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UV-Spectroscopic Studies on the Potential State of Spirulina Cultures using Varied Culture Media in Uncontrolled Laboratory Conditions in Ballari Region

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Abstract: *Spirulina platensis is a filamentous cyanobacteria obtained from the wild was subject to exposure to different concentrations of media namely Zarrouk's and BG-11. The hydrological parameters were maintained naturally in the arid regions of Ballari district. The increased temperature during summer months and dry air substantially had a negative effect on the quality of the filamentous algae. The UV Spectroscopy absorbance showed increased levels with increase in pigment concentration. The study revealed that highest absorbance patterns are due to greater number of chromophores found in the order of the samples A2>A>S_2> S_1 respectively.*

Keywords: Spirulina platensis, Zarrouk's media, BG-11, Spectroscopy

I. INTRODUCTION

India is a bio resource country and it is known for its rich biodiversity. Various aquatic resources, namely seas, brackish waters, fresh water systems; like lakes, ponds, irrigation tanks, seasonal irrigation tanks and reservoirs available in India have with fishery potential (Kannappan and Kartikeyan, 2013). Likewise, the aquatic plant resources, namely the phytoplankton, seaweeds and sea-grasses are also abundant that serve as photosynthetic industries (Patricia et al, 1999). From over 2000 species of Cyanobacters about 150 genera have wide range of shapes and sizes (Vincent W.F, 2009). *Spirulina* is a filamentous blue-green alga which has gained considerable popularity in the field of food industry and provides immense protein and vitamins supplements to aquaculture. It can be cultured indoors as well as outdoors with optimum environmental conditions. *Spirulina* is functionally a complete food for the present and has futuristic potentiality as strong food due to the total amino acid profile and high content of omega-3 fatty acids (Eladl Eltanahy and Aya Torky, 2021).

In 1981, FAO documented the possibilities of blue-green algae to replace chemical fertilizers and rebuild depleted soil structure soils. Low cost, ready availability and preferential use of inorganic fertilizers hinders the use of *Spirulina* based fertilizers. *Spirulina* used in combination with other fertilizers gave better yield of tomato (Zeenath et al, 1990).

Spirulina was studied as a feed supplement and found to significantly improve growth, survival and FCR. The supplement range from 5–20% resulted better compared to other percentage economically (Nakagawa et al, 1975). *Spirulina* is inexpensive feed ingredient than others of animal origin. It is found to promote growth, immunity and viability of prawns (*Penaeus monodon*) and scallops showed reduction in the cultivation time, mortality and increase in the shell thickness. Abalone (*Haliotis midae*) showed good growth when fed a diet containing *Spirulina* meal (Britz P.J, 1996).

The fatty acid profile of fish eggs fed on *Spirulina* supplements contained more linoleic acid, γ-linolenic acid, eicosatrienoic acid, eicosatetraenoic acid, docosapentaenoic acid showed significant differences than those of fish fed solely on *Spirulina*. Carotenoids and carotenoproteins are responsible for the various colors of crustaceans (Britton G et al, 1981). The present investigation was to study its quality of *Spirulina* culture during all seasons during 2021-22 using standard Zarrouk's media and BG-11 media under controlled laboratory conditions.

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II. MATERIALS AND METHODS

Spirulina platensis strain used in this work was obtained from CFTRI Mysore, Karnataka, India. Two different media were prepared for culturing of *Spirulina platensis* i.e., Zarrouk's media and BG-11. Both the media were prepared in the double distilled water, for the culture aspects to study morphological changes and ability to withstand higher temperature under laboratory conditions. The chemical compositions of nutrient media are shown in Table-1 and Table-2.

III. RESULTS AND DISCUSSION

The sterile culture tubes, trays and flasks were used throughout the experimentation to avoid microbial contamination. The cultures were inoculated as shown in table-3. The temperature parameter was subject to natural conditions that ranged between 28 ± 2 to 35 ± 2 °C. Natural illumination was provided continuously day and night with white fluorescent lights. Agitation was carried out using aquarium aerators for constant exposure and to prevent sedimentation. Subcultures were inoculated based on the appearance of distinct green coloration of the culture flasks thereby confirming after microscopy (Fig.-1 to Fig.-6).

