



## **Growth Performance and Nutritional Analysis of *Spirulina platensis* in Different Concentrations of Papaya Skin Powder Media**

**M. A. Toyub<sup>\*a</sup>, M. Z. Uddin<sup>a</sup>, M. I. Miah<sup>b</sup> and M. A. B. Habib<sup>b</sup>**

<sup>a</sup>*Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh and* <sup>b</sup>*Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh*

### **Abstract**

The growth performance of *Spirulina platensis* was studied in four different concentrations viz. 0.3, 0.4, 0.5 and 0.6 g/l of papaya (*Carica papaya*) skin powder media (PSPM) and in control, Kosaric Medium (KM) in triplicate. The initial cell weight of *S. platensis* was 6.0 mg/l which attained a maximum weight of 913.13 mg/l on the 10th day of culture in the control KM followed by 699.07, 612.13, 538.99 and 377.48 mg/l that grown in 0.40, 0.30, 0.50 and 0.60 g/l of PSPM respectively. Similar trend was observed in the case of chlorophyll a content ranging 3.57 to 8.15 mg/l. The specific growth rate (SGR,  $\mu$ /day) of *S. platensis* on the basis of cell weight and chlorophyll a content was recorded in the ranges of 0.41 to 0.50 and 0.41 to 0.49, respectively, for all the treatments. The SGR on the basis of cell weight and chlorophyll a was recorded significantly ( $p < 0.05$ ) higher in KM among all the treatments. On the other hand among the four different concentrations of PSPM, 0.40 g/l showed significantly ( $p < 0.05$ ) higher SGR than others. The pH and other physico-chemical factors were within suitable range of algae culture. The cultured microalga was nutritionally rich.

**Keywords:** *Spirulina*, Papaya skin, Cell weight, Chlorophyll a

### **Introduction**

The earliest attempts to culture algae started about more than a century ago with solutions of a few inorganic salts, actually devised for studies of vascular plants. A common feature of these media was their high contents of nutrients, particularly nitrogen, phosphorus and potassium (Rodhe, 1978). Kosaric medium (KM) is the most commonly used medium for *Spirulina* culture. However, it is expensive and not readily available in Bangladesh. Thus, for mass production of *Spirulina*, particularly in developing countries there is a need to find an effective, cheaper and readily available alternative media. Nutrients such as phosphorus and nitrogen present in agro-industrial effluents as well as in domestic wastewater may cause serious eutrophication in any water body. But these nutrients can be used to increase plant growth, such as phytoplankton, which can be utilized as natural fish food or for pharmaceutical purpose. *Spirulina* is one of the most promising microalgae for culture due to its high nutritional values. *Spirulina platensis* was successfully grown in digested sago starch factory waste water in Malaysia (Miah *et al.*, 2000). Suitable organic media may also be used for the culture of *S. platensis*. It may be cultured in other nutrient rich media collected from vegetables.

Bangladesh is an agro-based country. Here huge amounts of vegetables such as papaya, cabbage, potato, tomato etc. are producing every year. *Chlorella elepsoides* was successfully cultured in papaya skin powder media by Toyub *et al.* (2006). So, it may be possible to culture *S. platensis* using papaya skin powder (PSP) as inexpensive medium. The present experiment was conducted to study the growth performance of *S. platensis* in various concentrations of PSP and to analyze its nutritional value.

### **Materials and Methods**

#### **Collection and preparation of culture media**

For collection of papaya skin, papaya was collected from local market and sun dried primarily and then in an oven at 40°C for overnight. The dried skin was powdered and sieved with mesh size of 0.01 mm for getting fine particle. For preparation of different concentrations of papaya skin powder medium (PSPM) 0.3, 0.4, 0.5 and 0.6g of papaya skin powder was added per liter of distilled water and was then thoroughly mixed. Urea (0.2 g/l) was added to increase nitrogen content and NaHCO<sub>3</sub> (6.0 g/l) was added to increase pH

\* Corresponding author: E-mail: : [toyubma@yahoo.com](mailto:toyubma@yahoo.com)

of each concentration of the culture media since the initial pH were 7.6 - 7.8. Then the media were mixed well and sterilized at 120°C for a period of 15 minutes with moist heat by autoclave and cooled for a period of 24 hours.

