J. Bangladesh Agril. Univ. 3(2): 303–308, 2005 ISSN 1810-3030

Growth performance of *Spirulina platensis* in three different concentrations of banana leaf ash with added jackjfruit seed powder and urea

M.A. Toyub[1], M.M. Rahman[1], M.I. Miah[2] and M.A.B. Habib[3]
[1]Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh
[2]Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh
[3]Department of Aquaculture, Bangladesh Agricultural University, Mymensingh 2202

Abstract

The growth performance of *Spirulina platensis* was studied in three different concentrations of banana leaf ash (BLA) added with 0.4 g/l jackfruit seed powder (JSP) and 0.2 g/l urea in the laboratory. *S. platensis* was cultured in three different concentrations viz. 4.8, 7.2 and 9.6 g/1 of BLA and Kosaric medium (KM) as control with three replications for a period of three months. Each trial was done for a period of 12 days in the laboratory. The initial cell weight of *S. platensis* was 4 mg/1 which attained a maximum cell weight of 545 mg/1 in Kosaric medium followed by 523, 485, and 307 mg/1 in 7.2, 4.8, and 9.6 mg/1 of BLA media with added 0.4 g/l JSP and 0.2 g/l urea respectively, on the 8th day of culture. Similar trend was observed in case of chlorophyll a content where the highest amount was 8.12 mg/l in KM and among different concentrations of BLA highest amount was 7.53 mg/l in 7.2 g/l BLA with added 0.4 g/l JSP and 0.2 g/l urea. The percentage of crude protein, crude lipid, moisture, ash and NFE contents of JSP were recorded 12, 1.85, 10, 3.62, and 72.53%, respectively. The percentage of total nitrogen, phosphorus, sulpher, sodium and calcium of BLA were recorded 0.18, 0.09, 2.35, 0.10, 0.07 and 10.81%, respectively. The result showed that the growth rate of *S. platensis* was significantly ($p<0.05$) higher in 7.2 g/1 BLA medium with 0.4 g/l JSP and 0.2 g/l urea than other concentrations of BLA. It is suggested that BLA algal culture medium is as effective as commercially produced culture medium making it feasible to culture *Spirulina*.

Keywords: *Spirulina,* Culture media, Cell weight and Chlorophyll a

Introduction

Microalgae is an important factor in the food chain of aquatic organisms in aquaculture (Islam *et al.,* 2004). They play a vital role in aquaculture as live food for larval stages of many species of crustacean, shrimp and fin fish (Renaud *et al.*, 1991). *Spirulina* is a potential source of an alternative protein for animal feed. It contains about 60% protein, a good source of vitamins, essential fatty acids and pigments (Cohen *et al.*, 1985). *Spirulina* contains about 6-11% polyunsaturated fatty acids viz. palmitic, γ-linoleic acid (GLA), linoleic and oleic acids. The GLA is one of the most important essential fatty acids, which is rarely found in animal and human diets. The GLA is a precursor of cholesterol in human blood (Cohen *et al.,* 1987). Due to the presence of proteins, essential fatty acids and carotenoid, it is a good source of complementary diet to prevent malnutrition in developing countries (Borowitzka, 1988). At present *Spirulina* is used as health food and as a natural pigment (Borowitzka, 1988; Becker, 1988; Richmond, 1988). In addition, it is used as poultry duck and shrimp feed (Nguyen, 1988; Dang 1989). *Spirulina* is therefore, a good species for mass culture.

Kosaric Medium (KM) is the most commonly used expensive medium for *Spirulina* culture. Thus, for mass production of *Spirulina platensis*, particularly in developing countries, there is a need to find a more effective, cheaper and readily available alternative media. So, attempt was made to find out an inexpensive organic media for *Spirulina* culture. The main objective of the experiment is to develop inexpensive culture medium for large-scale production of *Spirulina platensis*.