Spirulina platensis was subjected to two synthetic culture media viz., Zarrouk's media (Zarrouk, 1966) and BG – 11 medium (Rippka R et al, 1979). According to (Ruma Arora Sani et al, 2019) the optimal temperature for the better growth performance of Spirulina is at 30°C, whereas temperature below 25°C and above 35°C affects the growth of algal filaments. It is true for the pH as well. The optimum pH range was maintained between 8.5 and 10.5. In the present study the experiments were carried out under uncontrolled laboratory conditions, in the sub arid region of Bellary. The increased temperature during summer months and dry air substantially had a negative effect on the quality of the filamentous algae. To quantify the efficacy of cyanobacter spectroscopic study was performed.

UV-V spectra have been made on *Spirulina* dissolved in double distilled water. The samples obtained from the above cultures, namely S_1(Grown in Zarrouk's media) & S_2 (grown in BG-11 media) along with the dried form of capsulated *Spirulina* (A) obtained from the market were used to analyze the absorbance. The three samples S_1, S_2 and A were diluted in the three different cuvette using the double distilled water. These were run individually in the UV spectrometer and the absorbance capacity of each samples were plotted.

As all cyanobacters include chlorophyll-a and varying amounts of the auxiliary phyco-biliprotein pigments, phycocyanin (blue) and phycoerythrin (red), which are photosynthetic pigments, they have a wide spectrum of coloration. The concentrations of these pigments have a significant impact on the final exterior colour of a specimen. All cyanobacteria have characteristically thin or thick gelatinous sheaths outside of their cell walls. Phycoerythrin, phycocyanin, and allophycocyanin are some of the pigments found in cyanobacteria collectively called phycobilisomes. Phycoerythrin absorbs energy between wave lengths of 500 and 600 nm, phycocyanin between 550 and 650 nm, and allophycocyanin between 600 and 675 nm. Their ability to absorb light will consequently cover the full visible spectrum when combined with chlorophyll-a (Md.Fuad Hussain, 2020).

In contrast to the blank sample, UV-Visible spectroscopy analytical technique counted the number of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample. One of the most crucial factors in the cultivation of Spirulina is optical density (OD) or light absorbance. Optical density measurement is typically used to estimate growth (S Rajendran, 2013). Following are the light absorption patterns for various Spirulina samples (A2, A, S1 & S2). The concentrated version of sample A is A2. The culture inoculated in the Zarrouk's media, after seven days showed change in color i.e., from pale green to pink color. This alteration may be attributed to the presence of high levels of phycoerythrin. Similar observations were made by Thanh Sang Vo et al, (2015). Different pigments and carotenoids are present in different quantities in different microalgal species. The absorbance of a substance increases with increase in pigment concentration. This is because a higher absorbance indicates less transmission because the amount of light that is absorbed depends on the number of molecules it interacts with.

From the Figure-7 it is deduced that, the samples with brighter in color showed the highest absorption pattern. According to Beer Lambert's law the absorbance of a solution increases as attenuation of the beam increases. Absorbance is directly proportional to the path length and the concentration of the absorbing species (chromophores). Therefore from the UV –Visible spectroscopic analysis of all the samples (Fig.-8-11) showed that the capsules of Spirulina obtained from the market showed the highest degree of absorbance indicating the greater number of

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chromophores (absorbing species) in the sample. Whereas other samples such as S1 and S2 showed the lower degree of absorbance indicating the fewer number of chromophores in the sample. Water was taken as the bare sample control (C) and showed no absorbance indicating no chromophores in the sample.

IV. CONCLUSION

The cyanobacter *Spirulina* is a non-toxic blue-green-alga abundant in minerals, proteins and essential fatty acids. It has beneficial energy supplement and works well as a low calorie food. *Spirulina* cultures thrived well both in Zarrouk's media as well as BG-11 media under uncontrolled laboratory conditions. There was optimum growth in the initial 06 months of period during monsoon and post- monsoon. In the later days BG-11 declined the dominance of *Spirulina* but *Chlorella*. Thus Zarrouk's media is highly suited for culture yet the alternative low cost BG-11 media could be used alternatively for a limited period of time without any contamination. UV Spectroscopic studies revealed that highest absorbance patterns are due to greater number of chromophores. Frequent sub-culturing procedure help in the retention of the quality and the concentrated cultures can be used in dried or live wet forms as food to the larval culture tanks in the hatcheries of fish and prawn.

V. ACKNOWLEDGEMENT

The author acknowledges Dept. of Studies in Zoology, Vijayanagara Sri Krishnadevaraya University, Ballari for providing the laboratory facilities and financial support for carrying out this research.

