International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **1(6),** 49-56, October **(2012)** *I. Res. J. Biological Sci.*

Impact of different Physical and Chemical Environment for mass Production of *Spirulina pletensis*- An Immunity Promoter

Mehta Jitendra*, Sharma Priyanka, Jakhetia Madhulika, Syedy Mohsina, Makhijani Komal and Khamora Neha
Algae Research Laboratory and Department of Biotechnology, Vital Biotech Research Institute, University of Kota, Kota, Rajasthan, INDIA

Available online at: www.isca.in
Received 16th August 2012, revised 24th August 2012, accepted 28th August 2012

Abstract

Spirulina is one of the most explored cyanobacteria. Since ancient time it is being used as source of protein. Spirulina pletensis was cultivated in different medium like; zarrouk's medium, CFTRI medium, OFERR medium, zarrouk's medium + PGR medium, agitation and without agitation medium, improved VITAL BIOTECH medium. Different temperature, light intensity and pH were monitored for 20 days on daily basis. pH was found in range from 9.1 to 10.1in different medium. Gradually increase in dry weight (dw) was noticed along with the age of culture, 0.40-1.25 dw/l was achieved in different medium respectively. Spirulina inoculated in improved VITAL BIOTECH medium was survived and growth was flourished, achieving dry weight of 0.82 dw/l on 20th day of cultivation. Different amount of $NaHCO_3$ and $NaNO_3$ shown significant impact on Spirulina growth. However results of present investigation could be consider for commercial cultivation of Spirulina using different physical and chemical environment for mass production of Spirulina pletensis.

Keywords: Cyanobacteria, spirulina pletensis, CFTRI media, OFERR Media.

Introduction

The potential of cyanobacteria to produce large number of chemicals and biological compound such as vitamins, carotenoid pigments, proteins, lipids and polysaccharides. Placed cyanobacteria in the list of quite interesting microorganisms[1] for commercialization purpose of cyanobacteria is cultivated in large amount. Researchers are also doing this on global level[2]. *Spirulina* is cultivated in tropical and subtropical bodies of water and filamentous form of cyanobacteria. The water bodies should have high levels of carbonate and bicarbonate and alkaline Ph values of up to 11. In Africa chad lack in maxico texcoco lack produces *spirulina* which is harvested as a source of food[3]. *Spirulina* is singal cell protein[4] to produce vitamins, minerals, proteins, and polyunsaturated fatty acid[5]. Therapeutic properties[6], antioxidant activity[7], tubular photobioreactors[8], glass panels are used to cultivation of cyanobacteria. Cost and composition media is challenging factors for viable production of cyanobacteria. Different media used for cultivation of *spirulina* such as Zarrouks media, Rao's media, CFTIR media, OFERR media Revised media (6) and Bangladesh medium. The present report is aimed to the study of impact of different physical and chemical environment for mass production of *spirulina pletensis.*

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* is used as nutrient source for fish larvae. It is used in fish diet as an ingredient for juveniles and adults common corp.

Material and Methods

Culture development and maintenance: The strain of *Spirulina pletensis* was obtained from IARI, New Delhi, which is previously maintained in Zarrouk's agar media slants in 4ºC. *Spirulina pletensis was* 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: i. Firstly we had taken sterilized 1000ml conical flask. ii. Then we had taken 500ml D/W in flask. iii. After that mix properly all components of Zarrouk's media. iv. Autoclaved 500ml media for 15 to 20 minutes.

Preparation of inoculums: 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

Growth results expressed in terms of dry weight of *S. pletensis* at different physical and chemical parameters viz. temperature condition-25˚C and 35˚C (room temperature) (table-1) described maximum bulk density (0.60gm) of S. pletensis obtained at 25˚C temperature 1200 lux. Subsequently on different light intensity viz. 435 lux, 650 lux, 975 lux, 1100 lux, 1300 lux in flask (table-2) described maximum bulk density (0.80gm) of *S. pletensis* in 1300 lux. Then Change in concentration of Carbon and Nitrogen source (table-3) described maximum bulk density of *S. pletensis* in 1.0 gm of $NaNO_3$ (0.89 gm) and 8 gm of $NaHCO_3$ (1.25gm). After that at different pH (7, 8, 9, 10, and 11) in flask culture showed the maximum bulk density about 0.81gm/ml when pH of culture medium was maintained at 11 with medium volume 250ml in a 500ml flask (table-4). And in further parameter of Growth results of *S. pletensis* at different medium viz. CFTRI, Bangladesh, RM-6, A-5, and OFERR showed maximum growth of *S. pletensis* in a flask with RM-6 medium volume 250ml in a 500ml flask (table-5). Finally *S. pletensis* was grown at different chemical and physical environment in flask culture viz. Zarrouk's Media+PGR, Zarrouk's Media, Agitation Zarrouk's Media, Without Agitation Zarrouk's' Media. Maximum growth of *S. pletensis* was noticed a flask which has Agitation Zarrouk's Media with medium (table-6).

