International Journal of Recent Biotechnology
Available online at www.ijrbp.net

ISSN: 2322 – 0392
Int. J. Rec. Biotech. **2013,** 1 (1): 17-20

Research Article

IJRB

Study of mass production of *Spirulina maxima* under different Physical and Chemical environment

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ABSTRACT

Spirulina maxima was cultivated in different mediums like Zarrouk's medium, CFTRI, Bangladesh media, A-5 solution etc. with different physical and chemical conditions like pH, light intensity, temperature etc. they were monitored for 20 days on daily basis. Maximum biomass was found in Zarrouk's medium at pH 8, 25^{0}C temprature, 1900 lux light intensity. However results of present investigation could be consider for commercial cultivation of Spirulina using different physical and chemical environment for mass production of Spirulina maxima.

Keywords: *- Zarrouk's medium, Spirulina maxima, CFTRI, Bangladesh media.*

INTRODUCTION

Spirulina is a microscopic blue-green aquatic plant and it is the nature's richest and most complete source of organic nutrition.[1] Spirulina is cultivated in tropical and subtropical bodies of water and filamentous form of cyanobacteria. Spirulina is type of filamentous blue green alga due to the capacity to produce bioactive components such as vitamins, minerals, polyunsaturated fatty acid, carotenes, and other pigments that have an antioxidant activity to receiving attention spirulina have a antioxidant capacities to attribute biliproteins called as phycocyanin. Proteins (60%-70%), vitamins, essential amino acid, minerals and essential fatty acid such as palmitic acid, linolenic acid and linoleic acid are produced by sprulina. Spirulina, a blue-green alga, is now becoming a health food worldwide.[2] It is a multicellular, filamentous cyanobacterium belonging to algae of the class Cyanophyta.To over come this problem, alternative protein sources, other than conventional cereal protein were explored. Amongst sources tried i.e. poultry and fish, the single cell proteins were found to be convenient and cheapest. This search led to the rediscovery of the Cyanobacterium spirulina, which proved to be the richest sources of protein and other essential nutrients.[3,4]

The free floating filaments of both S.maxima are densily granulated at the cross-walls because of the presence of gas vacuoles (aerotopes), but those of the latter species display a more regular disposition of this granulation.[5]

Transmission Electron Microscope observations show for Spirulina prokaryotic organization, capsules, pluri-stratifed cell wall, photosynthetic or thylakoid lamella system, ribosome and fibrils of DNA region and numerous inclusions. The capsule has fibrillar structure and covers each filament protecting it. The irregular presence of capsule around the filaments in S. maxima is a differencing morphological characteristic to compare with S. maxima. S. maxima has been usually cultured at various places in world under natural growth condition but the use of a culture pool, which has a relatively shallow depth for exposing the culture containing cyanobacteria to sunlight. S. maxima grows better in a liquid environment or culture medium of high pH and alkalinity. It forms massive population in tropical and bicarbonate and high pH. The fame of s. maxima is a result of its economical importance, which is due to its nutritional and biomedical values.[6]

Physico-chemical profiles of S. maxima is describing the relationship between growth and environmental factors especially irradiance flux, density and temperature, which are important in the evolution of microalgae and cyanobacteria for biomass production, as well as their general characterization. High alkalinity is mandatory for the growth of S. maxima and bicarbonate is used to maintain high pH. Source of nutrition also affect the growth rate of cyanobacteria.

MATERIALS AND METHODS

Sterilization

All glassware and equipment used were cleaned thoroughly before use. The glassware's were kept for an over night treatment with potassium dichromate solution-5gm, $K_2Cr_2O_7$, 100 ml D/W, 100 ml concentrate H_2SO_4 **.** They were then washed under running tap water with mild detergent and then finally rinsed with D/W prepared by vertical quartz distillater. The flasks, Petri dishes and other glassware's are sterilized in a hot air oven. The temperature required would depend upon the length of time during which the heat is applied. Temperature of 100 ± 2ºC or above for three hours are generally used. All the medias, distilled water and other wet materials used in different experimental set up were sterilized by autoclaving them at 121 ± 2ºC at 15 lb per square inch for 20 minutes.

Organism

The strain of *Spirulina maxima* was collected or obtained from IARI, New Delhi, which is previously maintained in Zarrouk's agar media slants in 4ºC.

