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Culture of *Spirulina platensis* in various concentrations of papaya skin powder medium

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Abstract

The growth performance of *Spirulina platensis* was studied in various concentrations *viz.*, 0.3, 0.4 and 0.5 g/L of papaya skin powder medium (PSPM) and Kosaric Medium (KM) in the laboratory for three months. Each trial was carried out for a period of 12 days with three replications. The growth rate of *S. platensis* was found to vary in different media. The initial cell weight of *S. platensis* was 4.0 mg/L which attained a maximum weight of 0.720 g/L in KM followed by 0.693, 0.578 and 0.430 g/L in 0.3, 0.4 and 0.5 g/L of PSPM, respectively on 8th day of culture. Similar trend was observed in the case of chlorophyll a content of *S. platensis*. The proximate composition of papaya skin powder was analyzed and found that the amount of protein was 20.25%, lipid 3.85%, ash 9.31%, NFE 50.49% and moisture 16.10%. The result indicated that the growth rate of *S. platensis* was significantly ($p<0.01$) higher in 0.3 g/L concentration of PSPM than other concentrations of PSPM. The physico-chemical parameters *viz.*, temperature (30.06 °C), light intensity (2110 lux/m^2/s), dissolved oxygen (4.8 mg/L), pH (12.08), nitrate-nitrogen (3.29 mg/L), phosphate-phosphorus (1.97 mg/L), and nitrite-nitrogen (0.063 mg/L) were observed in optimum levels.

Keywords: Culture, *Spirulina*, Papaya skin powder

Introduction

Spirulina is a potential source of an alternative protein for food and feeds. Besides a high protein content (50-60% on dry weight basis), it is a good source of amino acids, essential fatty acids, vitamins and pigments (Li and Qi, 1997). A major pigment in *Spirulina* is phycocyanin that could be utilized as natural blue colour for food, drug and cosmetic industries to replace the currently used synthetic pigments (Borowitzka, 1994 and Li and Qi, 1997). Interest in the use of algae as a source of polyunsaturated fatty acids has increased recently. A great diversity was found in the distribution of fatty acids in the various *Spirulina* strains which provide more unsaturated acids. *Spirulina platensis* (Gom.) is a filamentous, helicoidal cyanophyte and cosmopolitan in distribution. It flourishes very well in alkaline and saline waters in pH 9-11 is too high for most other species to thrive in. China is using *Spirulina* as a substitute of imported forage to promote the growth, immunity and viability of prawns (Ciferri and Tiboni, 1985). *Spirulina*-containing forage was found to reduce the cultivation time and mortality, and increase shell thickness of Scallop. Feeding on *Spirulina* helped to improve disease resistance of high value fish resulting in an improvement in their survival rate for 15% to 30%. When *Spirulina* was added to forage for poultry and livestock, their growth rate was improved. Other *Spirulina* products are fomulated for weight loss and as an aid for quitting drug addiction (Islam, 2004). Cosmetics containing *Spirulina* extracts are also available on the market.

Spirulina platensis has been used for the last ten years as a model organism in many studies on outdoor cultivation of alga biomass as a source of protein and chemicals (Richmond, 1987). Although much progress has been done in this field, resulting in a set-up of several commercial production sites all over the world (Israel, Thailand, Taiwan, USA), the original

goal of producing a cheap alternative source of protein has not yet been achieved, and the cost of production is still one order of magnitude higher than that of conventional sources of protein. So it is needed to identify a culture media for *S. platensis* to spare the high cost of inorganic media. So, attempts were made to find out any inexpensive organic media containing high amount of protein for *Spirulina* culture. Papaya skin is a waste product in our daily life and is easily available in Bangladesh. As, the skin is not used for anything and very green in colour and containing high amount of nitrate considered as an inexpensive organic algal culture medium can be used commercially and economically to culture *S. platensis*, which can be used as feed for fish fry, poultry, livestock, and also as human food. Therefore, the present work was undertaken to study the growth performance of *S. platensis* in various concentrations of papaya skin powder.

