Research Article

GROWTH PERFORMANCE AND DIFFERENT BIOCHEMICAL PARAMETERS OF *SPIRULINA PLATENSIS* IN COW DUNG ASH EXTRACT AT DIFFERENT CONCENTRATIONS TO MINIMIZE PRODUCTION COST

Mohammad Yasin Sofi, Samuel G. Singh, Navdeep S. Bain, Ishtiyak Ahmad Najar
Department of Botany. St. John's College, Agra-282002, U.P, India.
P. G. Department of Botany, Govt. Dungar College Bikaner-334001, Rajastan, India.
Indian Institute of Integrative Medicine, Jammu – 180001, J & K, India.
***Corresponding author's E-mail:** myasin698@gmail.com

Accepted on: 05-06-2012; **Finalized on:** 31-08-2012.

ABSTRACT

Photosynthetic cyanobacterium, *Spirulina platensis* has been cultivated for mass production in many countries in tropic, subtropical and temperate regions for use in human food, animal feed and for colorimetric use. It is rich source of proteins, minerals, vitamin B12, β- carotene and essential fatty acids like gamma linolenic acid. It is one of the most prominent microalgae for the extraction of valuable pigments such as, β- carotene, phycocyanin etc. It was found that modified medium i.e. CFTRI medium supplemented with cow dung ash extract showed great potential for cultivating *Spirulina platensis*. *Spirulina platensis* grown in cow dung ash extract in different concentrations from control to 50% in triplicates at pH- 9.0, light intensity 1700 Klux and temperature 35°C under 12/12 light/dark period. Agitation was done three times a day for three minutes at an interval of four hours. Harvesting was done after 28 days of inoculation and growth was measured in terms of dried biomass, Chlorophyll-a, phycocyanin, allophycocyanin, phycoerythrin, carotene, protein, carbohydrate, amino acids, and minerals i.e. copper zinc, manganese, iron and sodium. It was observed that the *Spirulina platensis* grown in 50% cow dung ash medium showed highest growth and other biochemical parameters followed by 40%, 30%, 20%, 10% and control respectively.

Keywords: *Spirulina platensis*, cow dung ash extract, proteins, carbohydrates, phycobiliproteins, chlorophyll- a, CFTRI medium.

INTRODUCTION

The filamentous cyanobacterium *Spirulina platensis* has been used as a food for centuries by native peoples from Lake Chad in Africa and Lake Texcoco in Mexico[1, 2], an observation which has led to the use of *Spirulina* as a food supplement for undernourished people in many parts of the world[8], due to its high protein content (65%), high digestibility[8] and specific amino acid content. Since centuries cyanobacteria have been receiving increasing interest due to their potential to produce a diverse range of chemicals and biologically active compounds, such as vitamins, carotenoids, pigments, proteins, lipids and polysaccharides[3].

Biotechnological processes based on cyanobacteria have been receiving increasing interest due to their potential to produce a diverse range of chemicals and biologically active compounds, such as vitamins, carotenoid pigments, proteins, lipids and polysaccharides[3]. For economic reasons, the culture system predominating in the large-scale commercial production of these types of organisms is the open-air system, closed systems being very expensive and often difficult to scale up[4].

The microalga *Spirulina* is produced commercially all over the world, and the dried product is a valuable food supplement[5, 6]. It is rich in proteins (60–70% by weight), vitamins (especially B12 and h-carotene), and minerals. It contains many essential amino acids and fatty acids, being one of the sources of dietary gama linolenic acid (GLA). Pre-clinical and clinical studies suggest it has certain therapeutic effects[7], such as reduction in blood cholesterol, protection against some cancers, enhancement of the immune system, increase of intestinal lactobacilli, reduction of nephrotoxicity by heavy metals and drugs, radiation protection, reduction of hyper-lipidemia, and obesity[6, 8] reported that an aqueous extract of *S. platensis* partially inhibited HIV-1 replication in human T-cell lines, peripheral mononuclear cells, and Langerhans cells. Consequently, there has been increasing interest in the production of microalgae for commercial use.