Maintenance and multiplication of culture

A Spirulina maximum was auxenically grown in Zarrouk's medium. Firstly, we have transferred our culture in Zarrouk's broth from Zarrouk's agar slants. Culture were incubated in a culture room at temperature of 30±2ºC and illuminated with day-light fluorescent tubes saving 4 Klux at a surface of vessels. During the process of growth the flask was shaken 3 to 4 time per day. The experiment was run in triplicates. All manipulation involving the transfer of culture in the liquid media or on agar plates were carried out under aseptic conditions on a laminar air flow.

Preparation of Zarrouk's media as following step.

Firstly we had taken sterilized 1000ml conical flask.Then we had taken 500ml D/W in flask. After that mix properly all component of Zarrouk's media which has described previously in table (1.1)Autoclaved 500ml media for 15 to 20 minutes.

Preparation of inoculum:

Inoculum preparation for culture maintenance taken well-developed biomass concentration of Spirulina culture, which has inoculated before 20 to 25 days in Zarrouk's media.

Filtration: - Cells were collected by filtration using filter paper 8mm 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 cyclomixture:- Diluted inoculum shaked in cyclomixture for making homogenized mixture.

Dry weight measurement:

For dry weight measurement homogenous suspension of known quantity of *Spirulina* sample were filtered through screen-printing paper and oven dried at 75ºC for 2 to 6 hours. The dried filter paper containing *Spirulina* biomass were cooled and weighted. 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 gm/litre.

RESULTS AND DISCUSSION

When the mass production of S.maxima is tested under different physical and chemical conditions following results are obtained-

Effect on growth of *S. maxima* at different pH

S. maxima was grown at different pH (7, 8, 9, 10, 11) in flask culture and monitored and expressed in term of dry weight. The maximum bulk density about 2.34gm was noticed when the pH of culture medium was maintained at 8.0 with medium volume 500ml in a 1000ml flask. The maximum bulk density was attained on 20th day after the inoculation of culture in medium. The increase in the production of *S . maxima* could have been due to the availability of mire space, oxygen and light to the culture flask.

Result described in fig 3.2 suggest that *S. maxima* was grown on different pH 7,8,9,10,11, but the maximum yield of *S. maxima* is obtained on 8>11>7>9>10. Thus 8 pH is optimum for the growth of *S. maxima*.Earlier results also demonstrated that optimum pH for maximum growth of *S. maxima* was 9 to 9.5 ranges *S. maxima* is considered to been alkalophilic organism by nature [Table 1]

Effect on growth of *S. maxima* at different light intensity-

Maximum bulk density of *S. maxima* obtained in 1900 lux. Culture was grown at different light intensity viz. 740 lux, 1200 lux, 1900 lux in flask and result expressed in term of dry weight. Maximum growth of *S. maxima* was noticed a flask which has maintained 1900 lux light with medium volume 500ml in a 1000ml flask. About 2gm of *S. maxima* biomass was measured at this phase in 20 days harvesting period. Result suggests 1900 lux is optimum light for the growth of *S. maxima* in subtropical region of rajasthan. [Table 2]. Earlier results were similar to the research paper. And also many researchers have been demonstrated at different-different light intensity in different-different region.

Effect on growth of *S. maxima* at Change in concentration of media i.e. $NaNO_3$-

Maximum bulk density of *S. maxima* obtained in 2.0 gm of **NaNO3**. Culture was grown in different media concentration viz. 0.75gm, 0.01gm, 2.0gm in flask and result expressed in term of dry weight. Maximum growth of *S. maxima* was noticed in a flask which has 2.0gm of **$NaNO_3$** with medium volume 500ml in a 1000ml flask. About 0.18gm of *S. maxima* biomass was measured at this phase in 20 days harvesting period. Result suggests that 2.0gm is optimum concentration for the growth of *S. maxima* in subtropical region of Rajasthan. [Table3].

Effect on growth of *S. maxima* at different temperature condition-

Maximum bulk density of *S. maxima* obtained at room temperature (35^0C) 1200 lux. Culture was grown at different temperature in flask and result expressed in term of dry weight. Maximum growth of *S. maxima* was noticed a flask which has maintained at room temperature (35*c*)1200 lux with medium volume 500ml in a 1000ml flask. About 1.18gm of *S. maxima* biomass was measured at this phase in 20 days harvesting period. Result suggests room temperature for the growth of *S. maxima* in subtropical region of rajasthan. [Table 4].

Effect on biomass of *S. maxima* in different media-

In the present study comparative growth of *S. maxima* was investigated on ZM media; A5; CFTRI media, and Bangladesh medium No. 3, Revised medium 6 media. The growth of S. *maxima* in flask culture was monitored and expressed in terms of growth response.