Materials and Methods

The experiment was conducted for three months from June to August 2002 in the wet laboratory of the Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Papaya skins were collected from the Kamal-Ranjit market of BAU, Mymensingh and were dried in an oven at 50°C for over night. For complete drying, these papaya skins were dried under sun for another seven days. Then Hamam Dista was used to make powder of the dried papaya skins. For getting very fine particle of papaya skin powder, it was sieved through a siever of 500 μm mesh size.

Collection of *Spirulina platensis*

Spirulina platensis under Order-Volvocales, Class-Chlorophyceae was collected from the laboratory of the Department of Aquaculture, BAU, Mymensingh.

Pure stock culture maintenance

For purity, the stock culture of *S. platensis* was maintained in the laboratory in Kosaric medium (KM). Growth of *S. platensis* was monitored and it was then checked under microscope to confirm its purity.

Preparation of papaya skin powder medium (PSPM) and Kosaric medium (KM) for *S. platensis* culture

For preparation of papaya skin powder medium, 0.3, 0.4 and 0.5g of papaya skin powder were taken into a 1.0 litre conical flask with three replications. Distilled water was added into the conical flask to dilute it and made 1.0 L volume. About 400 ml was taken in each 1.0 L flask. Then the medium was mixed well and sterilized at 120°C for 15 minutes with moist heat autoclave (Express Equipment, Dixon's Surgical Instrument LTD.).

Stock solutions of different chemical ingredients of KM (Phang and Chu, 1999) were prepared with distilled water and kept in freezer. The KM was prepared by taking required amount of solution from each stock in 1.0 L conical flask and volume was made up to one litre marked with distilled water. Then mixing, sterilization and cooling of the prepared media were done as followed the same procedure during the PSPM preparation.

Culture of *S. platensis* in papaya skin powder medium

At first, *S. platensis* were inoculated into three one litre culture bottle containing 0.3, 0.4 and 0.5 g/L of papaya skin powder medium respectively to produce a culture containing 10% *S. platensis* suspension (Optical density at 620 nm = 0.41) (Habib, 1998). 37.5 ml/L of *S. platensis* suspension was required for getting the density. Similarly, same inocula were taken into four culture bottles containing various concentrations (0.3, 0.4 and 0.5 g/L) of PSPM and KM. All the bottles were kept under fluorescent light (TFC, FL-40SD/38 Day light, Taiwan) in light: dark (12:12) conditions in the laboratory. These cultured bottles were continuously aerated using electric aerator (Davio Pump, Aquarium Pump NS-8200). Seven sub samplings were taken at every alternative day from each bottle to observe *S. platensis* cell density, chlorophyll a, water quality parameters (physical and chemical parameters) of culture media. All the glassware's used in the experiment were sterilized by dry heat in an oven at 70°C.

Experimental design of *S. platensis* culture

The two types of media *viz.* PSPM and KM were used for the culture of *S. platensis*. For PSPM 3 treatments each of 3 replications and for KM 1 treatment of 3 replications were used. For both the culture media 12 days culture period was maintained. Inoculum of *S. platensis* was collected from the pure stock culture.

Estimation of *S. platensis* cell weight (g/L)

For cell weight 10 ml of *S. platensis* sample from each treatment was taken and filtered with an electric filtration unit using filter papers (Whatman. GF/C) and shifted to the oven at 105°C for 24 hours. The samples were then transferred to the dessicator for colling and weight was measured using an electric balance. Before filtering the weight of the filter paper was taken.

Calculation

The cell weight of *S. platensis* (mg/L) were calculated by the following formula:

$W = (S+F) - F$

Where,

W = cell weight (mg/L)

S = weight of sample taken (1.0 ml) before filtering

F= filter paper weight

Estimation of chlorophyll a

Chlorophyll a of microalgal samples collected in different times were estimated. Ten ml of *S. platensis* samples was filtered with an electric filtration unit using filter papers (Whatman. GF/C), mixed with 10 ml of acetone, ground with glass rod and kept in a refrigerator (LMS, Laboratory Refrigerator) over night. The refrigerated samples were homogenized for 2 minutes followed by centrifugation at 4000 rpm for 10 minutes. The supernatant was separated and used for chlorophyll a determination.