The aim of the work was to study the growth of *Spirulina platensis* in CFTRI medium supplemented with cow dung ash medium in order to minimize the production cost of the test alga.

MATERIALS AND METHODS

Strain: *Spirulina platensis* was obtained from the Centre for Conservation and Utilisation of Blue-Green Algae, IARI, New Delhi. The culture was maintained by repeated transfer to liquid CFTRI medium (figure 1).

Culture Media

An attempt has been made to raise this alga in cow dung ash extract, a cheap but nutrient rich source. The cow dung was first sun dried and burned to form an ash. Take 50 gram of cow dung ash and dissolved in one litre of distilled water and filtered. The solution was used as a basic nutrient substrate. Different concentration levels of cow dung ash extract medium from 10%-50% were prepared using prescribed medium i.e. CFTRI medium. The culture was carried out in conical flasks, aerated and

photoperiod was given 12/12 alterative light and dark period with light intensity of 1500 Klux by fluorescent tubes. Initial pH was adjusted to 9, using sodium bicarbonate and cultures were incubated in a culture room at temperature 31°C. During the process of growth the flasks were shaken 3 times/day for 3 minutes at an interval of 4 hours. The experiments were run in triplicates.

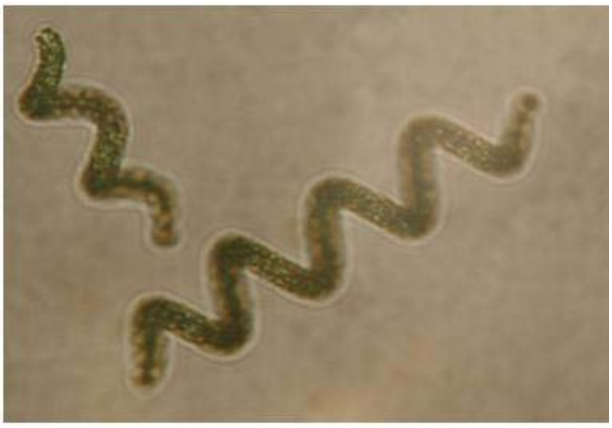


Figure 1: Microscopic view of *Spirulina platensis*

Filtration and washing

The cells were collected by filtration using filter paper of 8 micrometre (µm) pore size and these cells were washed with buffer solution (pH 7), diluted to known volume and processed for further inoculation, 10% (V/V) of the prepared inoculum were added to the flask and flasks were covered perfectly by cotton wool.

Growth Evaluation

Growth was measured as dried biomass and other biochemical parameters after 28 days culture. The results were compared with control of the same experiment.

Analytical methods

1. Dry weight determination was done by filtering 25ml homogenized algal sample through pre-weighed whatman filter paper no-4(diameter 10 cm). The difference between the initial and final weight was taken as the dry weight of algal biomass. The dry weight was expressed in terms of g/l.

2. Chlorophyll content was estimated and determined by procedure and equation[9]. Phycocyanin, allophycocyanin and phycoerythrin content was calculated by the series of equation[10].

3. The extraction and estimation of carotenoids was carried out[11].

4. Carbohydrate was estimated by simple Anthrone method[12].

5. Protein content was estimated by Lowry's method [13].

6. Quantitative estimated of amino acids was free amino acids was carried out[14].

Statistical Analysis

The data were analysed to analysis of variance (ANOVA) with confidence level of 95% ($p<0.05$) in order to verify significant difference among treatments, biomass and other biochemical parameters.

