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Evaluation of Biomass Production of *Spirulina maxima* on Different Reported Media

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ABSTRACT

In the present investigation the production of *Spirulina maxima* was optimized in terms of biomass and metabolites. In the present study, effect of different culture media for *Spirulina maxima* growth, protein and chlorophyll a content were studied. Zarrouk medium was found to be more favorable for the growth of alga. All together six culture media were included in this study.

Keywords: *Spirulina maxima*, Biomass, Chlorophyll a, Protein.

INTRODUCTION

Spirulina maxima is a microscopic unbranched, filamentous blue-green alga, rich in protein, vitamins especially vitamin B12 and pro-vitamin A (beta-carotene), iron, essential amino acids, minerals and essential fatty acids like gamma linolenic acid [1]. The *Spirulina maxima* has gained importance and international demand for its high value phytonutrients and pigments, which have applications in health foods, feed, therapeutics and diagnostics. It has been hailed as the ‘‘Food of the future’’, besides being considered as an ideal food for astronauts by NASA. It represents the second most important commercial

microalga for the production of biomass as health food and animal feed (after Chlorella) [2, 3].

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, a fact which means that *Spirulina maxima* deserves special attention both as a source of single cell protein (SCP) and because of its nutraceutical properties [4].

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

MATERIALS AND METHODS

Organism

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^{o}C. All the reagents used were of analytical grade, obtained from the Rankam Chemical Co. Sodium carbonate was added after autoclaving and pH was adjusted to 9.0. Growth and maintenance of the culture was done in an illuminated (5 Klux) growth room at 30±2 ^{o}C under 12/12 hour light-dark cycles. Manual shaking of cultures was done 3-4 times daily.

Media

Six media were included in this study. For this experiment we were take Zarrouk's media[5], Rao's media[6], CFTRI media[7], OFERR media[6], Revised Media (6)[3] and Bangladesh Medium No.(3)[8] (composition of all media described in Table 1, Table 2, Table 3, Table 4, Table 5,Table 6 respectively). Prepared 500ml each medium in 1000ml flask and inoculate the same amount of inoculums in each medium. After 20 days harvest the biomass of *Spirulina maxima* in the form of dry weight.

Table -1 Composition of Modified Zarrouk's Media

Ingredients	**g/l**
Sodium bicarbonate	16.8
Di-potassium hydrogen phosphate	0.5
Sodium Nitrate	2.5
Potassium Sulphate	1.0
Sodium Chloride	1.0
Magnesium Sulphate	0.2
Calcium Chloride	0.04
Ferrous Sulphate	0.01
Ethylene Diamine Tetra Acetate. Na	0.08
A_5 Solution	1 ml

Table -1.1 Composition of A_5 Solution

Ingredients	**g/l**
Boric Acid	2.860
Manganese Chloride	1.810
Zinc Sulphate	0.222
Sodium Molybedate	0.0177
Copper Sulphate	0.079

Table -2 Composition of Rao's Media

Ingredients	**g/l**
Sodium bicarbonate	15.0
Di-potassium hydrogen phosphate	0.50
Sodium Nitrate	2.50
Potassium Sulphate	0.60
Sodium Chloride	0.20
Magnesium Sulphate	0.04
Calcium Chloride	0.008
Fe Ethylene Diamine Tetra Acetate	0.20
A_5 Solution	1 ml

Table -3 Composition of CFTRI Media

Ingredients	**g/l**
Sodium bicarbonate	4.5
Di-potassium hydrogen phosphate	0.5
Sodium Nitrate	1.5
Potassium Sulphate	1.0
Sodium Chloride	1.0
Magnesium Sulphate	1.2
Calcium Chloride	0.04
Ferrous Sulphate	0.01

Table -4 Composition of OFERR Media

Ingredients	**g/l**
Sodium bicarbonate	8.0
Sodium Chloride	5.0
Urea	0.2
Potassium Sulphate	0.5
Magnesium Sulphate	0.16
Ferrous Sulphate	0.05
Phosphoric Acid	0.052ml

Table -5 Composition of Revised Medium 6

Ingredients	**g/l**
Calcium Chloride	0.04
Sodium Chloride	0.50
Single Super Phosphate	1.25
Muriate of Potash	0.89
Magnesium Sulphate	0.15
Sodium bicarbonate	8.0
Sodium Nitrate	2.50

Table -6 Composition of Bangladesh Medium No. 3

Ingredients	g/l
Sodium bicarbonate	2.0
Urea	0.05
Sodium Chloride	1.0
Gypsum	1.5

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^{o}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 culture was grown at 30±2 ^{o}C in 5 Klux. Samples were taken in triplicate and dry weight, chlorophyll and protein content were determination. The dry weights were expressed in terms of g/l. Chlorophyll a was estimated by the Mackinney method[9]. Protein was determined by the Lowry method[10].

Statistical analyses

The data recorded in triplicate for the parameters in various strains were subjected to ANOVA (analysis of variance).

RESULTS AND DISCUSSION

Large-scale production of cyanobacterial biomass is essentially a complex process involving a large number of variables and for their successful growth; the environment needs to be conditioned to meet as many of the essential requirements of the organism. Among the several constraints to the multiplication of cyanobacteria physical, physiological and economic limitations are of major importance. In tropical countries, especially developing countries such as India, emphasis is placed more on the production costs [11, 12].

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* describe the relationship between growths and environmental factors especially irradiance flux, density and temperature, 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. Sources of nutrition also affect the growth rate of cyanobacteria[18, 19]. The growth of *Spirulina maxima* is maximum at 30-35^{o}C. Because the *Spirulina maxima*

thallus had previously been adapted to the medium there was no lag phase.