Optical densities of the samples were determined at 664, 647 and 630 nm by using UV spectrophotometer (Milton Roy, Spectronic 1001 Plus) [Clesceri *et al.*, 1989]. A blank with 100% acetone was run simultaneously. Chlorophyll a content was calculated by the following formula:

Chlorophyll a (mg/L) = 11.85 (OD 664)-1.54 (OD 647)- 0.08 (OD 630).

Analysis of physico-chemical parameters of culture media

The physical parameters i.e. temperature and light intensity of the culture media were recorded in sampling day by a Celsius thermometer and a lux-meter [digital instrument, Lutron (LX-101)] respectively.

The chemical parameters i.e. dissolved oxygen and pH of the culture media were recorded in sampling day by using dissolved oxygen meter (YSI, Model 58) and an electric pH meter (Corning pH meter 445), respectively. Nitrate-nitrogen, phosphate-phosphorus and nitrite-nitrogen contents in the culture sample were estimated in the laboratory by using Hach Kit (DR/2010) and necessary reagent pillow.

Analysis of proximate composition

Proximate composition of sample was analyzed in the nutrition laboratory, Department of Aquaculture, BAU, following standard methods (Horwitz, 1984).

Statistical analysis

Analysis of variance (ANOVA) and DMRT of mean cell densities and chlorophyll a of *Spirulina platensis* cultured in different media were done to find wheather any significant different among treatments (Gomez and Gomez, 1976).

Results and Discussion

Cell weight and Chlorophyll a content of *S. platensis*

The range of cell weight of *Spirulina platensis* in different concentrations of papaya skin powder medium *viz.,* in concentration of 0.3 g/L was 0.004 g/L to 0.693 g/L; in concentration of 0.4 g/L was 0.004 g/L to 0.578 g/L; in concentration of 0.5 g/L was 0.004 g/L to 0.430 g/L and in KM was 0.004 g/L to 0.720 g/L (Table 1). The growth of cell was varied in different media and different concentrations. This variation might be due to different nutrient composition and concentrations of different media (Mario *et al.,* 1986). The growth rate of *S. platensis* was higher in KM than various concentrations of PSPM which might be due to the availability of more nutrients in KM than various concentrations of PSPM. On the other hand, the growth rate was higher in concentration of 0.3 g/L PSPM than other concentrations of PSPM. It might be due to the suitable amount of nutrients in PSPM (0.3 g/L) than other concentrations of PSPM.

Chlorophyll a contents were recorded in all media followed the similar trend like cell weight (Table 2). Similar type of work was carried out by Mario *et al.* (1986). They conducted a whole year experiment on the outdoor mass culture of *S. maxima* strain 4MX in fertilized seawater. They obtained the mean annual yield of biomass in seawater plus urea as nitrogen source was 7.35 g (dry wt.)/m^2/d, i.e. 0.394 g/L/d a value slightly lower than that obtained on the standard bicarbonate medium (8.14 g/m^2/d). Tanticharoen *et al.* (1990) reported that the addition of sodium bicarbonate ($NaHCO_3$) and nitrogen fertilizer in wastewater from the stabilization ponds of tapioca starch factory raised the productivity up to 7-10 g/m^2/d which was higher than the present findings. Li and Qi (1997) reported the biomass output rate in Chinese production plants was 7.0 g/m^2/d which was also much higher than the results of present study.