Materials and Methods

Preparation and analysis of Jackfruit seed powder (JSP) and banana leaf ash (BLA)

Jackfruit seed powder and banana leaf ash was used as organic ingredient. Collected seed was dried in an oven at 50 ^{o}C for over night. For complete drying of jackfruit seed, the sample was sun dried for another seven days. The sample was make powder and sieved through a sieve of 500 micro meter mesh size for getting very fine particle of jack-fruit seed powder. Proximate composition (moisture, crude protein, crude lipid and ash) of jackfruit seed sample was analyzed in the nutrition laboratory following the standard methods (Horwitz, 1984). Collected banana leaf was chopped with knife and dried in sunshine for seven days and dried banana leaf was burnt to make ash. Minerals (total nitrogen, P, S, K, Na and Ca) of sample was analyzed in the soil science laboratory following standard methods (Horwitz, 1984).

Preparation of media

Three different media of banana leaf ash (BLA) with added jackfruit seed powder (JSP) and urea, and Kosaric medium (KM) (Clesceri *et al.* 1989) were prepared for *Spirulina platensis* culture. For the preparation of the three different BLA media, 4.8, 7.2 and 9.6 g of BLA were weighed in 1.0 litre conical flask. Then 0.4 g JSP and 0.2 g urea were added in every flask and distilled water was added to make the volume up to 1.0 litre mark. Then the media were mixed well and autoclaved at 120°C for 15 minutes with moist heat. After autoclaving, flasks were kept for 24 hrs. for cooling before culture of microalgae. Mixing, sterilization and cooling of the prepared KM was done following the same procedures. Composition of KM is shown in Table 1.

Table 1. Composition of inorganic nutrients of Kosaric Medium (KM) modified after Zarrouk (1966); Phang and Chu (1999) for *Spirulina platensis* culture

SL. No.	Chemicals	Amount (g /l)
1.	$NaHCO_3$	9.00
2.	K_2HPO_4	0.25
3.	$NaNO_3$	1.25
4.	K_2SPO_4	0.50
5.	NaCl	0.50
6.	$MgSO_4$	0.10
7.	$CaCl_2$	0.02
8.	$FeSO_4$, $2H_2O$	0.005
9.	As micronutrient solution[a]	0.50 ml L^{-1}
	a) As micronutrient solution	g L^{-1}
	H_3BO_3	2.86
	$MnCl_2$, $4H_2O$	1.81
	$ZnSO_4$, $7H_2O$	0.22
	$CuSO_4$, $5H_2O$	0.08
	MoO_3	0.01
	$CoCl_2$, $6H_2O$	0.01

Culture of *Spirulina platensis*

The experiment was conducted in the laboratory of Department of Aquaculture, BAU, Mymensingh. Four treatments, three from three various concentrations of banana leaf ash containing 0.4 g/l JSP and 0.2 g/l urea and one from KM as control were used with three replications to culture *S. platensis* in 1.0 litre conical flask. *S. platensis* were then inoculated into the flasks to produce a culture containing 10% *S. platensis* suspension (OD at 620 nm = 0.20) (Habib, 1998). All the flasks were kept under fluorescent lights (light: dark = 12: 12) in the laboratory. The culture flasks were continuously aerated. Eight sub-sample were taken at every alternate day from each flasks to observe cell weight and chlorophyll-a of *S. platensis* and physico-chemical properties of culture media. Temperature, light intensity, dissolved oxygen and pH were determined by respective meters. Nitrite-nitrogen, nitrate-nitrogen and phosphate-phosphorus were determined by Hach kit (DREL/2000). *Spirulina* cells were weighed using GFC filter paper and chlorophyll a was determined by following Clesceri *et al.* (1989).

Statistical analysis

Differences among the treatments of *S. platensis* in different media were analyzed by one way ANOVA (Gomez and Gomez, 1976).

Results and Discussion

The initial cell weight of *S. platensis* was 4 mg/l, which attained to maximum cell weight of 545 mg/l in Kosaric medium. Among the three different concentrations of BLA, 7.2 g/l with added 0.4 g/l JSP and 0.2 g/l urea showed the maximum growth (523 mg/1). For all the treatments maximum cell weight of *S. platensis* was observed on 8^{th} day of culture. A decreasing trend of cell weight started from 10^{th} day of culture for all the treatments (Figure 1). The chlorophyll a content of *S. platensis* cultured in BLAM and KM was analyzed. The initial chlorophyll a content of *S. platensis* was 0.025 mg/l, which attained to maximum Chlorophyll a content of 8.12 mg/l. The highest content of chlorophyll a was recorded in Kosaric medium followed by that grown in 7.2, 4.8 and 9.6 g/l BLA media with added 0.4 g/l JSP and 0.2 g/l urea (Table 2). A decreasing trend in chlorophyll a content of *S. platensis* was also observed on 10^{th} day of culture for all the media. The growth of *S. platensis* cultured in 7.2 g/l BLA with added JSP and urea was significantly ($p<0.05$) higher than other concentration of BLA. Similarly, chlorophyll a content of *S. platensis* cultured in 7.2 g/l of BLA medium was also significantly ($p<0.05$) higher than other concentration of BLA. It was also found that the growth of *S. platensis* and the chlorophyll a content was significantly ($p<0.05$) higher in KM than three different concentrations of BLA with added 0.4 g/l JSP and 0.2 g/l urea.