In the present study we investigated comparative growth rate of *Spirulina maxima* on Zarrouk media, Rao's media, CFTRI media, OFERR media, Bangladesh Medium No. 3 and Revised Medium 6. The growth of *Spirulina maxima* in flask culture was monitored and expressed in terms of dry weight.

Table 7 shows that the specific dry weight of *Spirulina maxima* was 0.78g/500ml on Zarrouk medium, 0.42g/500ml on Rao' s medium, 0.38g/500ml on CFTRI medium, 0.60g/ml on OFERR medium, 0.056g/500ml on Bangladesh medium no. 3, 0.65g/500ml on Revised medium 6. The data shows that specific growth of *Spirulina maxima* is higher of Zarrouk medium. The Chlorophyll *a* content of *Spirulina maxima* was13.0mg/g on Zarrouk medium, 10.1mg/g on Rao's medium, 10.5mg/g on CFTRI medium, 12.90mg/g on OFERR medium, 6.0mg/g on Bangladesh medium no. 3 and 12.0mg/g on Revised medium 6. The protein content of *Spirulina maxima* was 62.0 % on Zarrouk medium, 55.2 %on Rao's medium, 61.0 % on CFTRI medium, 58.4 % on OFERR medium, 40.2 % on Bangladesh medium no. 3 and 60.4 % on Revised medium 6 (Fig 1). The Similar studies were done by Seshadri, *et al.*, (1978)[20].

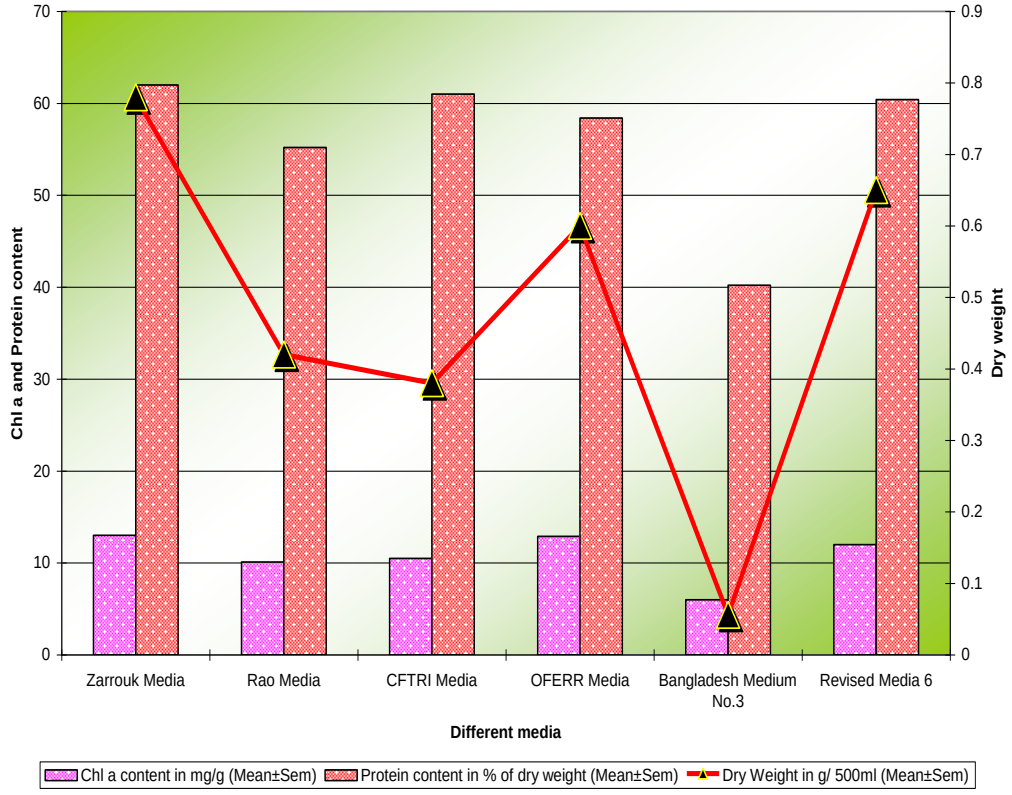
Table -7 Evaluation of Biomass Production of *Spirulina maxima* on Different Reported Media

S.No.	Reported Media	Dry Weight in g/ 500ml (Mean±Sem)	Chl a content in mg/g (Mean±Sem)	Protein content in % of dry weight (Mean±Sem)
1	Zarrouk Media	0.78±0.041	13.0±0.023	62.0±0.042
2	Rao Media	0.42±0.020	10.1±0.020	55.2±0.045
3	CFTRI Media	0.38±0.032	10.5±0.025	61.0±0.020
4	OFERR Media	0.60±0.011	12.90±0.020	58.4±0.015
5	Bangladesh Medium No.3	0.056±0.020	6.0±0.022	40.2±0.017
6	Revised Media 6	0.65±0.021	12.0±0.042	60.4±0.087

Growth Conditions – Light Intensity - 5 Klux; Inoculum - 1g/500ml; Relative Humidity - 75%

Room Temperature- 30±2 ^{o}C; Incubation Time- 20 days

Fig 1- Evaluation of Biomass Production of *Spirulina maxima* on Different reported Media



CONCLUSION

In the present course of investigation, we have optimized *Spirulina maxima* production in term of biomass and metabolites production. Production of *Spirulina maxima* was carried out *in vitro* cultivation. This investigation was taken up with the basic aim of providing a series of media for the production of *Spirulina maxima*, when evaluated in terms of chlorophyll, protein and dry biomass.

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