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RESEARCH ARTICLE

AN *IN VITRO* STUDY ON GROWTH PERFORMANCE OF SPIRULINA UNDER DIFFERENT LIGHT WAVE LENGTH

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ABSTRACT

The aim of this work is to investigate the growth performance of *Spirulina* under different light wavelength. *Spirulina* mother culture were collected and identified by microscope and it was cultivated using zarrouk's medium in 6 containers. The containers were covered with various color clothes to adjust the wavelength of the light passed into the container. The growth performance of *Spirulina* was studied at 10th day, 20th day, and 30th day by checking the various parameters such as microscopic observation, the amount of Chlorophyll a, Chlorophyll b, Total Chlorophyll, Total Carotenoids, and Phycocyanin. In commercial production units, the cultivation ponds should be made, in order to screen the specific wavelength of light.

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INTRODUCTION

Most groups of algae are obligatory photoautotrophs, in other words, they are entirely dependent on their photosynthetic apparatus for their metabolic needs, using sunlight as energy source and carbon dioxide (CO_2) as carbon source to produce the carbohydrates and ATP. The photoautotrophic, the light is an important factor for the survival of the algae. But the requirement of light intensity for growth is different for different organisms. *Spirulina* also requires a specific range of intensity for its growth (Gualtieri and Barsanti, 2006 and Lee, 2008). The dependence on light as energy source is an essential aspect in the design of photobioreactor that have high light intensity was the main characteristic of any photosynthetic system, and the correct distribution of light along the photobioreactor should be observed(Eriksen, 2008). *Spirulina* sp. is autotrophic, light intensity causes the metabolites to vary its biomass according to the incidence of light, *i.e.,* higher production under higher light intensities with shorter feed (Bezerre, 2006). Chlorophyll is a photosynthesis pigment which only find in autotrophic organisms or algae, chlorophyll content depends on biomass production (Norbert Wasmund and Dirk Schories, 2006).

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Carotenoid is provitamin A which prevents natural oxidation (Goodwin *et al.,* 1908). The accumulation and isomer of β-carotene were controlled by light intensity and quality (Senger and Wagner *et al.,* 1993). Temperature plays an important role in the growth of algae, biomass production, protein and chlorophyll concentration (Pandey *et al.,* 2010). *Spirulina* sp. grows well at pH from 9 to 11 (Dylan van Gerven, 2011). High pH leads to prevent the infection of other green algae (Richmond *et al.,* 1982).

MATERIALS AND METHODS

Collection of the Culture: Algal culture of *Spirulina* was collected from the Department of Microbiology, Ayya Nadar Janakiammal College, Sivakasi.

Maintenance of the Culture: The *Cyanobacterium Spirulina*, was cultivated in Zarrouk's medium at 25±2°C, pH 10 under photoautotrophic condition by continuous illumination using white fluorescent tubes and thrice daily shaking by hand for 15 days. The pH of the medium was maintained by using NaOH solution.

Composition of Zarrouk's medium

One liter of Zarrouk's medium consists of (part A) NaHCO 3 16.80 g and K2HPO4 0.50 g; (part B) NaNO3 2.50 g, K2SO4

1.00 g, NaCl 1.00 g, MgSO4.7H2O 0.20g, EDTANa 2.2H2O 0.08 g, CaCl2.2H2O 0.04g, and FeSO4.2H2O 0.01 g; trace elements mixture A (part C 10 mL/l): 1.00 mL, trace elements mixture B (part D 1.0 mL/l): 1.00 mL; part C mg/l: H3BO3 2.86, MnCl2.4H2O 1.810 g, ZnSO4.7H2O 0.222 MoO3 0.015, and CuSO4.5H2O 0.074 (the used amount is 10 mL/l); part D mg/l: NH4VO3 22.9, NiSO4.7H2O 47.8, NaWO2 17.9, Ti2(SO4)3.6H2O, and Co(NO3)2.6H2O 4.4 (the amount used was 1.0 mL/l) (Zarrouk's., (1966).

Cultivation of *Spirulina*

The light wavelength, preferred by the *Spirulina* for the production of pigment is 620 nm – 670 nm (Chronakis, *et al.,* 2000; Costa, *et al.,* 2000 and 2002; Colla, *et al.,* 2007a). In this experimental setup seven containers were taken, and one liter of zarrouk's medium was prepared for each containers. After that, 5 % of *Spirulina* culture was inoculated to each container. The container was placed under the light illumination and these were covered with commercial cotton cloths with different colors (Indigo, Blue, Green, Light green, Yellow, Orange & Red) to adjust the wavelength of light which is passed into the container. The pH of the medium in all containers was maintained as 10. The manual mixing of culture was done for 3 times in a day.