The range of temperature, light intensity, dissolved oxygen and pH were recorded which were in the ranges of 27.5 to 29.5°C, 1720 to 1910 lux/m^2/s, 3.15 to 5.12 mg/l and 9.41 to 11.31 respectively. Nitrate nitrogen, nitrite nitrogen and phosphate phosphorus of the culture media were found in the ranges of 3.01 to 7.71, 0.011 to 0.095 and 2.01 to 8.34 mg/l respectively.

Table 2. Mean values (± SD) of Chlorophyll a content (mg/l) of *S. platensis* grown in three different concentrations of BLAM with added 0.4 g/l JSP and 0.2 g/l urea and KM

Treatment/ Control	Original order					
	2nd day	4th day	6th day	8th day	10th day	12th day
1	$0.33^{c} \pm 0.01$	$1.23^{c} \pm 0.03$	$3.15^{c} \pm 0.05$	$7.30^{c} \pm 0.03$	$5.05^{c} \pm 0.04$	$4.31^{c} \pm 0.01$
2	$0.44^{a} \pm 0.03$	$2.01^{b} \pm 0.02$	$4.17^{b} \pm 0.03$	$7.53^{b} \pm 0.03$	$5.78^{b} \pm 0.02$	$5.11^{b} \pm 0.02$
3	$0.26^{c} \pm 0.01$	$0.79^{d} \pm 0.02$	$2.70^{d} \pm 0.04$	$4.13^{d} \pm 0.03$	$2.79^{d} \pm 0.04$	$2.32^{d} \pm 0.01$
4	$0.45^{a} \pm 0.01$	$2.07^{a} \pm 0.03$	$4.46^{a} \pm 0.04$	$8.12^{a} \pm 0.02$	$7.36^{a} \pm 0.04$	$6.21^{a} \pm 0.01$

Different superscripts in each row indicates significant differences ($p < 0.05$)
Treatment 1: 4.8 g/l BLAM with added 0.4 g/l JSP and 0.2 g/l urea
Treatment 2: 7.2 g/l BLAM with added 0.4 g/l JSP and 0.2 g/l urea
Treatment 3: 9.6 g/l BLAM with added 0.4 g/l JSP and 0.2 g/l urea, and
Treatment 4: KM (control medium)

The total nitrogen (N), phosphorus (P), potassium (K), sulpher (S), sodium (a) and calcium (Ca) of banana leaf ash were determined which were found 0.18, 0.094, 2.348, 0.098, 0.071 and 10.81 % respectively. The percentage of moisture, crude protein, crude lipid ash and nitrogen free extracts (NFE) of jack-fruit seed powder were recorded 10.00, 12.00, 1.85, 3.62 and 72.53 % respectively.