Table-1
Biomass production of *Spirulina pletensis* different different temperature

Temperature (*in ˚c*)	**Microorganism (Spirulina pletensis) (Temperature 25 ˚C, Duration 3 weeks) Initial pH 8 of Zarrouk's Media**			**Final pH of Zarrouk's Media**
25	**Biomass (gm/ 250 ml)**	**Frequency of biomass Growth (%)**	**Color**	9.82
	0.60	80	dark green, thick few clumps, no contamination	
35	0.49	65	green, thick few clumps, no contamination	9.80

Table-2
Biomass production of *Spirulina pletensis* different light intensity

Intensity of light (*in lux*)	**Microorganism (*Spirulina pletensis*) (Temperature 25 ˚C, Duration 3 weeks) Initial pH 8 of Zarrouk's Media**			**Final pH of Zarrouk's Media**
435	**Biomass (gm/ 250 ml)**	**Frequency of biomass Growth (%)**	**Color**	9.41
	0.70	65	light green, thick few clumps, no contamination	
650	0.67	60	green, thick few clumps, no contamination	9.38
975	0.65	57	light green, thick few clumps, no contamination	9.23
1100	0.75	70	Dark green, thick few clumps, no contamination	9.79
1300	0.80	80	Dark green, thick few clumps, no contamination, thin film of cells on flask wall	9.90

Table-3
Biomass production of *Spirulina pletensis* different carbon and nitrogen source

Different Carbon Source $NaHCO_3$ gm/250ml	Different Nitrogen Source $NaNO_3$ gm/250ml	Microorganism (*Spirulina pletensis*) (Temperature 25 ˚C, Duration 3 weeks) Initial pH 8 of Zarrouk's Media			Final pH of Zarrouk's Media
		Biomass (gm/ 250 ml)	Color	Frequency of biomass Growth (%)	
3.0	-	0.74	light green, thick few clumps, no contamination	60	10.1
8.0	-	1.25	green, thick few clumps, no contamination	85	10.0
10.0	-	1.11	Dark green, thick few clumps, no contamination, thin film of cells on flask wall	78	9.9
-	0.037	0.76	pale green, no contamination	62	9.9
-	0.5	0.80	green, thick few clumps, no contamination	66	10.0
-	1.0	0.89	Dark green, thick few clumps, no contamination	70	10.0

Table-4
Biomass production of *Spirulina pletensis* in different pH

Initial pH of Zarrouk's Media	Microorganism (*Spirulina pletensis*) (Temperature 25 ˚C, Duration 3 weeks)			Final pH of Zarrouk's Media
	Biomass (gm/ 250 ml)	Frequency of biomass Growth (%)	Color	
7	0.73	70	light green, thick few clumps, no contamination	9.23
8	0.76	75	green, thick few clumps, no contamination	9.30
9	0.70	65	green, thick few clumps, no contamination	9.67
10	0.77	80	Dark green, thick few clumps, no contamination	9.68
11	0.81	90	Dark green, thick few clumps, no contamination, thin film of cells on flask wall	9.88

Table-5
Biomass production of *Spirulina pletensis* different medium

Different media	Microorganism (*Spirulina pletensis*) (Temperature 25 ˚C, Duration 3 weeks)		
	Biomass (response)	Color	Frequency of biomass Growth (%)
CFTRI	+++++	green, thick few clumps, no contamination	70
Bangladesh	++	Pale green, no contamination	40
RM-6	+++++++	Dark green, thick few clumps, no contamination, thin film of cells on flask wall	80
A-5	-	-	-
OFERR	+++	light green, no contamination	60

Table-6
Biomass production of *Spirulina pletensis* in different chemical and physical environment