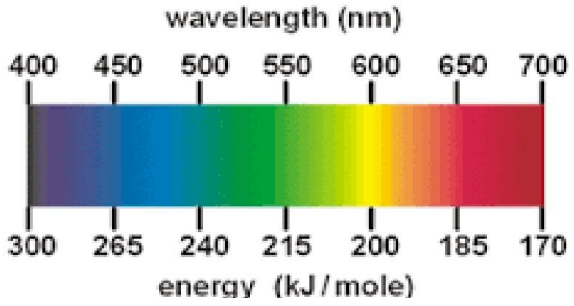

Fig. 1. Wavelength of Different colors

Growth performance of *Spirulina* in different wavelength

The following parameters were checked to study the growth performance of *Spirulina* such as microscopic observation, concentration of biomass, chlorophyll a, chlorophyll b, Total chlorophyll, carotenoids and phycocyanin pigments of the slurry formed.

Determination of biomass (Dried) concentration

10 ml of *Spirulina,* grown in the zarrouk's medium was filtered and washed several time with distilled water to remove soluble salts. Then the slurry was dried at 80°C for 30 min and weighed. The difference was compared and the dry weight mass was calculated using the given formula: Biomass (mg/L) = Wf – Wi × 1000/ V; Whereas, Wf - Final weight, Wi - Initial weight, V - Volume of the sample (Usharani *et al.,* 2014).

Determination of chlorophyll

Analytical grade acetone to 80% acetone was diluted. 1g of spirulina was taken and added with of 10ml acetone. Then centrifuged at 5000rpm for 5minutes and then the supernatant were collected. Read the absorbance of the solution at 663nm and 645nm against the solvent (80% acetone) blank in a spectrophotometer. The amount of chlorophyll was calculated by using the following equations:

Chlorophyll a (mg) = $12.7(A_{663}) – 2.69(A_{645}) \times V/1000 \times W$
Chlorophyll b (mg) = $22.9(A_{645}) – 4.68(A_{663}) \times V/1000 \times W$
Total chlorophyll (mg)= $20.2(A_{645}) + 8.02(A_{663}) \times V/1000 \times W$

Where, A = Absorbance at specific wavelength, V = Final volume of chlorophyll extract in 80% acetone, W = Fresh weight of the slurry (Thimmaiah, 1999).

Determination of Total Carotenoids

A known volume of homogenized algal suspension was centrifuged at 3000 rpm for 5 minutes. The pellet was washed with distilled water 2-3 times to remove traces of adhering salts. To the pellet, 2-3 ml of acetone (85%) was added and then subjected to repeated freezing and thawing. The suspension was centrifuged and the supernatant containing pigment was collected. The extraction was repeated till the supernatant became colorless, for complete recovery of carotenoids. The pooled fractions of supernatants were made-up to a final known volume. The absorbance was taken at 450nm using 85% acetone as blank and the total amount of carotenoids was calculated in μgml^{-1} as follows: C = D x V F / 2500 x 100, Where, D = OD at 450nm; V = Volume of the extract, and F = Dilution factor (Saleh, *et al.,* 2011).

Extraction and determination of Phycocyanin

An aliquot of algal culture was used as a source for extracting phycocyanin. Harvested Biomass was homogenized in hand homogenizer for 20 minutes in presence of phosphate buffer at pH 6.8 in 1:3 ratios. The homogenized culture was subjected to freezing and thawing for 3 days. Freeze thawed sample was subjected to centrifugation at 5000 rpm for 45 minutes. The supernatant raw phycocyanin was taken in sterile tubes covered with aluminum foil and stored at 4°C for further analysis. The phycocyanin concentration was calculated spectrophotometrically by measuring the absorbance at 615nm and 652 nm using the following formula: Phycocyanin mg/ml $=A_{615} - 0.047(A_{652}) / 5.34$; Whereas, A_{615} - absorbance at 615nm A_{652} - absorbance at 652nm, 5.34 - constant factor (Usharani *et al.,* 2014).