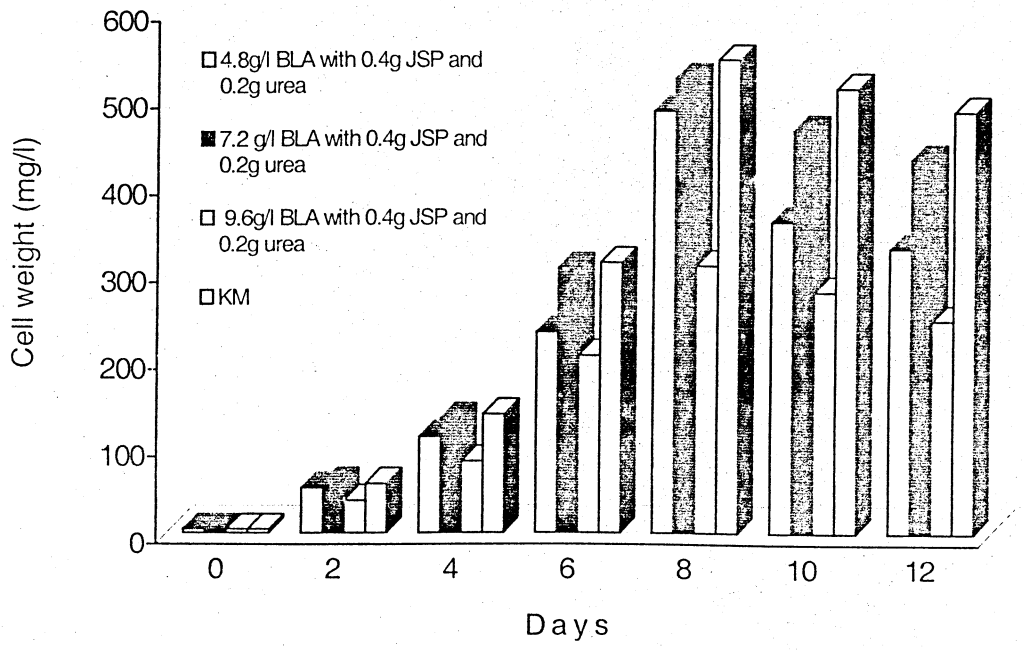

Figure 1. Mean values of cell weight (mg/l) of *S. platensis* grown in three different concentrations of BLA media with added 0.4 g/l JSP and 0.2 g/l urea and KM

Among the three concentrations of BLA (Banana leaf ash) media with added jackfruit seed powder and urea, the higher cell weight and chlorophyll a content of *S. platensis* was observed when grown in treatment II (7.2 g/l BLA ash with added 0.4 g/l JSP and 0.2 g/l urea) than other BLA media. It might be happened due the suitable amount of nutrients in this concentration than other concentrations of ash. Alom (2002) recorded significantly ($p<0.05$) higher cell weight and chlorophyll a content of *S. platensis* when grown in 0.2 g/l press mud with added 0.2 g/l urea than other two media (0.1 and 0.3 g/l press mud with added urea) which showed the similarity with the present findings. Mario *et al.* (1986) carried out similar type of work where the annual yield of biomass of *Spirulina maxima* strain 4MX grown in fertilized seawater in out door system was 0.394 g/l/d which was higher than the present study. In Bangladesh, Begum *et al.* (1998) worked on *Spirulina* in BCSIR laboratories, Dhaka with their developed culture media named as Bd-1, Bd-2, Bd-3, Bd-4 and Bd-5 (Bd = Bangladesh). For domestic production of *Spirulina* Bd-3 and Bd-5 media gave the highest yield of 664 mg/l and 665 mg/l respectively which agree with the present findings. The increasing trend of pH up to the stationary phase favoured the growth of *Spirulina*. The decreasing trend of pH was observed after the stationary phase might be due to the presence of dead cells and other organic loads. A similarity was found with the findings of Richmond (1986), Begum *et al.* (1998) and Alom (2002).

Temperature and light intensity are the most important physical factor for the growth of microalgae. During the present study, the temperature and light intensity were varied from 27.5 to 29.5°C and 1720 to 1910 lux/m^2/s respectively which are almost similar the observations recorded by Alom (2002). The dissolved oxygen values were found 3.15 to 5.12 mg/l. This fluctuation in dissolved oxygen value might be due to alteration rate of photosynthesis in the culture media. A decreasing trend of O_2 was observed after stationary phase, which might be due to the decomposition of dead cells. The finding is similar to Alom (2002).

The range of mean values of nitrite-nitrogen in cultured media was 0.011 to 0.095 mg/l. Nitrite-nitrogen was found lowest on the initial day and highest on the 12th day of culture. An increasing trend of nitrite-nitrogen was observed in culture media, may be due to the decomposition of dead *S. platensis* cells. Nitrate-nitrogen availability has been considered very important for microalgae production in cultured media. Nitrate-nitrogen was found maximum on the initial day and these values were gradually decreased. The range of mean value of nitrate-nitrogen was 3.01 to 7.71 mg/l of the present study. The availability of phosphate-phosphorus also has been considered very important for microalgae production. The phosphate-phosphorus was found higher on initial day and found minimum on 8th day of culture and the range of mean value was 2.01 to 8.34 mg/l. However, the over all chemical parameters of the culture media were found in the suitable range for the culture of *S. platensis*.

