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Optimization of Biomass Production of *Spirulina maxima*

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ABSTRACT-

Spirulina maxima is known to be useful to man in virtually all aspects of life including health, food and cosmetics. In the present study, we set out to investigate how a combination of a set of parameters, namely temperature, light intensity, pH and agitation, affect maximum production of biomass, chlorophyll a and protein. Through manipulating environmental condition of the algal growth, one can modify the biomass production. In the present investigation the production of *Spirulina maxima* was optimized in terms of biomass and metabolites. The dry weight of *Spirulina maxima* was 0.73g/500ml and protein and Chlorophyll a content were 63.8% and 13.1mg/gm respectively at pH 9. At 5 Klux light intensity the dry weight of *Spirulina maxima* was 0.72g/500ml while protein content and Chlorophyll a were 64.2% and 9.5mg/gm respectively. Aeration is also very important factor for production of *Spirulina*. Continuous mixing of the culture medium is required to prevent cell sinking and thermal stratification to maintain the even nutrient distribution and to remove excess oxygen. In terms of aeration and

non-aeration the values were 2.35 g/500ml in aeration system and 1.81 g/500ml for non-aeration system.

Key words - Biomass, Chlorophyll a, Light intensity, Aeration, *Spirulina maxima*.

INTRODUCTION

Spirulina maxima is a planktonic photosynthetic filamentous cyanobacterium that forms massive populations in tropical and subtropical bodies of water which have high levels of carbonate and bicarbonate . For centuries, native peoples have harvested *Spirulina maxima* from Chad Lake in Africa and Texcoco Lake in Mexico for use as a source of food [1], a fact which means that Spirulina deserves special attention both as a source of single cell protein (SCP) [2] and because of its nutraceutical properties.

Chemical composition of *Spirulina* indicates that it has high nutritional value due to its content of a wide range of essential nutrients, such as provitamins, minerals, proteins and polyunsaturated fatty acids such as gamma-linolenic acid [3]. More recently, *Spirulina* has been studied because of its therapeutic properties[4] and the presence of antioxidant compounds [3, 5], such as phenolics. The occurrence of phenolic compounds in plants is well documented and these compounds are known to possess antioxidant activity in biological systems but the antioxidant characteristics of algae and cyanobacteria are less well documented, although decreased cholesterol levels have been reported in hypercholesterolemic patients fed Spirulina 6 and the antioxidant activity of phycobiliproteins extracted from Spirulina maxima has also been demonstrated[5].

The influence of growth conditions on the chemical composition of *Spirulina* has been studied by many researchers with the purpose of optimizing the production of economically and nutritionally interesting compounds, especially gamma-linolenic acid (GLA) and phycocyanin [7, 8.] Although the use of *Spirulina* for the production of phycocyanin for use as a natural pigment seems commercially efficient, the production of GLA from this cyanobacterium presents higher costs when compared to production using other sources [7.] The extraction of nutritionally active compounds in pure form is expensive, but

the direct consumption of *Spirulina* as a nutraceutical food is a viable alternative. Growth conditions optimized for biomass production and productivity are usually used in the commercial production of *Spirulina* without considering chemical composition, but higher concentrations of potentially useful compounds such as polyunsaturated fatty acids, proteins and phenolics can be obtained by manipulating growth conditions.

The objective of the work presented in this paper was to evaluate the physical factors for growth of *Spirulina maxima* and temperature on the protein, lipid and chlorophyll a, maximum specific growth rate and productivity of *Spirulina maxima* at different cultural condition.

MATERIALS AND METHODS-

Microorganism and culture medium

The strain of *Spirulina maxima* was obtained from School of Studies in Biotechnology Jiwaji University Gwalior M.P., which is previously maintained in Zarrouk's agar media slants in 4°C [9]. All the reagents used were of analytical grade, obtained from the Rankam Chemical Co.

Cultivation

Spirulina maxima was axenically grown in Zarrouk's medium. Cultures were incubated in a culture room at temperature of 30 ± 2°C and illuminated with day- light fluorescent tubes saving 4Klux at the surface of the vessels. During the process of growth the flask was shaken 3 to 4 times per day. The experiments were run in duplicates. All manipulation involving the transfer of cultures in the liquid media or on agar plates were carried out under aseptic conditions in a laminar flow.

Filtration: - Cells were collected by filtration using filter paper 8 mm pore size (Screen printing paper).

Washing: - Cells were washed with buffer solution (pH 7), diluted to known volume and processed for further inoculation.

Shaking in cyaclomixture:- Diluted inoculum shaken in cyaclomixture for making homogenized mixture.

Analysis of variance (ANOVA) was used to compare the data during experiments.

