures showed enlarged cells, thinner cell walls and lower starch content than red light cells. Under blue light, the degree of stacking of the thylakoid membranes was significantly lower than under white or red light conditions. The blue LED is an efficient energy source for high density

culture of *C. vulgaris* as compared to white fluorescent light because it reduce the cultivation time to 8 days compared to 10 days under white fluorescent light in addition to its low power consumption, less heat dissipation and deep penetration in the cultivation system (Atta *et al.*, 2013).

### **Conclusion**

Color of growth container effect on different cell metabolites was varied due to the received light by grown algal cell and the high obtained biomass under define exposure light is not conditioned by the increasing of other determined cell metabolites including chlorophyll and carotenes. The highest obtained biomass with adequate oil content is more efficient rather than those lower biomass with high oil content.

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## تأثير الاطوال الموجية المختلفة للضوء على نمو ومحتوى الزيت لطحلب *Chlorella vulgaris*

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تم زراعة طحلب *Chlorella vulgaris* في زجاجات بولي إيثيلين شفافة تماما (20 لتر) ثم تعرضت لألوان مختلفة من الضوء (الأصفر والأخضر والأزرق والأحمر والأبيض) من خلال تغطيتها برفائق النايلون الملونة ذات الشفافية الكاملة مع كمية الضوء الثابتة (120  $\mu\text{e}$ ). تم تنفيذ النمو على وسط النمو BG-11. وفي نهاية فترة النمو (17 يوما)، تم تحديد الزيوت أيضا. تم تقييم معاملات النمو بما في ذلك الوزن الجاف، والكلوروفيل الكلي والكاروتين. وتفاوتت معاملات النمو بسبب نوع لون الحاويات المستخدمة. عززت الألوان الصفراء والبيضاء تراكم الوزن الجاف، على الرغم من تثبيط تكوين الكلوروفيل. تم الحصول على أقصى محتوى للكلوروفيل للزجاجات التي نمت في الضوء الأخضر تليها التي نمت في الضوء الأزرق والأحمر. وزادت الكاروتينات مع كل الاطوال الموجية الضوء الملون المستخدم، ولكن الضوء الأخضر مثل أدنى زيادة بالمقارنة بالنمو تحت الظروف الطبيعية. وفيما يتعلق بتقييم نمو الوزن الجاف، وجد أن معدل النمو الأقصى هو الأعلى مع الضوء الأصفر يليه اللون الأبيض. على النقيض من ذلك، لوحظ وجود علاقة سلبية مع النمو التي تتعرض للضوء الأحمر. أما بالنسبة للكلوروفيل الكلي، لوحظت اختلافات طفيفة. وفيما يتعلق بالكاروتين، كانت النموات المعرضة للألوان الخضراء والحمراء تمثل أدنى مستوى، في حين أن النموات المعرضة للألوان البيضاء والصفراء والزرقاء كان لها اختلاف بسيط. كما وجد أن محتوى الزيت مرتبط قليلا بالكاروتينات. فقد تبين أن الزجاجات المعرضة للضوء الأبيض تمثل محتوى الزيت الأقصى يليها تلك التي تعرضت للضوء الأزرق والأصفر. وكان محتوى الزيت مرتبطا ارتباطا وثيقا بمحتوى الكاروتين، كما لوحظ محتوى الزيت الأقصى مع النموات المعرضة للضوء الأصفر (12.7%)، تليها 10.02% مع الضوء الأزرق مقارنة و 9.4% مع الضوء الأبيض و 9.34% مع الضوء الأحمر. وقد لوحظ أن أدنى محتوى من الزيت كان مع النموات المعرضة للضوء الأخضر الذي أعطى 8.22%.