Table 1. Mean (± S.E.) of cell weight (g/L) of *S. platensis* grown in papaya skin powder medium (PSPM) and Kosaric Medium (KM)

Sampling day	Concentration 0.3 g/L	Concentration 0.4 g/L	Concentration 0.5 g/L	KM
0	0.004	0.004	0.004	0.004
2	0.067±0.01	0.059±0.01	0.051±0.01	0.072±0.01
4	0.220±0.02	0.152±0.02	0.168±0.02	0.190±0.01
6	0.375±0.02	0.325±0.02	0.220±0.02	0.410±0.03
8	0.693±0.03	0.578±0.03	0.430±0.03	0.720±0.04
10	0.580±0.02	0.483±0.02	0.380±0.02	0.633±0.03
12	0.543±0.02	0.442±0.02	0.318±0.02	0.581±0.02

Table 2. Mean (± S.E.) of Chlorophyll *a* content (mg/L) of *S. platensis* grown in papaya skin powder medium (PSPM) and Kosaric Medium (KM)

Sampling day	Concentration 0.3 g/L	Concentration 0.4 g/L	Concentration 0.5 g/L	KM
0	0.030	0.030	0.030	0.030
2	0.585±0.03	0.270±0.02	0.220±0.01	0.710±0.05
4	0.975±0.06	0.460±0.02	0.410±0.02	1.120±0.09
6	1.535±0.08	0.673±0.03	0.470±0.02	1.810±0.08
8	2.743±0.13	1.043±0.09	0.737±0.05	2.870±0.16
10	2.260±0.02	0.938±0.06	0.640±0.03	2.540±0.11
12	1.843±0.07	0.773±0.04	0.570±0.03	2.040±0.09

Pulz *et al.* (1992) recorded the productivity levels of 1.3 g/L/d, corresponding to 28 g/m^2/d on a basis of illuminated surface area and to 120 g/m^2/d of occupied land, had been obtained under natural illumination, which is also higher than the KM of present study. Zhiyong *et al.* (2000a) used tubular air-lift photobioreactor to culture *S. platensis* and found that when the cultural volume was 12L, under the condition of 5000 lux/m^2/s, 30°C and 200 KA/m, the maximum cell dry weight of *S. platensis* cultured in batch could reach 2.4 g/L which was also higher than the findings of the present study.

Physico-chemical characteristics

Light intensity is an important physical factor for the growth of microalgae. The best growth of *S. platensis* is found at light intensity of 2110 and 2120 lux/m^2/s in PSPM and KM, respectively (Fig 1). Zarrouk (1966) found that the growth of *S. platensis* was saturated at levels of 25-30 Klux/m^2/s. This variation might be occurred due to the difference of strain of species and composition of nutrient in different media.

For normal growth of microalgae, dissolved oxygen is one of the most important chemical parameter. During the period of experiment, the dissolved oxygen was found to range from 3.2 to 5.3 mg/L (Fig 2). The fluctuation in dissolved oxygen value might be due to alteration rate of photosynthesis in the culture media. Marquez *et al.* (1995) and Singh *et al.* (1995) reported that high oxygen level results in reduced growth rate and bleaching of the pigment.

The pH of the medium is one of the most important chemical factors in culturing *Spirulina*. Maintaining pH of over 9.5 is mandatory in *Spirulina* cultures in order to avoid contamination by other algae. In contrast, at present study the pH range was found at 9.48 to 12.18 which is suitable for the growth of *Spirulina* (Fig 3). The maximum cell weight followed at pH value 12.08 and 12.18 in PSPM and KM, respectively on 8th day of culture. Temperature is the most important physical factor for the growth of all living organisms. More or less similar temperature was recorded during the culture period. The maximum cell growth at 30.06°C in 0.3 g/L concentration of PSPM and KM respectively on 8th day of culture (Fig 4). Torzillo and Vonshak (1994) reported that the optimal temperature for photosynthesis of *Spirulina* strain marked M-2 was 35-38°C which is more or less similar to the present study. The minimum mean values of nitrate-nitrogen contents were recorded in 3.102, 3.440, 3.320 and 4.320 mg/L in 0.3, 0.4, 0.5 g/L concentrations of PSPM and KM, respectively on 10th day of culture which was maximum on first day of culture (Fig 5). The minimum mean values of phosphate-phosphorus contents were recorded in 1.968, 2.513, 4.250 and 157.500 mg/L in 0.3, 0.4, 0.5 g/L PSPM and KM, respectively on 8th day of culture when *Spirulina* attended to high growth (Fig 6). The amounts of phosphate-phosphorus in KM in all the sampling days compared to the different concentrations of PSPM were higher because Kosaric medium (KM) contains high amount of phosphate-phosphorus on the basis of their chemical composition. The maximum mean values of nitrite-nitrogen contents in various concentrations of PSPM and KM were 0.090, 0.075, 0.080 and 0.040 mg/L in 0.3, 0.4, 0.5 g/L concentrations of PSPM and KM respectively on 10th day of culture (Fig 7). The ranges of mean value of nitrite-nitrogen in cultured media was 0.010 mg/L to 0.125 mg/L. Nitrite-nitrogen was found lowest on the initial day and highest on the 12th day of culture. An increased trend of nitrite-nitrogen was observed in cultured media due to the decomposition of dead *S. platensis* cells.