Sl. No	*Chemical name*	*gm / liter*	*medium*
1	Sodium bi-carbonate	16.80	Dist. Water
2	Di-potassium hydrogen phosphate	0.500	Dist. Water
3	Ferrous sulphate	0.010	Dist. Water
4	Sodium nitrate	2.500	Dist. Water
5	Potassium Sulphate	1.000	Dist. Water
6	Magnesium sulphate	1.200	Dist. Water
7	Sodium chloride	1.000	Dist. Water
8	Calcium chloride	0.040	Dist. Water
9	EDTA	0.100	Dist. Water
10	Manganese chloride	1.810	Dist. Water
11	Zinc sulphate	0.222	Dist. Water
12	Sodium molybdate	0.039	Dist. Water
13	Copper sulphate	0.079	Dist. Water
14	Cobalt nitrate	0.049	Dist. Water
15	Boric acid	2.860	Dist. Water

Table 1: Zarrouk's media compostion (Source: CSIR-CFTRI)

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Sl. No	Chemical name	gm /liter	medium
1	Sodium nitrate	1.500	Dist. Water
2	Potassium hydrogen phosphate	3.050	Dist. Water
3	Magnesium Sulphate	7.500	Dist. Water
4	Calcium Chloride	3.600	Dist. Water
5	Sodium Carbonate	0.020	Dist. Water
6	Disodium EDTA	0.100	Dist. Water
7	Hydrated Citric Acid	0.600	Dist. Water
8	Ferric Ammonium Citrate	0.600	Dist. Water
9	Boric acid	2.860	Dist. Water
10	Manganese Chloride	1.810	Dist. Water
11	Zinc Sulphate	0.220	Dist. Water
12	Copper Sulphate	0.080	Dist. Water
13	Cobalt Chloride	0.050	Dist. Water
14	Sodium Molybdate	0.390	Dist. Water
	Acidified to 7.5 pH		

Table 2: BG-11 media

Test Tube	1	2	3	4	5	6	7	8	9	10
Spirulina Mother Culture (ml)	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
Media A (ml)	0.5ml	1.0ml	1.5ml	2.0ml	2.5ml	3.0ml	3.5ml	4.0ml	5.0ml	0.0ml
Media B (ml)	4.5ml	4.0ml	3.5ml	3.0ml	2.5ml	2.0ml	1.5ml	1.0ml	0.0ml	5.0ml
Ratio	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1

Table 3: Inoculation of cultures in Zarrouk's media (A) and BG-11(B).

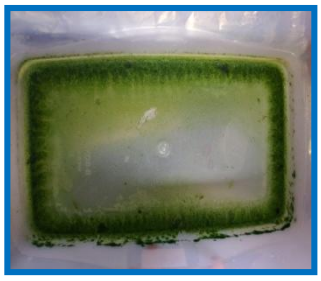

Fig.-1. Culture in trays

Fig.-2. Serial dilution

Fig.-3. Sub cultures

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Fig.-4. Microscopic view of *Spirulina*

Fig.-5. Dilution in flasks

Fig.-6. 2 week old cultures

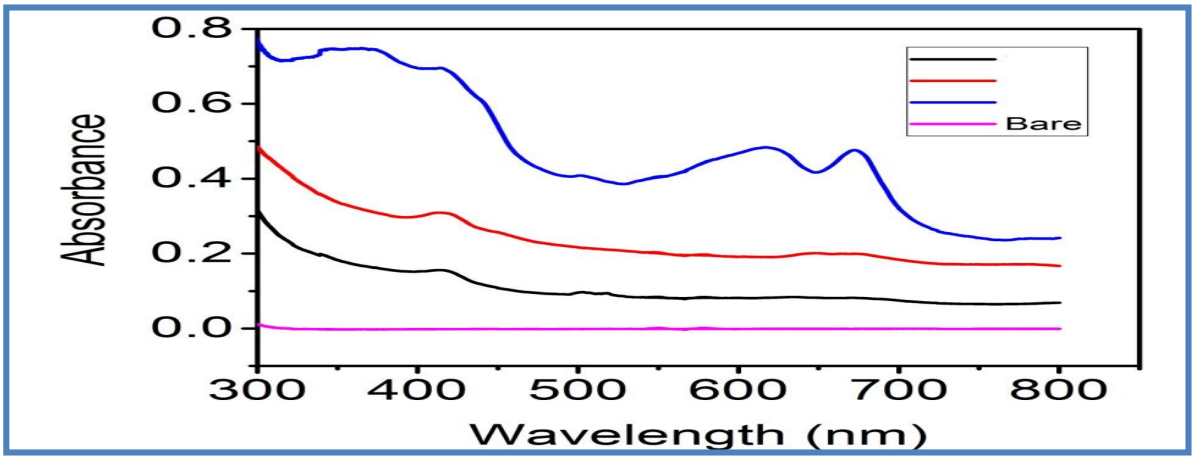

Figure 7: Spectroscopic light absorbance graph of all samples (A, S_1, S_2 and C=bare).