#### Preparation of control medium

Kosaric medium (KM) was used as control medium. Composition of KM modified form of Zarouk (1966) and Phang and Chu (1999) is shown in Table I. The prepared control medium was sterilized at 120°C for a period of 15 minutes with moist heat by autoclave and cooled for a period of 24 hours.

**Table I: Different composition of Kosaric medium (KM), modified form of Zarouk (1996); and Phang and Chu (1999) for culture of *Spirulina platensis*.**

Sl. No.	Chemicals	Amount
1.	NaHCO <sub>3</sub>	9.00 g/l
2.	K <sub>2</sub> HPO <sub>4</sub>	0.25 "
3.	NaNO <sub>3</sub>	1.25 "
4.	K <sub>2</sub> SPO <sub>4</sub>	0.50 "
5.	NaCl	0.50 "
6.	MgSO <sub>4</sub>	0.10 "
7.	CaCl <sub>2</sub>	0.02 "
8.	FeSO <sub>4</sub> , 2H <sub>2</sub> O	0.005 "
9.	As micronutrient solution <sup>a</sup>	0.50 ml/l
	a) As micronutrient solution	
	H <sub>3</sub> BO <sub>3</sub>	2.86 g/l
	MnCl <sub>2</sub> , 4H <sub>2</sub> O	1.81 "
	ZnSO <sub>4</sub> , 7H <sub>2</sub> O	0.22 "
	CuSO <sub>4</sub> , 5H <sub>2</sub> O	0.08 "
	MoO <sub>3</sub>	0.01 "
	CoCl <sub>2</sub> , 6H <sub>2</sub> O	0.01 "

#### Culture of *S. platensis*

Stock culture of *Spirulina platensis* (Initially collected from Malaysia) maintained in the Department of Aquaculture, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh was used for this study. The cells were separated with the help of micro-capillary using compound microscope. *Spirulina platensis* was inoculated initially in prepared KM to have a pure stock culture. Five treatments, four from different concentrations (0.3, 0.4, 0.5 and 0.6 g/l) of papaya skin powder medium (PSPM) and one from KM

(control) were used to grow *S. platensis*. One liter volumetric flasks were used to grow the microalga with three replications of each treatment. The microalga was inoculated into each culture flask from the stock having OD 4.8 at 620 nm to get 10% suspension of *S. platensis* (optical density at 620 nm = 0.20). All the flasks were kept under fluorescent lights (light: dark = 12 h: 12 h) in the Live Food Culture Laboratory of BAU (Habib, 1998). The culture flasks were continuously aerated using electric aerator. Samplings were carried out at every alternate day from each flask to observe cell weight, chlorophyll a, optical density and physico-chemical properties of culture media viz., temperature (°C), light intensity, dissolved oxygen (DO), pH, phosphate-phosphorus (PO<sub>4</sub>-P), nitrate-nitrogen (NO<sub>3</sub>-N), nitrite-nitrogen (NO<sub>2</sub>-N) and ammonia-nitrogen (NH<sub>3</sub>-N). All the glasswares used in the experiment were sterilized with dry heat in an oven at 70°C for 12 hrs. After successful completion of laboratory culture, mass culture was performed for a period of 10 days. At the 10th day of mass culture, the microalga was harvested and centrifuged at 3000 rpm for 15 minutes to separate the microalga. The separated microalga was dried and used for proximate composition analysis.

#### Estimation of physico-chemical properties of culture media

Dissolved oxygen (DO) and pH of the culture media were measured by oxygen meter (YSI, Model 58) and electric pH meter (Jenwey Model 3032), respectively. Temperature and light intensity of the culture media were recorded by using a Celsius thermometer and lux meter, respectively. Nitrite-nitrogen, nitrate-nitrogen and phosphate-phosphorus were determined by Hach kit (DREL/2000) following Clesceri *et al.* (1989).

#### Estimation of *S. platensis* cell weight

The samples were filtered with filter paper (Whatman, GF/C) and shifted to the oven at 105°C for 24 hours. The samples were then transferred to the desiccators for cooling and weight was measured using an electric balance. The weight of the dried filter paper was taken.