The biochemical compositions of the papaya skin powder analysed in the present study i.e. protein- 20.25, lipid- 3.85, ash- 9.31, NFE- 50.49 and moisture-16.10 (Table 3) was more or less similar to the findings of Fouzder *et. al.*, 1999. The cell weight of *S. platensis* from PSPM at concentration of 0.3 g/L was significantly ($p<0.01$) higher than other concentrations of PSPM (Table 4). Similarly Chlorophyll *a* content of PSPM (0.3 g/L) was also significantly ($p<0.01$) higher than other concentrations of PSPM (Table 5).

Table 3. Proximate composition of papaya skin powder medium (on dry matter basis)

Proximate composition	Percentage (%)
Moisture	16.10
Protein	20.25
Lipid	3.85
Ash	9.31
Nitrogen free extract	50.49

Table 4. Duncan 's New Multiple Range Test (DMRT) of mean values of cell weight of *S. platensis* grown on from 2nd day to 12th day in various concentrations of papaya skin powder medium (PSPM) and Kosaric medium (KM)

Original order						
Treatment/ control	Mean values					
	2^{nd} day	4^{th} day	6^{th} day	8^{th} day	10^{th} day	12^{th} day
1	0.067^{a}	0.220^{a}	0.375^{b}	0.693^{b}	0.580^{b}	0.543^{b}
2	0.059^{b}	0.152^{d}	0.325^{c}	0.578^{c}	0.483^{c}	0.442^{c}
3	0.051^{c}	0.168^{c}	0.220^{d}	0.430^{d}	0.380^{d}	0.318^{d}
4	0.072^{a}	0.190^{b}	0.410^{a}	0.720^{a}	0.633^{a}	0.581^{a}

Figures in the same column with same superscripts are not significantly different ($p<0.01$)
*Treatment 1- 0.3 g/L
*Treatment 2- 0.4 g/L
*Treatment 3- 0.5 g/L
*Treatment 4- KM (which was used as control medium)

Table 5. Duncan 's New Multiple Range Test (DMRT) of mean values of Chlorophyll a contents of *S. platensis* grown on from 2nd day to 12th day in various concentrations of papaya skin powder medium (PSPM) and Kosaric medium (KM)

Original order						
Treatment/ Control	Mean values					
	2^{nd} day	4^{th} day	6^{th} day	8^{th} day	10^{th} day	12^{th} day
1	0.585^{b}	0.975^{b}	1.535^{b}	2.743^{b}	2.260^{b}	1.843^{b}
2	0.270^{c}	0.460^{c}	0.673^{c}	1.043^{c}	0.938^{c}	0.773^{c}
3	0.220^{d}	0.410^{c}	0.470^{d}	0.737^{d}	0.640^{d}	0.570^{d}
4	0.710^{a}	1.120^{a}	1.810^{a}	2.870^{a}	2.540^{a}	2.040^{a}

Figures in the same column with same superscripts are not significantly different ($p<0.01$)
*Treatment 1- 0.3 g/L
*Treatment 2- 0.4 g/L
*Treatment 3- 0.5 g/L
*Treatment 4- KM (which was used as control medium)