RESULTS AND DISCUSSION

Growth performance of *Spirulina* under the exposure of light at varying wavelength

Growth performance of *Spirulina* on 10th Day

The presence and growth rate of *Spirulina* was observed under microscope daily for 10 days. On 10th day 20th day and 30th day, the growth performance of *Spirulina* culture covered with red, orange & yellow colour cloth was studied by microscopic observation, concetration of biomass, Chlorophyll a, Chlorophyll b, Total chlorophyll, Total Carotenoids and Phycocyanin content of the slurry formed. The result was recorded, calculated and tabulated (Table 1).

Biomass of *Spirulina* cultivated under the exposure of different wavelength on 30th Day: The biomass of *Spirulina* cultivated in zarrouk's medium under different wavelength was calculated and Tabulated (Table 2)

Table 1. The growth performance of *Spirulina*

Day	Parameters	Control	Red	Yellow	Orange
10th Day	Chlorophyll a (mg/l)	0.0123	0.0067	0.0195	0.0171
	Chlorophyll b (mg/l)	0.0286	0.0122	0.0188	0.0677
	Total chlorophyll (mg/l)	0.0413	0.0189	0.0383	0.0848
	Total Carotenoid (µg/ml)	1.28×10^{-3}	8.196×10^{-6}	7.141×10^{-6}	8.532×10^{-6}
	Phycocyanin (mg/ml)	0.093	5.01×10^{-4}	2.45×10^{-5}	3.4×10^{-4}
20th Day	Chlorophyll a (mg/l)		0.031	0.0345	0.0205
	Chlorophyll b (mg/l)		0.0015	0.0473	0.0765
	Total chlorophyll (mg/l)		0.0300	0.0818	0.0959
	Total Carotenoid (µg/ml)		7.452×10^{-6}	8.952×10^{-6}	9.636×10^{-6}
	Phycocyanin (mg/ml)		9.5×10^{-3}	3.9×10^{-3}	4.6×10^{-2}
30th Day	Chlorophyll a (mg/l)		0.0820	0.0837	0.1029
	Chlorophyll b (mg/l)		0.0470	0.0576	0.0779
	Total chlorophyll (mg/l)		0.1291	0.1413	0.1808
	Total Carotenoid (µg/ml)		1.314×10^{-5}	1.792×10^{-5}	1.951×10^{-5}
	Phycocyanin (mg/ml)		4.52×10^{-2}	1.11×10^{-2}	7.91×10^{-2}

Table 2. The biomass production of *Spirulina* on 30th day

S.No	Color	Biomass (mg/l)
1.	Indigo	252 mg/l
2.	Blue	252 mg/l
3.	Green	284 mg/l
4.	Light green	299 mg/l
5.	Yellow	345 mg/l
6.	Orange	350 mg/l
7.	Red	303 mg/l

In this study, an attempt was made to compare the growth analysis of *Spirulina* under different light wavelength. The absorption of light spectrum was studied by observing the growth performance of *Spirulina* at 10th day, 20th day, and 30th day by checking the various parameters such as microscopic observation, biomass, and concentration of Chlorophyll a, Chlorophyll b, Total Chlorophyll, Total Carotenoids & Phycocyanin content of the slurry formed. The presence and growth rate of *Spirulina* was observed under microscope every day. Based on the microscopic observation, the *Spirulina* shows the better growth in the containers covered with three colors such as yellow, orange and red. So, we selected the *Spirulina* cultivated container covered with these colors for further studies. The growth performance of *Spirulina* culture covered with red, orange & yellow colour cloth was studied by microscopic observation, biomass concentration, and the amount of Chlorophyll a, Chlorophyll b, Total chlorophyll, Total Carotenoids & Phycocyanin content of the slurry formed. The result of microscopic observation shows, the presence of maximum level of *Spirulina* in the container covered with orange color (Table 2). At the same time, the quantity of pigments presence is varying within these three colors (Table 1). The light wavelength observed by these colors is between 570 nm to 700 nm. The light wavelength, preferred by the *Spirulina* for the production of pigment is 620 nm – 670 nm (Chronakis, *et al.,* 2000; Costa, *et al.,* 2000 and 2002; Colla, *et al.,* 2007a).

Conclusion

As per the results observed, the biomass concentration, the quantity of Total chlorophyll and Total Carotenoids formed in *Spirulina* has increased in the container covered and opened with orange color (620 nm - 645nm). The quantity of Phycocyanin formed in the *Spirulina* has increased in the container covered with Red color (650 nm -700 nm). In commercial production units, the cultivation ponds should be made, in order to screen the specific wavelength of light.

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