#### Before filtering

#### Calculation

Cell weight (mg/l) = Weight of the filter paper with sample - Weight of the filter paper without sample before filtering

### Estimation of chlorophyll *a*

Optical densities of the prepared samples were determined at 664, 647 and 630 nm wave length by using UV spectrophotometer. A blank with 100% acetone was run simultaneously. Chlorophyll *a* content was calculated by the following formula (Clesceri *et al.*, 1989):

$$\text{Chlorophyll } a \text{ (mg/l)} = 11.85 (\text{OD } 664) - 1.54 (\text{OD } 647) - 0.08 (\text{OD } 630).$$

### Estimation of specific growth rate (SGR, $\mu$ /day) of microalgae

The specific growth rate (SGR,  $\mu$ /day) of cultured microalgae was calculated by the following equation (Clesceri *et al.*, 1989):

$$\text{SGR } (\mu/\text{day}) = \ln (X_1 - X_2) / t_2 - t_1$$

Where,

$X_1$  = Biomass concentration at the end of selected time interval,

$X_2$  = Biomass concentration at the beginning of selected time interval, and

$t_2 - t_1$  = Elapsed time between selected time in day.

### Analysis of proximate composition

Proximate composition of algal samples were analyzed in the nutrition laboratory, Faculty of Fisheries, Bangladesh Agricultural University, following standard methods (Horwitz 1984).

### Statistical analysis

Mean and standard deviation were calculated from the experimental data. Then the data were analyzed through one-way analysis of variance (ANOVA) using SPSS followed by Duncan's Multiple Range Test (DMRT) whether any significant difference among the treatment means (Zar 1984).

### Results and Discussion

The highest cell weight 913.13 mg/l was recorded on the 10th day of culture in the control KM followed by that grown in 0.40, 0.30, 0.50 and 0.60 g/l of PSPM (Fig. 1). Kosaric medium showed significantly higher ( $P < 0.05$ ) growth than all other treatments. On the other hand 699.07

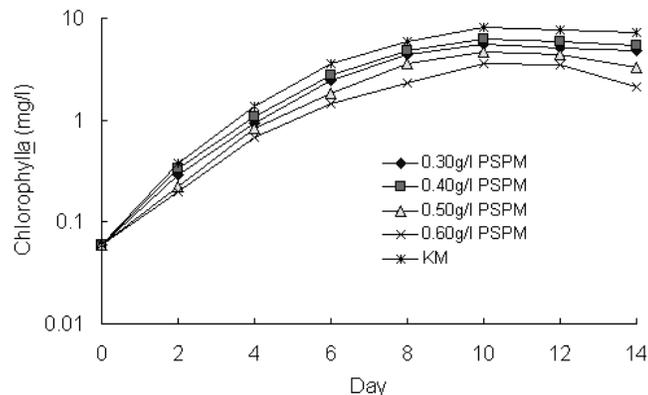
mg/l *S. platensis* was recorded in 0.40 g/l PSPM that was significantly ( $P < 0.05$ ) higher than that of grown in other concentrations of PSPM. Chlorophyll *a* content of *S. platensis* grown in KM also showed significantly ( $P < 0.05$ ) higher growth (8.15 mg/l) than all other treatments. But *S. platensis* grown in 0.40 g/l PSPM showed significantly ( $P < 0.05$ ) higher chlorophyll *a* content (6.27 mg/l) than that of grown in other concentrations of PSPM. The chlorophyll *a* content and optical density were recorded in the range of 3.57 to 8.15 mg/l and 1.02 to 2.05 respectively at 620 nm for all the treatments (Figs. 2 and 3). Miah *et al.* (2000) got higher cell weight in the control KM then the organic media of sago wastewater. Begum *et al.* (1998) cultured *S. platensis* in their developed media. In domestic production of Spirulina they found the yield of 664 mg/l and 665 mg/l in their media named Bd-3 and Bd-5 respectively which is more or less similar with present findings. The specific growth rate (SGR,  $\mu$ /day) of *S. platensis* on the basis of cell weight and chlorophyll *a* content was recorded in the ranges of 0.41 to 0.50 and 0.41 to 0.49, respectively for all the treatments. Toyub *et al.* (2005) cultured *S. platensis* in different concentrations (4.8 g/l, 7.2 g/l and 9.6 g/l) of banana leaf ash with added 0.4 g/l jack fruit seed powder and 0.2 g/l urea. They recorded SGR in the range of 0.41 - 0.49 on the basis of chlorophyll *a* which somehow agreed with the present findings. The SGR on the basis of cell weight and chlorophyll *a* was recorded significantly ( $p < 0.05$ ) higher in KM among all the treatments. On the other hand among the four different concentrations of PSPM, 0.40 g/l showed significantly ( $p < 0.05$ ) higher SGR than others. The total biomass on the basis of chlorophyll *a* content was recorded 238.97 to 546.05 mg/l among the all treatments (Table II). The total biomass on the basis of chlorophyll *a* was significantly ( $p < 0.05$ ) higher in KM than all other treatments. On the other hand 0.40 g/l PSPM showed significantly ( $p < 0.05$ ) higher total biomass than other concentrations (Table II). It might be due to the difference of nutrient released in the media. The highest pH was observed on the 10th day of culture and the recorded range was 10.04 to 10.56. The increasing trend of pH (Fig. 4) up to the stationary phase favoured the growth of *S. platensis*. The decreasing trend of pH at the death phase might be occurred due to dead cells and other organic loads. The pH value supported the findings of Richmond (1986), Begum *et al.* (1998) and Toyub *et al.* (2005).

