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Environmental Factors for Optimisation of *Spirulina* Biomass in Laboratory Culture

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Abstract: The study attempts to investigate the contribution of selecting optimal environmental factors as optimal temperature, light and pH condition obtained from previous experiments for growth and biochemical changes in *S. platensis* and *S. fusiformis*. Zarouk medium was used for the experiment in distilled water. For *S. platensis*, temperature was adjusted to 32°C, light to 2500 lux and pH 9 while for *S. fusiformis*, temperature was adjusted to 37°C, light to 2500 lux and pH 10. The specific growth rate and biomass of both *S. platensis* and *S. fusiformis* achieved in the present study are significantly higher than those achieved in previous cultures ($p<0.05$). Protein content of *S. platensis* was 58.6%. This value is vaguely lower than that achieved in previous experiments and the difference is not statistically significant. Protein content of *S. fusiformis* was 61.8%. This value is significantly higher ($p<0.05$) than that attained in temperature effect culture but statistically same to pH and light effect culture ($p>0.05$). These results suggested that favorable environmental conditions during *Spirulina* culture could be instrumental for good biomass production and protein production as well.

Key words: *Spirulina*, optimization, dry biomass, growth, biochemical composition

INTRODUCTION

The *Spirulina* is exceptionally high in protein and it appears to have the highest vitamin B_{12} content of any unprocessed plant or animal food[1]. It has relatively low percentage of nucleic acids which makes *Spirulina* a potential food items for persons suffering from coronary illness and obesity. In last 20 years *Spirulina* has the world wide acceptance and has been taken by millions of people as a natural food in the USA, Japan and Europe[2].

Spirulina grows better in a liquid environment or culture medium of high pH and alkalinity. It forms massive population in tropical and sub-tropical water bodies, characterized by high level of carbonate and bi-carbonate and high pH[3]. The fame of *Spirulina* is a result of its economic importance, which is due to its nutritional value[4]. It mass culture becomes attractive as a source of food feed and fine chemicals[5].

Physico-chemical profiles describing the relationship between growth and environmental factors especially irradiance flux, density and temperature are important in the evaluation of microalgae and cyanobacteria for biomass production, as well as for their general characterization. Such profiles are rarely found in the literature[6]. One difficulty lies in maintenance of the optimum temperature in the culture throughout the diurnal cycle and the year round[7]. During the winter *Spirulina* cannot be grown in open ponds except in the tropics. In many algal species, prolonged exposure to high light may cause photooxidative death[8]. Outdoor mass culture is not illuminated during the night. Preliminary measurements of the dark respiration losses in outdoor culture of *Spirulina* have revealed that up to 35% of the total biomass produced during the day may be lost through respiration at night[9,10]. The pH determines the solubility of carbon dioxide and minerals in the medium and directly and indirectly influences the metabolism of algae[11]. Different algae have different pH optima. High alkalinity is mandatory for the growth of *Spirulina* and bicarbonate is used to maintain high pH. Nevertheless, the whole array of limitations and their interactions exerted on growth and productivity of *Spirulina* cultures in outdoor raceways throughout the year has yet to be elucidated. The present study attempts to investigate the contribution of selecting optimal environmental factors as optimal temperature, light and pH condition obtained from previous experiments in laboratory culture for the production of *Spirulina* biomass.

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MATERIALS AND METHODS

Strain: In the present study two species of *Spirulina* were studied. *S. platensis* strain was collected from the stock available in the University College of Science and Technology, Malaysia. *S. fusiformis* was collected from Marugappa Chettair Research Centre, Chennai, India. The culture was maintained by repeated transfer to liquid Zarouk medium.

Culture condition: This experiment was done by selecting optimal environmental factors as optimal temperature, light and pH condition obtained from previous experiments. Zarouk medium was used for the experiment in distilled water. For *S. platensis* temperature was adjusted to 32°C, light to 2500 lux and pH 9. For *S. fusiformis* temperature was adjusted to 37°C, light to 2500 lux and pH 10. Both species was cultivated in 500 mL Erlenmeyer flask containing 250 mL sterilized media. Ten percent (v/v) of the prepared inoculums were added to the flask. The flasks were covered perfectly by cotton wool and aluminum foil and sealed with laboratory sealing film. Growth was measured as chlorophyll-a concentration every two days of 20 days culture. The results were compared with those found highest in previous experiments of light, temperature and pH effects[12,13].

Analytical procedure: Chlorophyll-a was determined spectrophotometrically after absolute methanol extraction utilizing the absorption coefficient factor reported by Vonshak and Richmond[7]. Dry weight determination was done by 20 mL algal sample of suspension that was filtered through a Whatman GF/C filter of 47 mm diameter. The filter was dried in an oven for overnight at 70°C, put in desiccators for 20 min for cooling and weighed. The Specific growth rate was measured by using the formula,

$$M = \frac{\ln x_2 - \ln x_1}{t_2 - t_1} \quad (1)$$

Where, M = growth rate, x_1 and x_2 are biomass concentration at time interval t_1 and t_2, respectively.

For protein determination, 6 mL 0.5 N NaOH was added to filtered algal samples then sonicate for two minutes and 80°C at water bath for 20 min. Then the sample was centrifuged in 3000 rpm for 10 min. The protein content was then measured by Bradford method[14] using BSA as protein standard. Carbohydrate was determined based on method of Kochart[15] using glucose as a standard. Total Lipid determination was done based on the method of Bligh and Dyer[16] as modified by Kates and Volcani[17].

RESULTS AND DISCUSSION

Results presented in Fig. 1 shows the specific growth rate of *S. platensis* and *S. fusiformis*. Figure 1 also shows comparison of the results with those found highest in previous experiments of light, temperature and pH effects.