Different media	**Microorganism (*Spirulina pletensis*) (Temperature 25 ˚C, Duration 3 weeks) Concentration of PGR (1 mg/l BAP)**			**Final pH of Zarrouk's Media**
	Biomass (gm/ 250 ml)	**Frequency of biomass Growth (%)**	**Color**	
Zarrouk's Media+PGR	0.53	70	green, few clumps, no contamination	9.60
Zarrouk's Media	0.56	75	Dark green, thick few clumps, no contamination	9.50
Agitation Zarrouk's Media	0.59	80	Dark green, thick few clumps, no contamination	9.60
Without Agitation Zarrouk's media	0.55	73	green, thick few clumps, no contamination, thin film of cells on flask wall	9.40

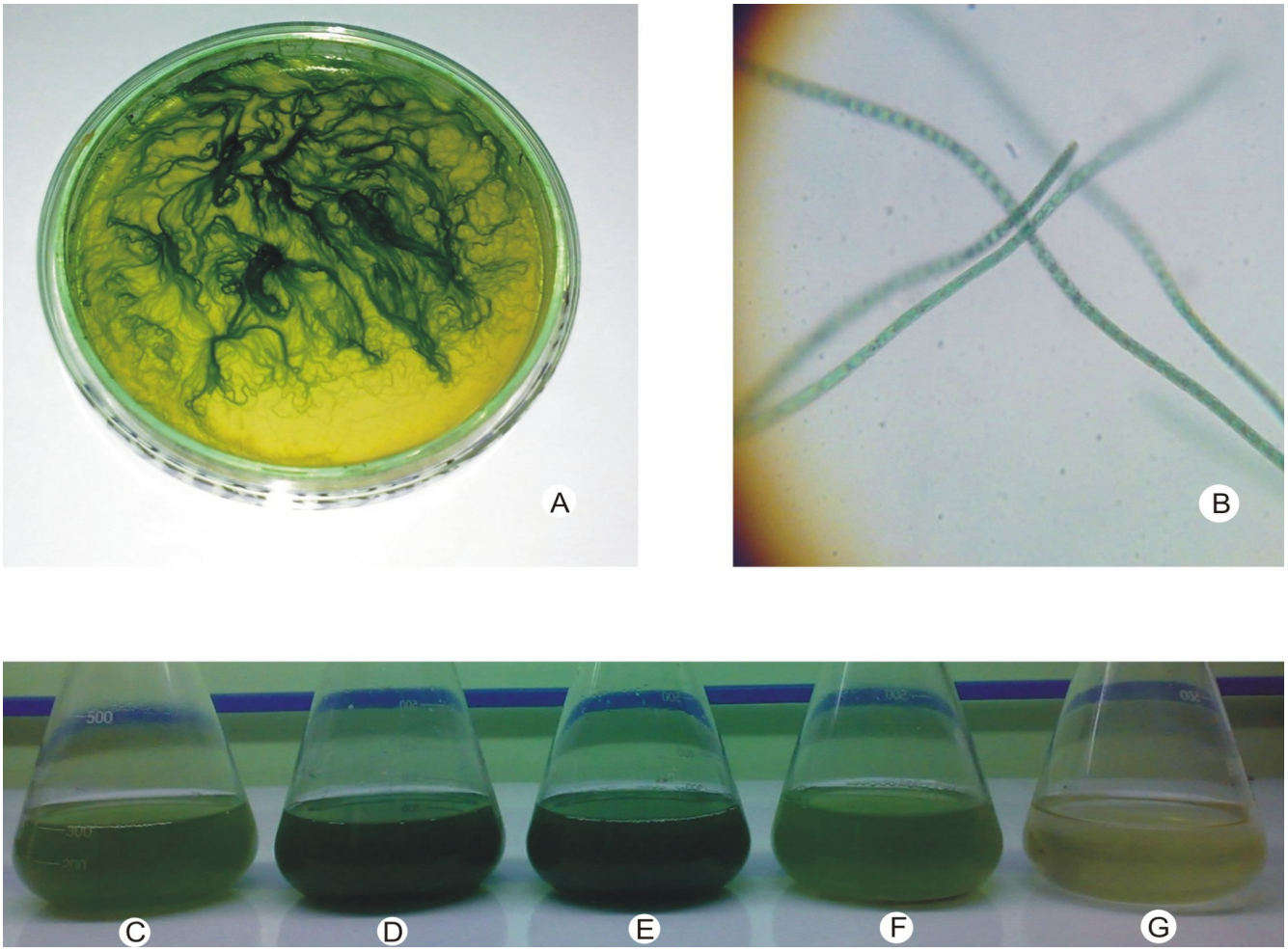

Figure-1 (A-G)
A. *S. Platensis* on Zarrouk's agar media, B. Microscopic view of *S. Platensis,* C. *S. Platensis in* CFTRI Media, D.*S. Platensis in* Bangladesh Media, E. *S. Platensis in* RM-6 Media, F. *S. Platensis in* OFERR Media, G. *S. Platensis in* A-5 Media