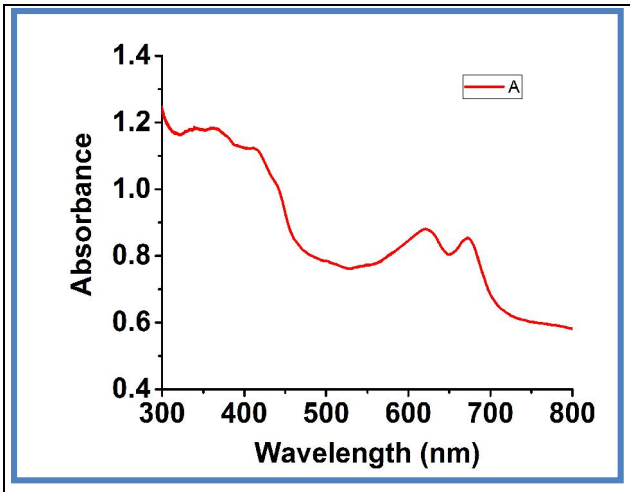

Fig.8. Absorption curve of Sample A

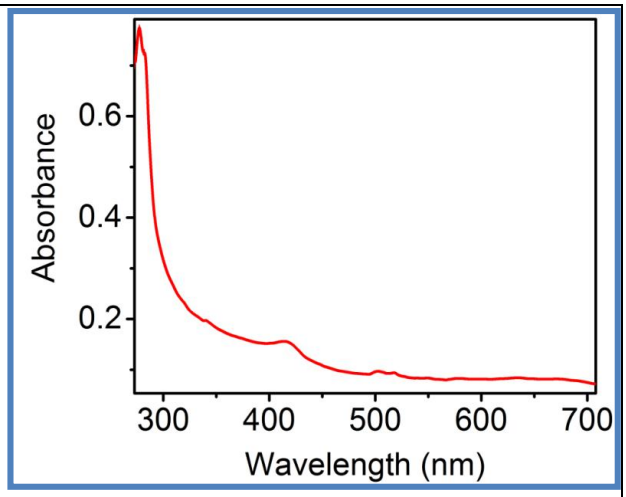

Fig.9. Absorption curve of Sample A_2

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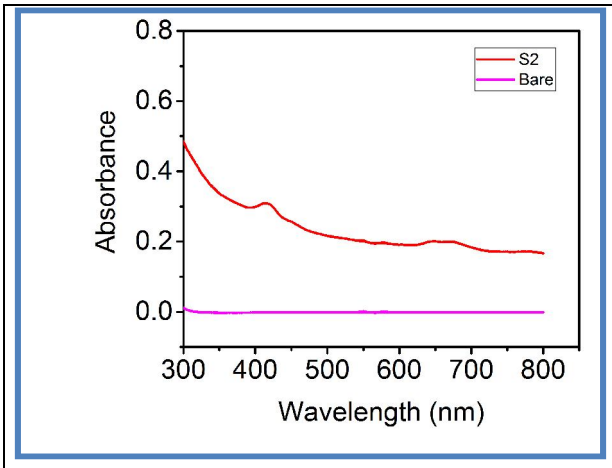

Fig.10. Absorption curve of Sample S_2

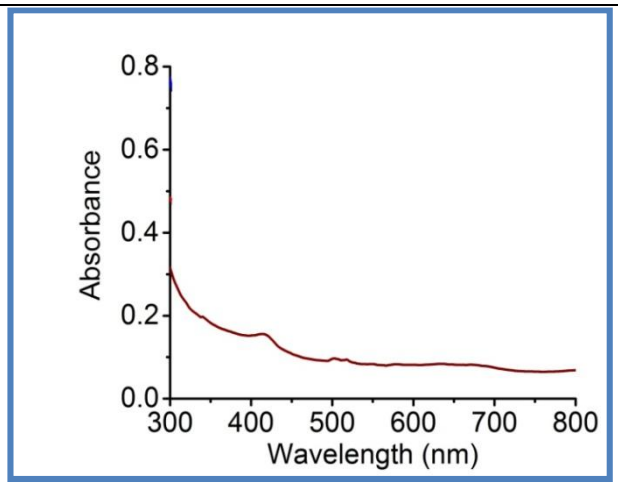

Fig.11. Absorption curve of Sample S_1

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