Analytical methods

Biomass concentration (gl^{-1}) was calculated by measuring dry weight. For dry weight measurement homogenous suspensions of known quantity of *Spirulina* sample were filtered through screen-printing paper and oven dried at 75°C for 4 to 6 hours. The dried filter paper containing

Spirulina biomass were cooled and weighed. The difference between the initial and final weight were taken as the dry weight of *Spirulina* biomass. The dry weights were expressed in terms of g/l.

Chlorophyll a was estimated by the Mackinney method [10].

Protein was determined by the Lowry method[11].

As pH is important for the growth of *Spirulina* for biomass, different pH levels *viz.* 7, 8, 9, 10, 11, 12 were set for the experiment. The pH was set with the help of 8m NaOH and 1N HCl solution. Flasks were prepared as described above and inoculated equal biomass concentration of *Spirulina maxima* in 500ml-modified Zarrouk's media. Subsequent harvest for biomass estimation and above pH the test *Spirulina maxima* of after 20 days.

As light is important for the photosynthesis of *Spirulina maxima*, different light intensities such as 3 Klux, 3.5 Klux, 4 Klux, 4.5 Klux and 5 Klux light were set for the light intensity test (The culture was prepared in flasks as explained before). The flasks were taken in triplet for each light intensity.

Growth of Spirulina maxima in laboratory : Six 1500ml transparent plastic pots each containing 1000ml of the growth medium were set up. One set of three pots was aerated using an aquarium pump which pumped air at 150 bubbles per minutes through a drip set (plastic tubing) fitted with a regulator. Another set was kept in non-aerated condition. The same amount of culture of *Spirulina maxima* was inoculated in both set of pots and harvested after 25 days [12.]

RESULTS AND DISCUSSION-

Culture of *Spirulina maxima* in conical flask has its limitation in providing complete information related to growth, development and production of value added chemicals viz. vitamins, amino acids, fatty acids, protein and polysaccharides both in quantity and quality and disposing of carbon dioxide one of the major causes of global warming [13,14]. Extensive research has been conducted on production of *Spirulina maxima* living at salt lakes in the tropical regions [15-17.]

Physico-chemical profiles of *Spirulina maxima* describes the relationship between growth and environmental factors especially irradiance flux, density and temperature [18], which are important in the evolution of micro algae and cyanobacteria for biomass production, as well as their general characterization. High alkalinity is

mandatory for the growth of *Spirulina maxima* and bicarbonate is used to maintain high pH[19-21] . Sources of nutrition also affect the growth rate of cyanobacteria[22.] The growth of *Spirulina maxima* is maximum at 30-35ºC. Because the *Spirulina maxima* thallus had previously been adapted to the medium there was no lag phase. It has been shown by previous workers[23] that the optimal growth temperature for *Spirulina maxima* is between 30 and 35 °C.

The pH value of the culture medium combined with dry cell weight may be an indirect method for determining the degree of cell growth of *Spirulina maxima*. This is because the pH gradually rises as bicarbonate added to the culture medium is dissolved to produce CO_2, which releases OH- during cultivation of *Spirulina maxima*[24,25]. *Spirulina maxima* was grown at different pH (7, 8, 9, 10, 11, and 12) in flask culture and monitored and expressed in term of dry weight (Table 1).

Table 1- Effect of different medium pH on biomass production of Spirulina maxima

S. No.	Initial pH of ZM media	Dry weight in g/500ml (Mean±Sem)	Final pH of culture (Mean±Sem)	Chl a content in mg/g (Mean±Sem)	Protein content in % of dry weight (Mean±Sem)
1	7	0.64±0.042	9.34±0.16	11.0±0.65	60.5±0.15
2	8	0.68±0.035	9.53±0.13	11.4±0.20	58.5±0.15
3	9	0.73±0.056	10.02±0.18	13.1±0.45	63.8±0.20
4	10	0.25±0.015	10.16±0.09	6.2±0.22	55.2±0.16
5	11	0.11±0.020	10.20±0.04	6.5±0.15	47.4±0.20
6	12	0±0.0	11.95±0.02	0±0.0	0±0.0

Growth Condition –Light Intensity- 3.5 Klux; Inoculum - 1g/500ml; Relative Humidity- 75%; Room Temperature- 28±2 ºC ; Incubation Time- 25 days

The maximum bulk density about 0.73 g/500ml was noticed when the pH of culture medium was, maintained at 9.0 with medium volume 500 ml in a 1000 ml flask. The maximum bulk density was attained on 25th day after the inoculation of culture in medium. The increase in the production of *Spirulina maxima* could have been due to the availability of mire space, oxygen and light to the culture flask. Earlier results also demonstrated that optimum pH for maximum growth of *Spirulina maxima* was 9 to 9.5 ranges[19]. *Spirulina maxima* is considered to be a alkalophilic organism by nature[20]. Chlorophyll a content and protein content were also maximum in pH 9. The Chlorophyll a content was 13.1 mg/g and protein content was 63.8 % of dry weight. Similar studies have also been done by various workers of cyanobacteria [26, 27].