So, it is concluded that the growth of *S. platensis* was maximum in 7.2 g/l BLA medium added with 0.4 g/l JSP and 0.2 g/l urea than other media. Thus, this concentration may be recommended for culture of *S. platensis* though the control KM gave the highest ($p<0.05$) growth.

References

Alom, M.I. 2002. Culture of *Spirulina platensis* (VAR, ISRAEL) in press mud medium adding urea in different concentration. *M.S. thesis*, Dept. of Aquaculture, BAU, Mymensingh

Becker, E.W. 1988. MIcroalgae for human and animal consumption. In Borowitzka, M. A., Borowitzka, L. (eds). *Microalgal Biotechnology*. Cambridge U. P., Cambridge. pp. 222-256.

Begum, S., Noor, P., Akhtar, N. and Majid, F.Z. 1998. *Spirulina* culture in Bangladesh VI. Domestic scale production of *Spirulina*. *Bangladesh J. Sci. Ind. Res.* 33 (3): 473-478.

Borowitzka, M.A. 1988. Vitamins and fine chemicals from microalgae, In Borowitzka, M. A. and Borowitzka, L. (eds.), *Microalgal Biotechnology*. Cambridge U.P., Cambridge. pp. 153-196.

Clesceri, L.S., Greenberg, A.E. and Trussell, R.R. 1989. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association and Water Pollution Control Federation; New York. USA. pp. 92-1110.

Cohen, Z., Vonahak, A. and Richmonds, A. 1987. *Phytochemistry*, 26(8): 225-254.

Dang Dinh, K. 1989. The biotechnology of mass culture of microalgae. *Rev. of Biology*. National Center for Scientific Research of Vietnam. pp. 1-1989.

Gomez, K.A. and Gomez, A.A. 1976. Stastical procedures for agricultural research. 2nd edition. Published by the International Rice Research Institute, Philipines, 641 pp.

Habib, M.A.B. 1988. Culture of selected microalgae in rubber and Palm oil mill effluents and their use in the production of enriched rotifers. *Ph. D. thesis*, Faculty of Science and Environmental Studies, University of Putra, Malaysia. 532 pp.

Horwitz, W. 1984. Official Methods of the Analysis of the Association of Official Analytical Chemists (14th ed.). Association of Official Analytical Chemists; Washington D. C., USA. 1018 pp.

Islam, M.R., Habib, M.A.B., Miah, M.I. and Khan, A.N..M.A.I. 2004. Growth performance and organic nutrients of *Chlorella ellipsoidea* grown in cabbage powder media. *J. Asiatic Soc. Bangladesh Sci.*, 30(1); 71-78.

Mario, R., Papuzzo, T. and Tomaselli. 1986. Outdoor mass culture of *Spirulina platensis* in sea-water, *Appl. Microbiol. Biotech.*, 24: 47-50.

Nguyen Huu, T. 1988. The alga *Spirulina*- a good source for nutrition and for medicine. Sci. and Tech. edition, 109 pp.

Phang, S.M. and Chu, W.L. 1999. Algae Culture Collection, Catalogue of Strains. Institute of Postgraduate Studies and Research, University of Malaya, Kuala Lumpur, Malaysia. 77 pp.

Renaud, S.M., Parry, D.L., Thinh, L.V., Kov, C. and Padovan, A.S.N. 1991. Effect of light intensity on the proximate biochemical and fatty acid composition of *Isochroyis* sp. and *Nannochlopsis oculata* for use in tropical Aquaculture. *J. Appl. Phycol.*, 3: 43-53.

Richmond, A. 1988. *Spirulina*. In: Borwitzka, M. A., Borowitzka, L. (eds.), *Microalgal Biotech*. Cambridge U.P., Cambridge. pp. 85-121.

Zarrouk, C. 1996. Contribution a Letude d une Cyano-phycee de Divers Facteurs Physique et Chimiques sur la Croissane et Photosynthese de *Spirulina maxima* Zarrouk Geitler, *Ph.D. Thesis*, University of Paris, France.