RESULTS AND DISCUSSION

At pH 9, the highest mean dry biomass, chlorophyll, phycocyanin, allophycocyanin, phycoerythrin, carotenoids, proteins, carbohydrate and amino acids content of alga was observed in 50% 0.710 g/l, 8.89 mg/g, 7.65%, 3.55%, 1.350%, 0.296%, 65%, 9.50% and 1.16% respectively followed by 40%, 0.698 g/l, 8.39 mg/g, 7.60%, 3.41%, 1.340%, 0.241%, 60%, 9.1% and 1.13% respectively, 30%, 0.695 g/l, 8.22 mg/g, 7.48%, 3.24%, 1.139%, 0.235%, 57%, 8.5% and 1.10% respectively, 20%, 0.682 g/l, 8.18 mg/g, 7.27%, 3.11%, 1.139%, 0.230%, 55%, 8.1%, 1.05%, respectively 10%, 0.675 g/l, 8.05 mg/g, 7.03%, 3.08%, 1.119%, 0.210%, 53%, 7.8%, 1.02% respectively whereas the lowest growth performance was observed in control, 0.668 g/l, 7.78 mg/g, 6.85%, 3.00%, 1.101%, 0.205%, 50%, 7.5%, 0.98%, 0.047 ppm, 0.024 ppm, 0.043 ppm, 0.037 ppm and 0.658% respectively (Table 1 and Fig. 2 and 3). *Spirulina platensis* grown in cow dung ash extract in different concentrations from control to 50% in triplicates at pH- 9.0, light intensity 1700 Klux and temperature 35°C under 12/12 light/dark period. Agitation was done three times a day for three minutes at an interval of four hours.

Our findings are also supported by many workers, Animal waste constitute was an excellent medium for growing algae[15]. In CFTRI medium the growth of *Spirulina platensis* was rapid and exponential[16]. The final yield with soda as a substitute to analytical NaHCO3 was only 0.3 times less than the original CFTRI medium. Cultivation of *Spirulina* in swine dung to check the utilization of swine dung and growth of *Spirulina* was estimated by direct microscopic count and they found best growth on twentieth day of inoculation in swine waste medium [17]. Effluents from chicken and cow sheds and from piggeries were tested for growth of *Spirulina*[18]. Half the strength of Zarrouk's medium was used as control and it was observed that effluents from piggery and cow sheds could be used as good substitutes for *Spirulina* cultivation. It was found experimentally that 16:8 hrs light dark regime at light intensity of 2000±200 lux temperature of 30±1°C and pH of 9.2 are the optimal growth conditions for this microorganism 1[9]. Our results were also in agreement with that on optimum photoperiod for the growth of *Spirulina platensis* (isolated from Jalmahal Lake Jaipur) was subjected to different photoperiod i.e. continuous illumination at 16 h d^{-1} light condition was optimum photoperiod for that strain[20].

Table 1: Growth and different biochemical analysis of *Spirulina platensis* at pH-9 in different concentrations of Cow dung ash medium in 2010.

Treatment	Drybiomass (mg/l)	Chlorophyll (mg/g)	PC (%)	APC (%)	PE (%)	Carotenoids (%)	Proteins (%)	Carbohydrates (%)	Amino acids (%)
Control	0.668	7.78	6.85	3.00	1.101	0.205	50	7.5	0.98
10%	0.675	8.05	7.03	3.08	1.119	0.210	53	7.8	1.02
20%	0.682	8.18	7.27	3.11	1.139	0.230	55	8.1	1.05
30%	0.695	8.22	7.48	3.24	1.239	0.235	57	8.5	1.10
40%	0.698	8.39	7.60	3.41	1.340	0.241	60	9.1	1.13
50%	0.710	8.89	7.65	3.55	1.350	0.296	65	9.5	1.16

Figure 2: Growth performance of *Spirulina platensis* in different concentration of CDAM in CFTRI medium at pH 9.0