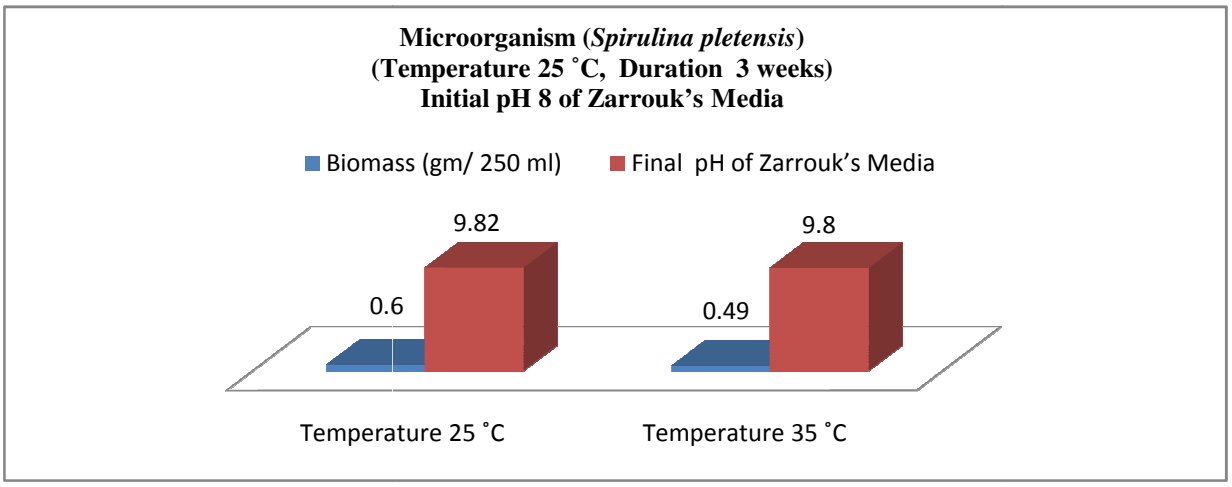

Figure-2
Biomass production of *Spirulina pletensis* different temperature

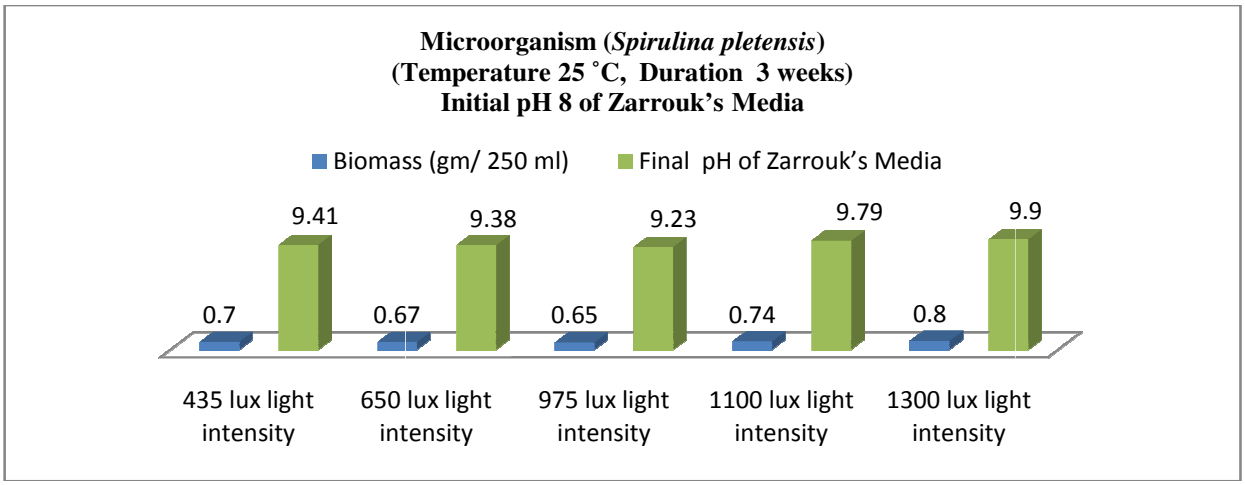

Figure-3
Biomass production of *Spirulina pletensis* different light intensity

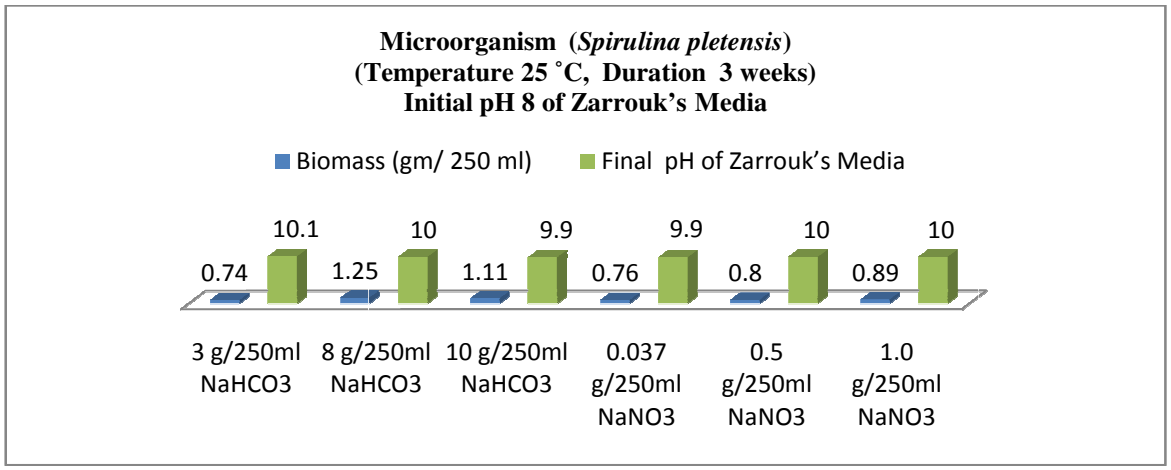

Figure-4
Biomass production of *Spirulina pletensis* different carbon and nitrogen source