**Table II: Mean ( $\pm$ SD) of specific growth rate ( $\mu$  /day) of cell weight, chlorophyll *a* (chl.-*a*) and total biomass (mg/l) of *Spirulina platensis* grown in different concentrations of PSPM and KM**

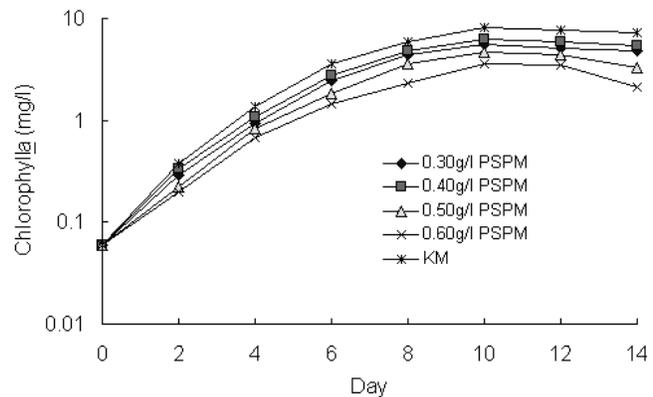
Parameters	0.30 g/l PSPM	0.40 g/l PSPM	0.50 g/l PSPM	0.60 g/l PSPM	KM
SGR of cell wt.	0.46 $\pm$ 0.00 <sup>c</sup>	0.48 $\pm$ 0.01 <sup>b</sup>	0.45 $\pm$ 0.00 <sup>d</sup>	0.41 $\pm$ 0.01 <sup>e</sup>	0.50 $\pm$ 0.00 <sup>a</sup>
SGR of chl.- <i>a</i>	0.45 $\pm$ 0.00 <sup>c</sup>	0.46 $\pm$ 0.01 <sup>b</sup>	0.43 $\pm$ 0.01 <sup>d</sup>	0.41 $\pm$ 0.00 <sup>e</sup>	0.49 $\pm$ 0.00 <sup>a</sup>
Total biomass (Chl.- <i>a</i> x 67)	370.51 $\pm$ 11.62 <sup>c</sup>	420.09 $\pm$ 13.45 <sup>b</sup>	309.99 $\pm$ 10.06 <sup>d</sup>	238.97 $\pm$ 7.71 <sup>e</sup>	546.05 $\pm$ 13.75 <sup>a</sup>

Different superscripts in each row indicates significant differences ( $p < 0.05$ )