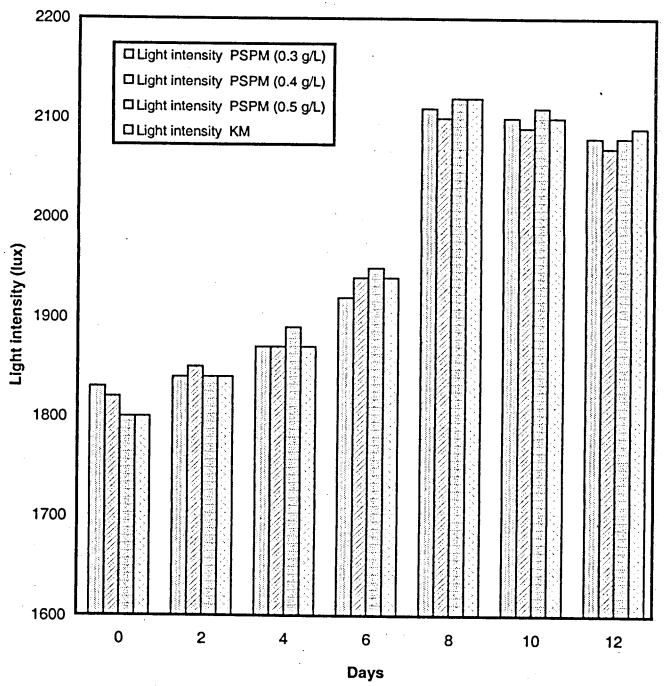

Fig. 1 Mean light intensity (lux) of different concentrations of papaya skin powder medium (PSPM) and kosaric medium (KM) contained *Spirulina platensis*

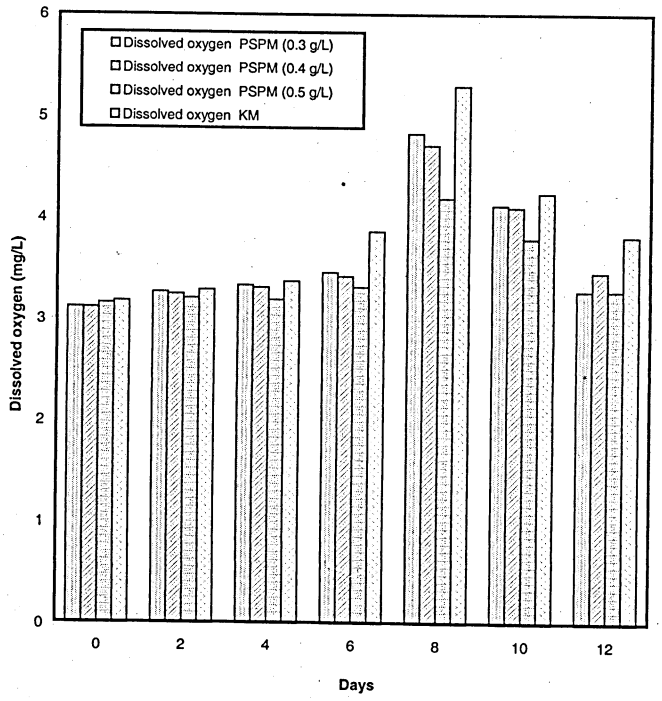

Fig. 2 Mean dissolved oxygen (mg/L) of different concentrations of papaya skin powder medium (PSPM) and kosaric medium (KM) contained *Spirulina platensis*