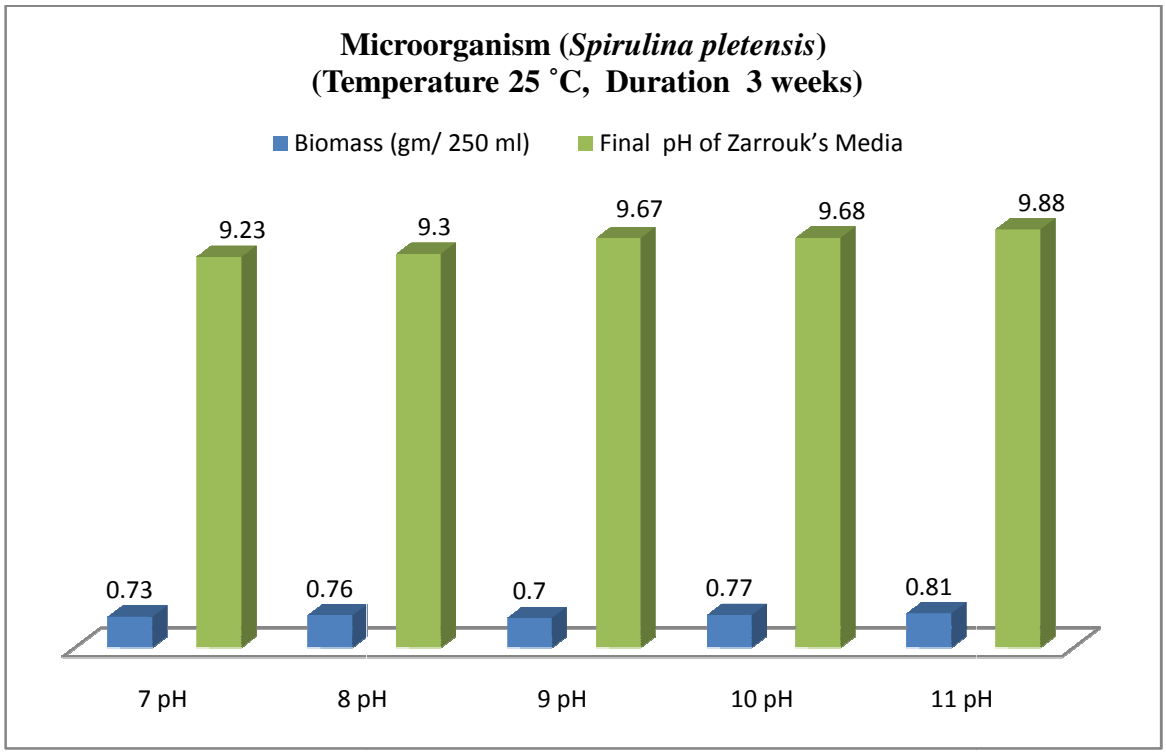

Figure-5
Biomass production of *Spirulina pletensis* in different pH

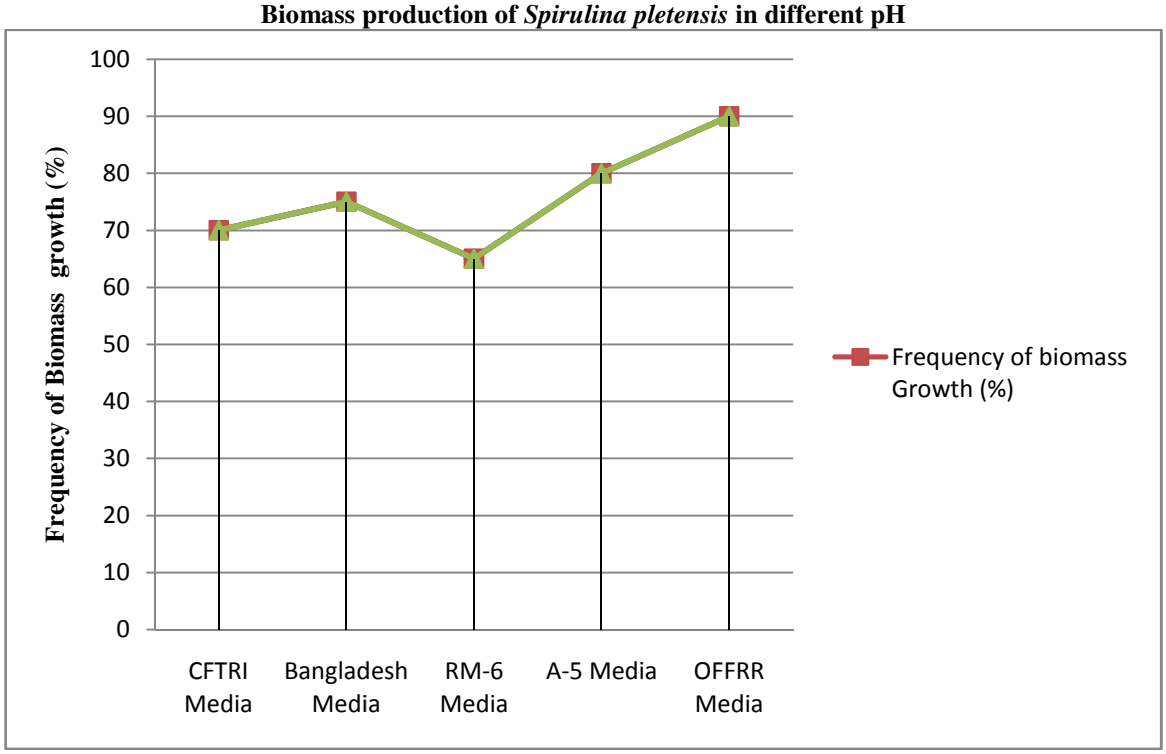

Figure-6
Biomass production of *Spirulina pletensis* different medium

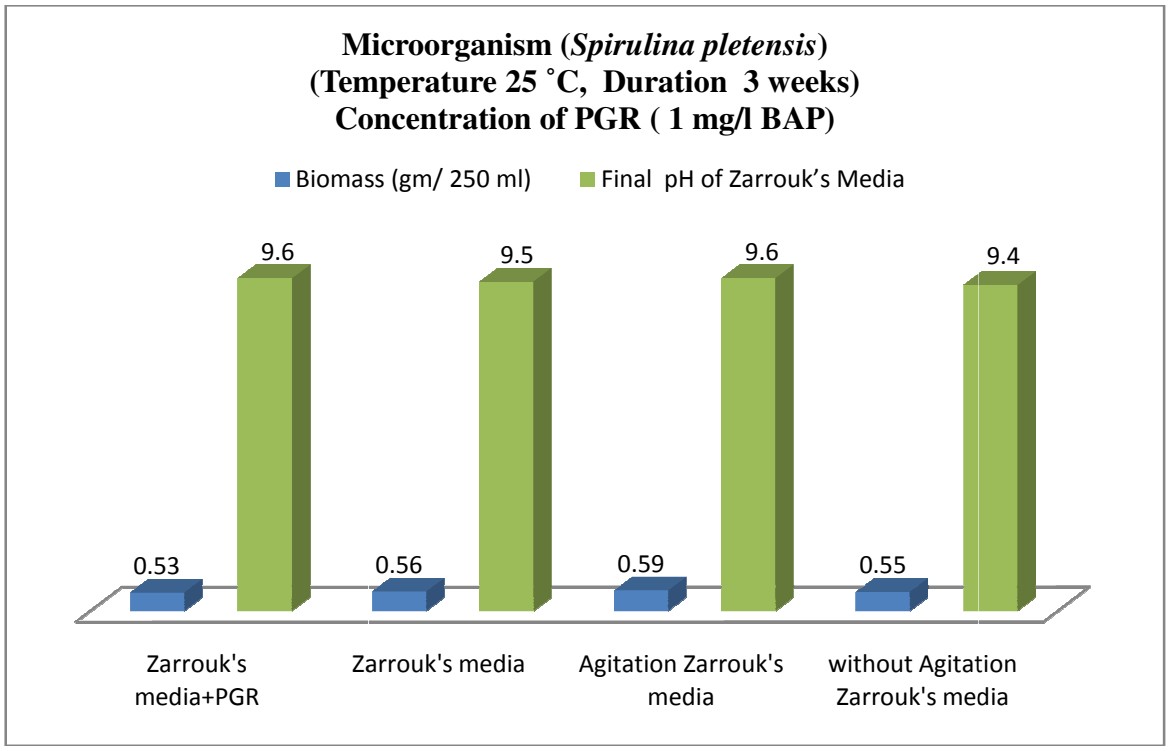

Figure-7
Biomass production of *Spirulina pletensis* in different chemical and physical environment

Improved Vital Biotech (2012) Media for production of *S. pletensis:* Culture development and maintenance: *Spirulina pletensis was* grown in improved vital biotech (2012) Media. Firstly, we have transferred our culture in improved vital biotech (2012) Media. Cultures were incubated in a culture room at temperature of 25^{0}C. During the process of growth the flask was shaken 3 to 4 time per day and colour of improved vital biotech (2012) Media was Dark green and no contamination, thin film of cells on flask wall.