The protein content of *S. maxima* on Zarrouk's medium was 62.5%; 61.4% CFTRI medium, 60% on Revised medium 6 and 40.7% on Bangladesh medium No.3. The result shows maximum bulk density is obtained in Zarrouk's media in comparison to other media.

Table 1: Different pH concentration

S.No.	pH	Volume *(in ml)*	Intensity of light (*in lux*)	Temperature (*in c*)	Duration *(in days)*	Biomass (*in grms*)
1	7	500	840	25	20	1.82
2	8	500	840	25	20	2.34
3	9	500	840	25	20	1.26
4	10	500	840	25	20	1.4
5	11	500	840	25	20	2.16

Table 2: Different Light condition

S.No.	pH	Volume *(in ml)*	Intensity of light (*in lux*)	Temperature (*in c*)	Duration *(in days)*	Biomass (*in grms*)
1	8	500	740	25	20	1.2
2	8	500	1200	25	20	1.24
3	8	500	1900	25	20	2

Table 3: Change in concentration of media i.e. $NaNO_3$

S.No.	pH	Vol. (*in ml*)	Intensity of light (*in lux*)	Con. of $NaNO_3$ (*in gm*)	Duration (*in days*)	Biomass (*in gm*)
1	8	500	840	0.75	20	0.14
2	8	500	840	1.01	20	0.14
3	8	500	840	2.0	20	0.18

Table 4: Different temperature condition

S.No.	pH	Vol. (*in ml*)	Intensity of light (*in lux*)	Temp. (*in c*)	Duration (*in days*)	Biomass (*in grms*)
1	8	500	1200	25	20	1.4
2	8	500	1200	35	20	1.18

Table 5: Response of various Medias on Spirulina growth

Medias	Response
Zarrouks Media	+++++
Zarrouks media with 0.5ml – BAP+NAA	+++
Zarrouks media without autoclaving	++
Zarrouks media with tap water	+++
CFTRI	--
Improved CFTRI	+
Bangladesh media	++
A-5 solution	--
RM-6 Medium	+++

- - No Response

\+ - Average

++ - Good

+++++ - Very Good

CONCLUSION

It is cultivated on a large scale as a monoculture in intensive outdoor cultivation system. Standardization of Spirulina in different media was summarized maximum growth noticed in Zarrouk's media. As it is after the treatment of different pH the best growth resulted in pH 9. Aeration effect was important for Spirulina cultivation.

Aeration agitates the growth medium and this gives homogenous distribution of Spirulina filaments throughout the growth vessel for adequate exposure to illumination uniformly and removes some inhibitory substances produced such as carbon dioxide. This phenomenon is similar in outdoor cultivation of Spirulina strain. Aeration is essential for the cultivation of the Spirulina maxima it is 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.

In conclusion, the result of this investigation shows that pH and light intensity are very important factors in biomass production.

REFERENCE

1. Annapurna, V. et al. National Institute of Nutrition, India. **1991,** 10: 145-151.
2. Baojiang. G et al. South China Normal University. Pub. In Proceed of second Asia Pacific Conference on Algal Biotechnology, University of Malaysia, **1994,** p. 33-38.
3. Others, S., and Pire, R. Fatty acid composition of Chlorella and Spirulina Microalgae species. *J. AOAC Int.* **2001,** 84:1708-1714.
4. Lacaz, R., and Nascimento, E. Producao de biomassade Spirulina maxima para alimentacao humana e animal. *Rev Microbial.* **1990,** 21:85-97.
5. Mazo, V. K., Gmoshinski, I.S. Microalgae Spirulina in humain nutrition. Vopr. Pitan. **2004,** 73(1): 45-53.
6. Puyfoulhoux, G., Rouanet, J.M., Besancon, P., Baraoux, B. Iron availability from Iron modified Spirulina by an in vitro digestion / Caco-2 cell culture model. Agaric Food Chem. **2001,** 49:1625-29.
7. Stanier, R. Y. and Van Niel, Y. The Concept of a Bacterium. Mikrobiol. **1962,** 42:17-35.
8. Gustafson, K. et al.,Pub. In journals of the National Cancer Institute, **1989,** 81: (16) p.1254, USA.
9. CIFERRI, O. *Spirulina*, the edible microorganism. Microbial. **1983,** 47: 551-578.
10. Hayashi,K. Calcium-Spirulinan, an inhibitor of enveloped virus Replication, from a blue-green alga Spirulina. *J Nat Proud.* **1996,** 59:83-87.
11. Jung, T., and Dailey, M. A novel and inexpensive source of Allophycocyanin for multicolor flow cytometry. *J Immunol. Meth.* **1989,** 121: 9-18.