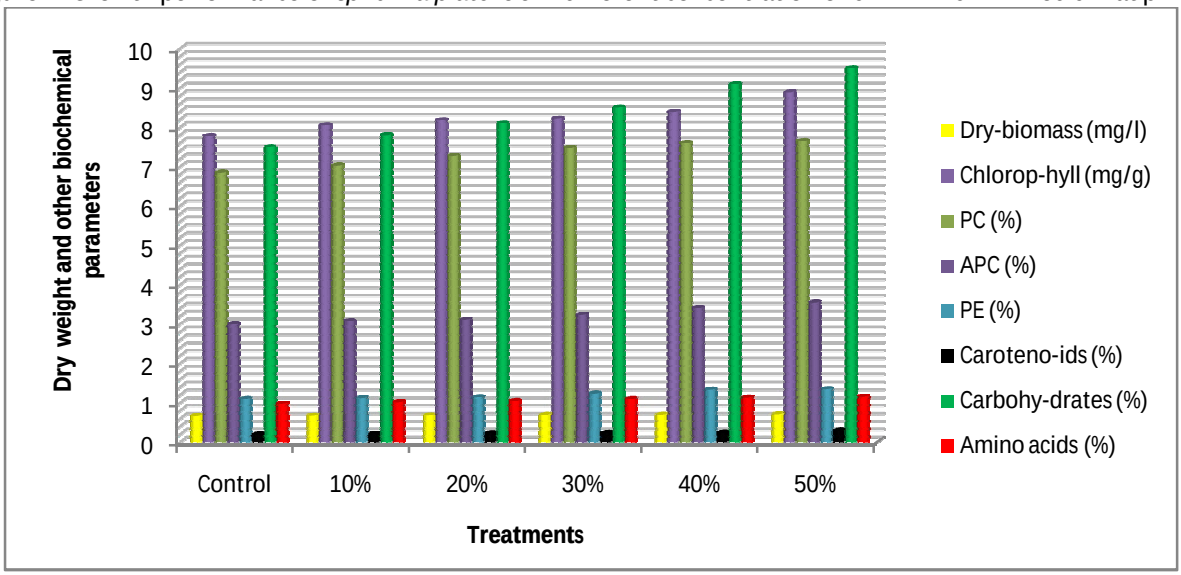
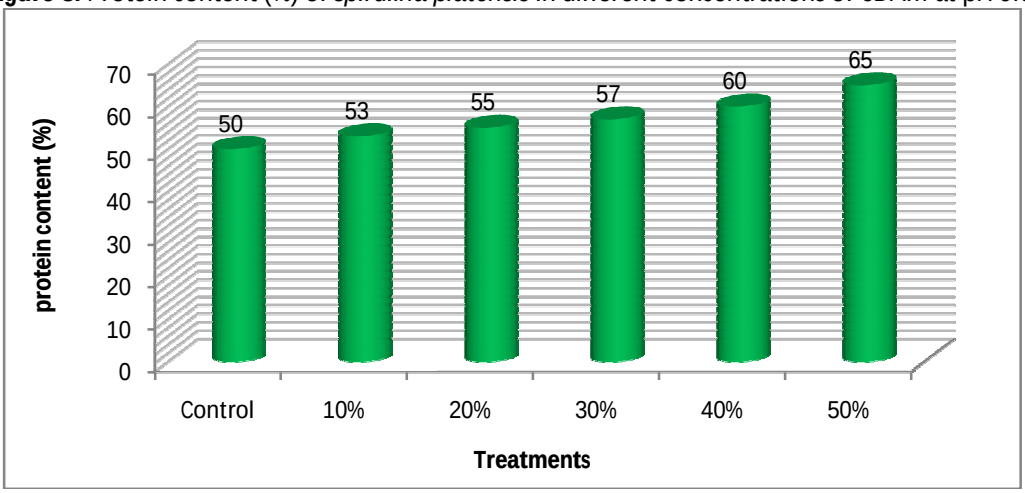


Figure 3: Protein content (%) of *Spirulina platensis* in different concentrations of CDAM at pH 9.0.



It was also recorded experimentally that pH value above 10.3 were shown to be harmful for the culture. pH of the medium played a very important role in growth of microorganism *Spirulina platensis*[21]. During the course of investigation, it was observed that the new media formulated for mass cultivation of *Spirulina platensis* by incorporating the nutrients of standard CFTRI medium with cheap nutrients by adding different kinds of wastes found to be heavily economical. Therefore, the commercial production of *Spirulina platensis* was cost effective by reducing the input costs and replacing costly inputs with cheap and readily available materials without sacrificing the production efficiency.