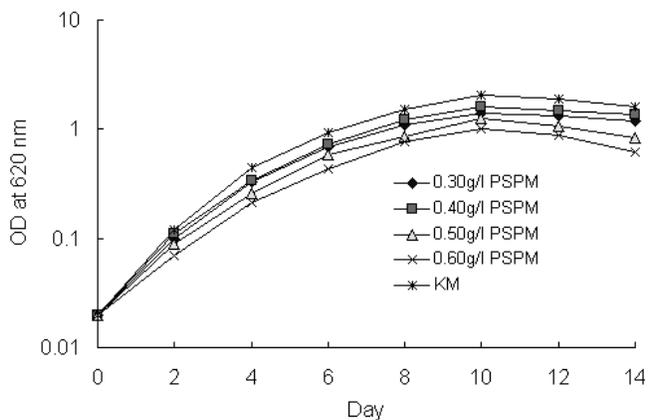
Both the content of PO<sub>4</sub>-P and NO<sub>3</sub>-N of the media were decreasing with the age of the culture up to the stationary phase and then these were increasing again. Use of these nutrients by growing cells in the media may cause the decreasing trend of the amount of PO<sub>4</sub>-P and NO<sub>3</sub>-N. At the stationary phase, the content of PO<sub>4</sub>-P and NO<sub>3</sub>-N was recorded in the range of 2.14 to 4.21 mg/l and 2.47 to 4.38 mg/l, respectively for all the treatments (Figs. 5 and 6). The content of NO<sub>2</sub>-N and NH<sub>3</sub>-N of the media was the lowest at the beginning and increased with the age of the culture. Decomposition of dead cells of the alga may cause it. These were recorded in the range of 0.10 to 0.23 mg/l and 0.31 to 0.60 mg/l, respectively among all the treatments on the 10th day of culture when the growth of *S. platensis* was the highest. Toyub *et al.* (2005) observed same trend of PO<sub>4</sub>-P, NO<sub>3</sub>-N, NO<sub>2</sub>-N and NH<sub>3</sub>-N contents when they cultured *S. platensis* in banana leaf ash with added jack fruit seed powder and urea. Islam (2004) also recorded same trend of these parameters when cultured *S. platensis* in press mud and cabbage powder media.



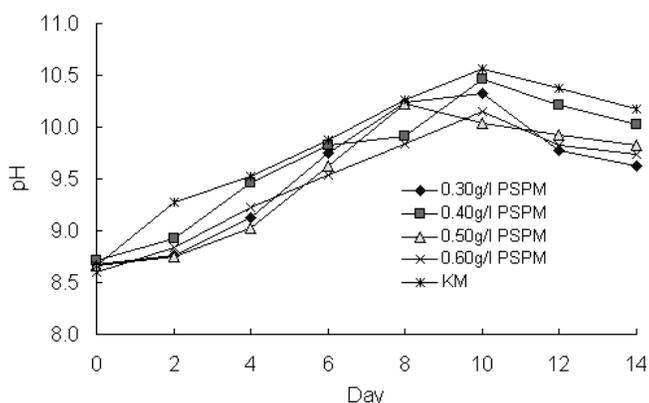
**Fig. 1: Semilogarithmic growth curves based on cell weight (mg/l) of *Spirulina platensis* grown in different concentrations of PSPM and KM**



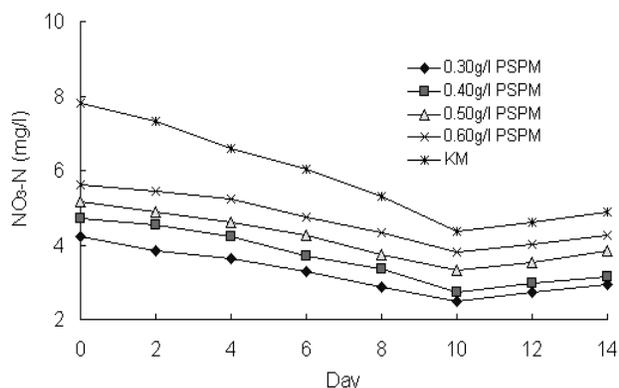
**Fig. 2: Semilogarithmic growth curves based on chlorophyll *a* content (mg/l) of *Spirulina platensis* grown in different concentrations of PSPM and KM**