The duration, intensity and quality of light are the most important factors in the success of photosynthetic organism. The synthesis of various cell components is known to be influenced by light intensity. Sorokin, *et al.*, (1965)[28] had reported that an increase in light intensity first favors cell division then, after the optimal light intensity was attained, a further increase in light intensity inhibited cell division. Dubey (2006)[29] had reported moderate light intensity in the cultivation of *Spirulina*, suggesting low light intensity at the beginning to avoid photolysis. He also noted that exposing *Spirulina* to high light intensity photolysis them. Result of the present study suggests 5 Klux is optimum light for the growth of *Spirulina maxima* in subtropical region of Madhya Pradesh (Table 2).

Table 2 – Effect of different light intensity on biomass production of *Spirulina maxima*

S.No.	Light intensity	Dry weight in g/500ml (Mean±Sem)	Chl a content in mg/g (Mean±Sem)	Protein content in % of dry weight (Mean±Sem)
1	3Klux	0.54±0.050	14.0±0.072	58.8±0.056
2	3.5Klux	0.58±0.030	13.2±0.042	61.0±0.080
3	4Klux	0.66±0.070	11.2±0.035	63.3±0.075
4	4.5Klux	0.69±0.084	11.4±0.072	60.2±0.035
5	5Klux	0.72±0.022	9.5±0.075	64.2±0.039

Growth Condition – Initial pH - 8.25; Inoculum - 1g/500ml; Relative Humidity- 75%; Room Temperature - 28±2 °C; Incubation Time- 25 days

The Chlorophyll a and protein content were 9.5mg/g and 64.2 % at 5 Klux light intensity. Chlorophyll a content was maximum at 3 Klux light intensity, which was 14.0 mg/g. The similar studies were done by Danesi, *et al.*, (2004)[30], Richmond, *et al.*, (1986) [25]; Ogbonda, *et al.*, (2007)[12].

Aeration provides agitation of growing cells to maintain the cells in suspension has been described as very necessary in good quality and better yield of Spirulina species. Richmond, (1986)[25] also noted that continuous mixing of the culture medium is required to prevent cell sinking and thermal stratification, maintain even nutrient distribution and to remove excess oxygen. The present study demonstrated that the *Spirulina maxima* produced higher biomass when the growth medium was bubbled with air (aerated) than when the medium was not bubbled with air (non aerated). At the end of 25 days incubation period, biomass obtained was 2.35 g/l in aerated condition and 1.81 g/l in non-aerated condition. The Chlorophyll a content was also more in aerated condition. In aerated condition Chlorophyll a content was 13.5 mg/g and in non-aerated condition it was 12.8 mg/g. The higher protein content had been observed in aerated condition (61.3%) and 58.6 % in non-aerated condition (Table 3).

Table 3- Effect of Aeration on biomass production of *Spirulina maxima*

Days	Non-Aeration Condition			Aeration Condition		
	Dry Weight in g/l (Mean±Sem)	Chl a content in mg/g (Mean±Sem)	Protein content in % of dry weight (Mean±Sem)	Dry Weight in g/l (Mean±Sem)	Chl a content in mg/g (Mean±Sem)	Protein content in % of dry weight (Mean±Sem)
5 days	0.50±0.040	11.5±0.04	56.0±0.52	1.02±0.40	12.5±0.04	60.0±0.52
10 days	1.10±0.035	11.8±0.02	57.5±0.30	1.26±0.35	12.8±0.02	60.2±0.20
15 days	1.38±0.02	12.2±0.05	58±0.64	1.65±0.20	13.1±0.05	60.5±0.40
20 days	1.65±0.025	12.6±0.04	58.5±0.32	1.98±0.25	13.4±0.04	60.8±0.82
25 days	1.81±0.030	12.8±0.06	58.6±0.58	2.35±0.30	13.5±0.06	61.3±0.62

Growth Condition – Light Intensity- 3.5 Klux; Inoculum- 1g/l; Relative Humidity - 75%; Room Temperature - 28±2 °C ; Initial pH - 8.25

SUMMARY

This paper has demonstrated that temperature has an important influence on the production of biomass, proteins and chlorophyll a by *Spirulina maxima*. In the present study, we set out to investigate how a combination of a set of parameters namely temperature, light intensity, pH and agitation affect maximum production of biomass and protein. On the basis of utility, *Spirulina maxima* can be cultured under variable natural, artificial and laboratory conditions. Nutrients content of *Spirulina maxima*

depends on the location and environment in which the cyanobacterium grows. Percentage of specific components of *Spirulina maxima* can increase or decrease according to need by growing under regulated growth conditions

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