CONCLUSION

In the present study, the investigator had cultured the *Spirulina platensis* in cow dung ash medium in different concentration form 10%-50%, the alga shows great potentiality to grow in this medium. 50% shows highest growth followed by 40%, 30%, 20%, 10% and control. Growth was measured in terms of dried mass and other biochemical parameters. It was concluded that the cow dung ash medium used as growth medium minimized the production cost of *Spirulina platensis*.

REFERENCES

1. Vonshak A: *Spirulina platensis* (*Arthrospira*). Physiology, Cell-kiBiology and Biotechnology. Taylor & Francis, London, UK 1997.
2. Henrikson R:, Microalga *Spirulina.* Superali- mento del futuro. Ediciones S. A. Urano, Barcelona, Spain. 1994.
3. Zhang, X.-W., Zhang, Y. M., Chen, F: Application of mathematical models to the determination optimal glucose concentration and light intensity for mixotrophic culture of *Spirulina platensis. Process Biochem.* 34:1999; 477–481.
4. Borowitzka M: Commercial production of micro- ponds, tanks, tubes and fermenters. J. Biotechnol. 70,1999; 313-321.
5. Cifelli, O: Spirulina: the edible organism. Microbiol. Rev. 47,1983; 551–578.
6. Belay, A., Ota, Y., Miyakawa, K., Shimamatsu, H: Current knowledge on potential health benefits of Spirulina. J. Appl. Phycol. 5, 1993;235–241.
7. Fox, R.D: Spirulina. Production and Potential. Edisud, Aix-en-Provence, France 1996; 232.
8. Ayehunie, S., Belay, A., Baba, T.W., Ruprecht, R.M: Inhibition of HIV-1 replication by an aqueous extract of Spirulina platensis (Arthrospira platensis). J. Acquir. Immmune Defic. Syndr. Human Retrovirol. 18,1998; 7 – 12.
9. Parsons, T.R. and Strickland, J.D.H: Particulate organic matter III. I. Pigment analysis III, Determination of Phytoplankton pigments. J. Fish.Res. 18: 1965; 117-127.
10. Bennett, A. and Bogard, L: Properties of sub units and aggregates of blue-green algal biliproteins. 10:1971; 3625-3634.
11. Jensen, A: Chlorophylls and carotenoids. In : Handbook of phycological methods, physiological and biochemical methods. J. A. Hellebust and J.S. Craige (edt.) 1978; 59-70 Cambridge University Press, Cambridge.
12. Roe, J.H: The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol. Chem.* 212:1955; 335-343.
13. Lee, Y.P. and Takahasi, T: An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem* 1966; 14-71.
14. Gedaliah Shelef: The combination of algal and an anaerobic waste treatment in a biogenerative farm system. Pub. Department of environmental and water resources engineering, Technion-Israel Institute of Technology, Haifa, Israel 2006; 1-8.
15. Srivastava, P. and Gajraj, R.S: *Spirulina* biotechnology of Rajastan-Jaipur. Recent Adv. Biotech.1993; 47-56.
16. Manikandavelu, D. and Murugan T: Utilization of swine dung in *Spirulina* production and isolation of phycocyanin. TN. J. Veterinary and animal sciences. 5:2009; 171-173.
17. Yunus: Effect of chicken, pig and cow dung mediaon the population growth of *Spirulina*. J. Pneteitian-Budidaya-Pantai (Indonasia). 6 :1990; 81-86.
18. Jetley, J.K., Meenakshi, C. and Tasneema F: The impact of physical stresses on the growth of Cyanobacterium *Spirulina platensis* S5. Jour. of Env. Sci. and Engg. 46:2004; 303-311.
19. Pareek, A. and Srivastava, P: Optimum photoperiod for the growth of *Spirulina platensis*. J. Phytol. Res 14: 2001; 219-220.
20. Richmond, A., Vonshak, A. and Arad, S.M: Environmental limitations in outdoor production of algal biomass. In : G. Shelef and Soeder, C.J. (eds.) Algae biomass 1980; 65-72. Elsevier/North-Holland Biomedical Press. Amsterdam.

****