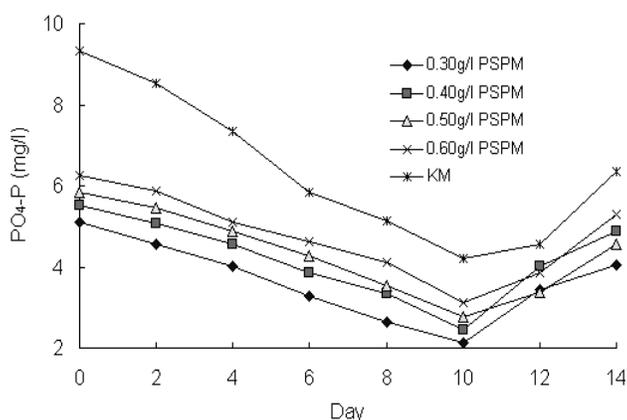
**Fig. 3: Semilogarithmic growth curves based on optical density (OD) at 620 nm of different concentrations of PSPM and KM containing *Spirulina platensis***



**Fig. 4:** pH of different concentrations of PSPM and KM containing *Spirulina platensis*



**Fig. 6:** Nitrate-nitrogen (NO<sub>3</sub>-N) mg/l of different concentrations of PSPM and KM containing *Spirulina platensis*



**Fig. 5:** Phosphate-phosphorus (PO<sub>4</sub>-P) mg/l of different concentrations of PSPM and KM containing *Spirulina platensis*

At the beginning the range of dissolved oxygen was 3.12 to 3.28 mg/l but on the 10th day of culture it was recorded in the range of 4.81 to 5.41 mg/l among all the treatments. The range of average temperature and light intensity was record-

ed 28.50 to 30.50°C and 2142.51 to 2174.61 lux/m<sup>2</sup>/s, respectively during the whole culture period. The range of light intensity, temperature and dissolved oxygen recorded during the study period were found within the favourable ranges for culture of micro algae and supported by Miah *et al.* (2000), Khan (2003), Islam (2004), Islam *et al.* (2004) and Toyub *et al.* (2005).

Proximate compositions of cultured *S. platensis* were studied to know the nutritional values. The highest protein content of *S. platensis* 58.42% was recorded when grown in the control KM followed by which grown in 0.40, 0.30, 0.50 and 0.60 g/l PSPM. The content of lipid was recorded in the range of 10.44 to 12.25% among all the treatments. The ash and crude fiber content was recorded in the range of 6.46 to 8.51% and 7.04 to 7.83%, respectively. On the other hand the moisture and nitrogen free extract (NFE) content was recorded in the range of 8.12 to 8.83% and 5.19 to 15.75%, respectively among all the treatments (Table III). The protein percentage of *S. platensis* grown in KM was significantly ( $P < 0.05$ ) higher than that of grown in other treatments. Crude lipid, ash,

**Table III:** Mean values (± SD) of proximate composition (%) of cultured *Spirulina platensis* in different concentrations of PSPM and KM

Composition	0.30 g/l PSPM	0.40 g/l PSPM	0.50 g/l PSPM	0.60 g/l PSPM	KM
Protein	53.67 ± 0.58 <sup>bc</sup>	54.87 ± 1.29 <sup>b</sup>	52.65 ± 0.98 <sup>cd</sup>	51.49 ± 0.80 <sup>d</sup>	58.42 ± 1.07 <sup>a</sup>
Crude lipid	11.52 ± 0.28 <sup>b</sup>	12.23 ± 0.32 <sup>a</sup>	10.77 ± 0.54 <sup>c</sup>	10.44 ± 0.33 <sup>c</sup>	12.25 ± 0.39 <sup>a</sup>
Ash	7.21 ± 0.31 <sup>b</sup>	8.19 ± 0.30 <sup>a</sup>	7.30 ± 0.33 <sup>b</sup>	6.46 ± 0.37 <sup>c</sup>	8.51 ± 0.42 <sup>a</sup>
Crude fiber	7.44 ± 0.37 <sup>b</sup>	7.83 ± 0.26 <sup>a</sup>	7.15 ± 0.29 <sup>b</sup>	7.04 ± 0.25 <sup>b</sup>	7.53 ± 0.30 <sup>ab</sup>
Moisture	8.12 ± 0.24 <sup>b</sup>	8.50 ± 0.23 <sup>ab</sup>	8.47 ± 0.27 <sup>ab</sup>	8.83 ± 0.26 <sup>a</sup>	8.13 ± 0.24 <sup>b</sup>
NFE*	12.03 ± 1.57 <sup>b</sup>	8.41 ± 0.77 <sup>c</sup>	13.62 ± 0.73 <sup>ab</sup>	15.75 ± 1.72 <sup>a</sup>	5.19 ± 0.67 <sup>d</sup>