Preparation of Improved vital biotech (2012) media as following step: Firstly we had taken sterilized 500ml conical flask. Then we had taken 250ml D/W in flask. After that mix properly all components of improved vital biotech (2012) media. Result described in table-7.

Table-7
Improved Vital Biotech (2012) Media for production of *S. pletensis*

Ingredients	gm/lit
Sodium bicarbonate	8.0
Sodium chloride	5.0
Urea	0.2
Sodium nitrate	2.5
Potassium sulphate	0.5
Magnesium sulphate	0.16
Ferrous sulphate	0.05
Potassium di hydrogen phosphate	0.052

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 improved vital biotech (2012) 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.

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 0.82 gm/liter. Final pH was observed of Improved VITAL BIOTECH (2012) Media is pH 10. We have observed that improved vital biotech (2012) Media is good media for biomass production of *Spirulina platensis.*

Conclusion

The genus *spirulina*, is the most important commercially cultivated cynobacterium, due to its high nutritional value, chemical composition and safety of its biomass for human

consumption. 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 11. 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 pletensis* 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. The biomass production of *Spirulina* species was lower in light intensity (435lux) and higher when the growth was illuminated in 1300 lux. *Spirulina* inoculated in improved VITAL BIOTECH medium was survived and growth was flourished achieving dry weight of 0.82 dw/L on 20th day of cultivation.

In conclusion, the result of this investigation shows that agitation and light intensity are very important factors in biomass production and also protein biosynthesis in the *Spirulina* species.