Specific growth rate were 0.134 and 0.138/day, respectively for *S. platensis* and *S. fusiformis*. The biomass was 2.7 and 2.9 g L^{-1} for *S. platensis* and *S. fusiformis*, respectively. It is found from the findings that the specific growth rate and chlorophyll concentration achieved for *S. fusiformis* in the culture was significantly higher than that of *S. platensis* ($p<0.05$). This might be state that *S. fusiformis* has the genetic properties to grow fast and could be utilized more in culture. The specific growth rate and biomass of both *S. platensis* and *S. fusiformis* achieved in the present study are significantly higher than those achieved in other cultures ($p<0.05$). These results suggested that whether favorable environmental conditions could be maintained a good biomass production is expected, which also favors protein production. As biomass produced in this experiment is high than previous experiment, protein content attained per unit area is also high. Paoletti *et al.*[18] reported chlorophyll content of *Spirulina* was 8-15 g^{-1}. Henrickson[19] obtained 10 mg g^{-1} chlorophyll-a in *S. platensis* at the Earthrise farm. In present study chlorophyll content found higher than these findings.

Protein, carbohydrate and lipid content attained in the experiment are plotted in the Fig. 2. The results also indicate the comparison of protein, carbohydrate and lipid content attained in previous experiments of environmental factors. Protein content of *S. platensis* was 58.6%. This value is vaguely lower than that achieved in previous experiments and the difference is not statistically significant. Carbohydrate content achieved in the present study of *S. platensis* is significantly lower than that attained in temperature and pH effect culture but significantly higher than that attained in light effect culture ($p<0.05$). Lipid content obtained for *S. platensis* was 7.4%. Lipid content achieved in the present study of *S. platensis* is statistically same with that attained in pH, temperature and light effect culture ($p>0.05$). Protein content of *S. fusiformis* was 61.8%. This value is significantly higher ($p<0.05$) than that attained in temperature effect culture but statistically same to pH and light effect culture ($p>0.05$). Carbohydrate content achieved of *S. fusiformis* was 18.2%. Carbohydrate content achieved of *S. fusiformis* is significantly higher than that achieved in pH effect culture but significantly lower than that attained in temperature effect culture

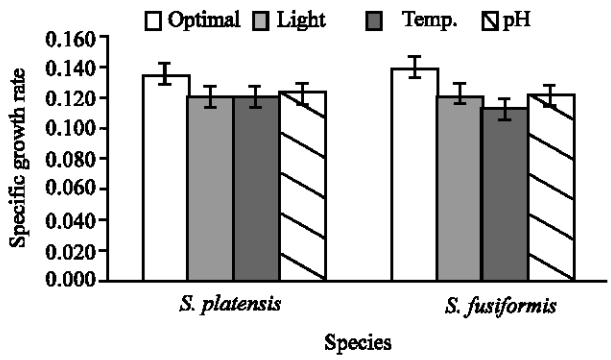

Fig. 1: Specific growth rate of *S. platensis* and *S. fusiformis* grown in optimum environmental condition. The values are means of three replicates.

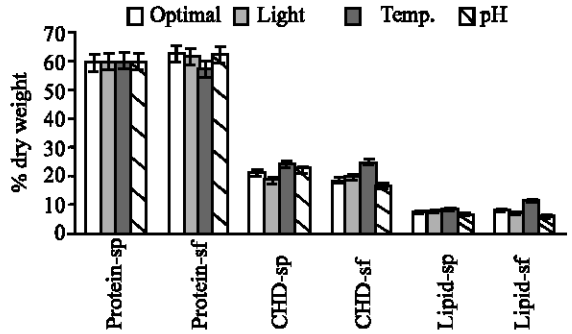

Fig. 2: Biochemical composition of *S. platensis* and *S. fusiformis* grown in optimum environmental condition. The values are means of three replicates. sp = *S. platensis*, sf = *S. fusiformis*, CHD = carbohydrate

($p<0.05$). Carbohydrate content achieved is statistically same compared to that attained in light effect culture ($p>0.05$). Lipid content obtained of S. *fusiformis* was 6.9 %. Lipid content achieved of *S. fusiformis* is significantly higher than that achieved in pH and light effect culture but significantly lower than that attained in temperature effect culture ($p<0.05$).

Ciferri[20] claimed that the culture conditions, as temperature, light intensity and pH etc were known to change the protein content of the blue-green algae *S. platensis*. Protein content of *Spirulina* grown commercially for health food may range from 55 to 70% dry weight[21] according to different effort of optimization. These findings agree favorably with the present study as because protein content gained in this experiment was 58.6 and 61.7% in *S. platensis* and *S. fusiformis*, respectively. Carbohydrates are energy-yielding nutrient, which are mainly branched polymer of glucose forming 15-20% of the dry weight of *Spirulina*[20]. Belay *et al.*[2] also reported the value of carbohydrate in this blue green algae as in the range of 15-25%. Hence the present findings (20.4 and 18.2%) are in accordance with earlier observation. Typical of cyanobacteria, which are poor in lipids, *Spirulina* contains only 6-13% lipids half of which are fatty acids. Tatichareon *et al.*[22] stated that fatty acids and lipids of *Spirulina* depend on strain and on the environmental conditions. Tedesco and Duerr[23] studied the effects of some factors such as light, temperature and nitrogen to optimize the production on the total lipid content of *S. platensis* and recorded the total lipid content of this strain as 7.2%. These results compare favorably with the present study since lipid content found 7.4% *S. platensis* and 8.2% for *S. fusiformis*, which is significantly higher than previous studies.

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