Different superscripts in each row indicates significant differences ( $p < 0.05$ )

\* Nitrogen free extract.

moisture and crude fiber percentage of *S. platensis* cultured in KM and 0.40 g/l PSPM was insignificantly ( $P < 0.05$ ) different. The NFE content of *S. platensis* grown in different concentration of PSPM was higher ( $P < 0.05$ ) than that of grown in KM. There is some how similarity of the present study regarding chemical composition of *Spirulina* with the findings of Olguin (1986); Miah *et al.* (2000).

So, it is concluded that the growth of *S. platensis* was maximum in 0.4 g/l PSPM than other media of the experiment. Thus, this concentration may be recommended for culture of *S. platensis* though the control KM gave the highest ( $P < 0.05$ ) growth.

#### Acknowledgement

The authors are indebted to the Director, Bangladesh Agricultural University Research System, Bangladesh Agricultural University, Mymensingh, Bangladesh for providing the fund for successful completion of the research.

#### References

- Begum S., Noor P., Akhter N. and Majid F. J. (1998). *Spirulina* culture in Bangladesh VI. Domestic scale production of *Spirulina*. *Bangladesh J. Sc. and Ind. Res.*, **23**(3): 273-278
- 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.
- Habib M. A. B. (1998). Culture of selected microalgae in rubber and palm oil effluents and their use in the production of enriched rotifers. Doctoral Thesis, University of Putra, Malaysia, 532pp.
- 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. (2004). Culture and chemical composition of three important microalgae using different agro-based organic media. PhD Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh, 337pp.
- 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.
- Khan A. I. A. N. M. (2003). Culture of Live Food Organisms in Sugarcane Industry Waste and their Use as Food for *Clarias batrachus* Fry. PhD thesis submitted to the Faculty of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh, 89pp
- Miah M. S., Phang S. M., Chu W. L. and Hashim M. A. (2000). *Spirulina* culture in digested sago starch factory wastewater. *J. Appl. Phycol.*, **12**: 395-400.
- Olguin E. J. (1986). Applied biotechnological systems in the arid environment. *Appl. Microbiol.*, 111-134.
- Phang S. M. and Chu, W. L. (1999). Algae Culture Collection, Catalogue of Strains. Institute of Postgraduate Studies & Research, University of Malaya, Kuala Lumpur, Malaysia, 77pp.
- Phang, S. M. and Ong, K. C. (1988). Algal biomass production in digested palm oil mill effluent. *Biol. Wastes*, **25**: 177-191.
- Richmond A. (1988). *Spirulina*. In: Borowitzka, M.A. Borowitzka, L. (eds.), Microalgal Biotechnology. Cambridge U. P., Cambridge, UK, pp. 85-121.
- Rodhe W. (1978). Algae in culture and nature. *Mitteilungen International Vereinigung Limnol.*, **21**: 7-20.
- Toyub M. A., Rahman M. M., Miah M. I. and Habib, M. A. B. (2005). Growth performance of *Spirulina platensis* in three different concentrations of banana leaf ash with added jackfruit seed powder and urea. *J. Bangladesh Agril. Univ.*, **3**(2): 303-308.
- Toyub M.A., Uddin, M. Z., Miah M. I. and Habib M.A.B. 2006. Growth performance of *Chlorella ellipsoidea* in different concentrations of papaya skin powder media. *Bangladesh J. Agric.*, **31** (1& 2): 35-43.
- Zar J. H. (1984). Biostatistics. prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA, 718pp.
- Zarouk C. (1996). Contribution a Letude d une Cyano-phycee de Divers Facteurs Physique et Chimiques sur la Croissane et Photosynthese de *Spirulina maxima* Geitler, Ph.D. Thesis, University of Paris, France, pp. 45-47.

Received : September 27, 2009;

Accepted : January 13, 2011