Acknowledgement

We are grateful to algae Research Laboratory and Department of Biotechnology, Vital Biotech Research Institute, Kota for providing laboratory facilities and also thankful to Mr. Jitendra Mehta of Vital Biotech for sincere efforts in writing this research paper. We are also grateful Priyanka Sharma, Madhulika Jakhetia, Mohsina Syedy, Komal Makhijani, and Neha Khamora for their continuous team work. We would also like to thank staff members of Vital Biotech Research Institute for encouragement and we would like to thank the reviewers of this paper for their excellent comments.

Reference

1. Zhang X.W., Zhang Y.M. and Chen F., *Process Biochem.,* **34,** 477–481 **(1999)**

2. Belay A., Mass culture of Spirulina outdoors–the Earthrise experience. In: Vonshak A (ed) *Spirulina platensis* (Arthrospira): physiology, cell-biology and biotechnology, Taylor and Francis, London, 131–158 **(1997)**

3. Vonshak A., *Spirulina platensis* (Arthrospira), Physiology, Cellbiology and Biotechnology, Taylor and Francis, London **(1997)**

4. Anupama P.R., Value-added food: single cell protein, *Biotechnology Advances*, **18,** 459–479 **(2000)**

5. Miranda M.S., Cintra R.G., Barros S.B.M. and Filho J.M., Antioxidant activity of the microalga *Spirulina maxima, Brazilian J. of Medical and Bio. Res.,* **31,** 1075–1079 **(1998)**

6. Belay A., Ota Y., Miyakawa K. and Shimamatsu H., Current knowledge on potential health benefits of Spirulina, *Journal of Applied Phycology,* **5,** 235–241 **(1993)**

7. Estrada J.E., Bescós P. and Villar Del Fresno A.M., Antioxidant activity of different fractions of *Spirulina platensis* protean extract, *Farmaco,* **56,** 497–500 **(2001)**

8. Torzillo G., Pushparaj B. and Bocci F., Production of Spirulina biomass in closed photobioreactors, *Biomass.,* **11,** 61-74 **(1986)**

9. Mishra P.C., Dash A.K. and Pradhan Khageswar, Metals in Environmental segments at Hirakud of Odisha, India, *ISCA J. Biological Sci.,* **1(1),** 7-23 **(2012)**

10. Khandkar-Siddikur Rahman, Nazmul Alam D.M. and Md. Nazrul Islam, Some Physical and Mechanical Properties of Bamboo Mat-Wood Veneer Plywood, *ISCA J. Biological Sci.,* **1(2)** 61-64 **(2012)**

11. Singh A., Ahmed F. and Ahmed S., Bandey, *ISCA J. Biological Sci.,* **1(2)** 69-72 **(2012)**

12. Patil J. and Patil H.M., *Res. J. Recent Sci.,* **1(ISC-2011),** 333-366 **(2012)**

13. Shaziya Bi and Goyal P.K., *ISCA J. Biological Sci.,* **1(1)** 2-6 **(2012)**

14. Sainkhediya Jeetendra and Aske Dilip Kumar, *ISCA J. Biological Sci.,* **1(2)** 77-79 **(2012)**

15. Rani C.R., Reema C., Singh A. and Singh P.K., Salt tolerance of Sorghum bicolor cultivars during germination and seedling growth, *Res J Recent Sci*., **1(3)** 1-10 **(2012)**

16. Bhattacharya Anjanabha, Power John B. and Davey Micheal R., Genetic Manipulation of Gibberellin (GA) Oxidase Genes in Nicotiana sylvestris using constitutive promoter to modify Plant Architecture**,** *Res J Recent Sci.,* **1(5)** 1-7 **(2012)**

17. Eman A. Alam, *ResJ Recent Sci.,* **1(ISC-2011)** 398-404 **(2012)**

18. Bora A., Science Commmunication through Mass media, *Res J Recent Sci.,* **1(1)** 10-15 **(2012)**

19. Mehta J., Sain M., Sharma D.R., Gehlot P., Sharma P. and Dhaker J.K., *I. Res. J. Biological Sci.,* **1(3),** 37-42 **(2012)**

20. Mehta J., Dhaker J.K., Kavia A., Sen P., Kaushal N., Datta S., *I. Res. J. Biological Sci.,* **1(4),** 12-16 **(2012)**