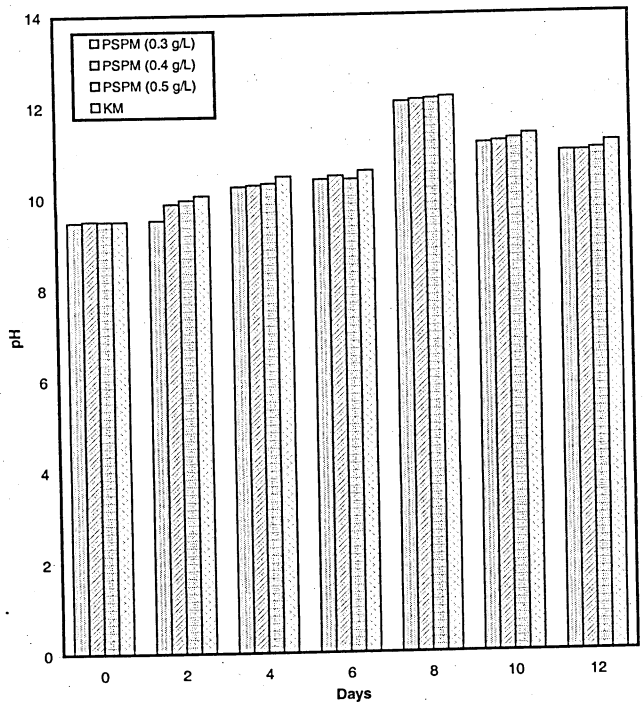

Fig. 3 Mean of pH of different concentrations of papaya skin powder medium (PSPM) and kosaric medium (KM) contained *Spirulina platensis*

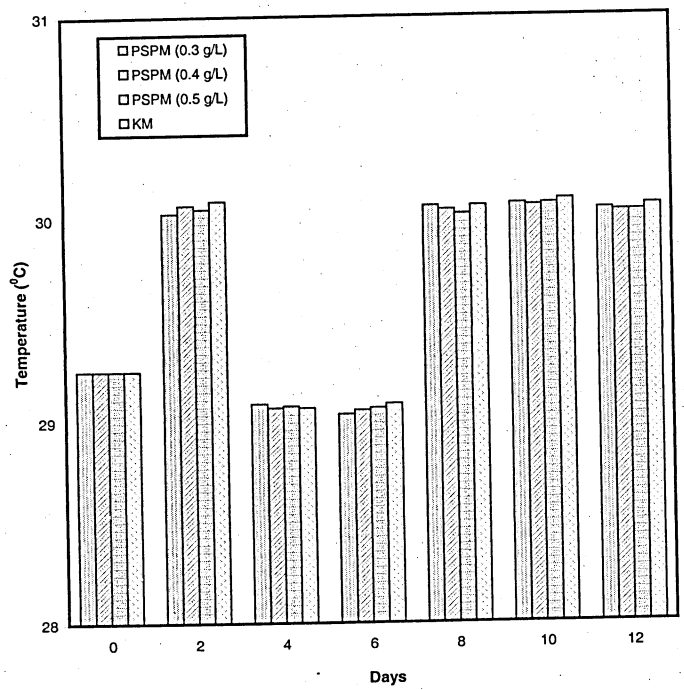

Fig. 4 Mean of temperature (^{0}C) of different concentrations of papaya skin powder medium (PSPM) and kosaric medium (KM) contained *Spirulina platensis*

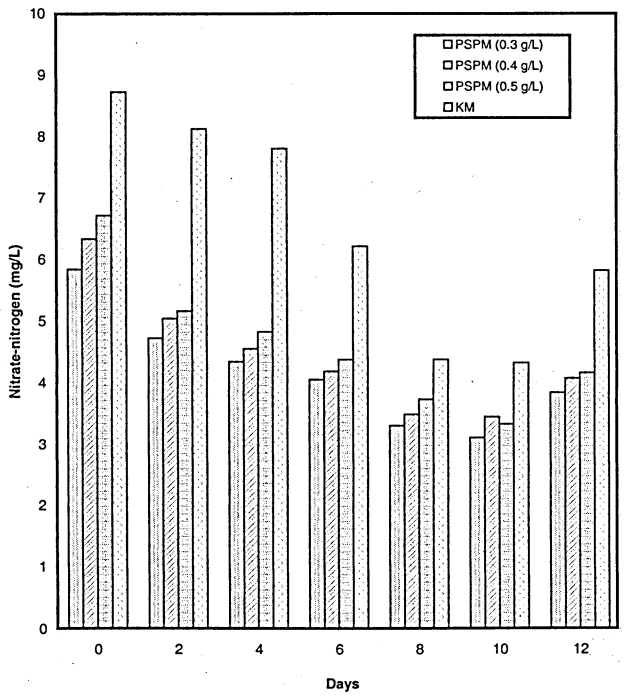

Fig. 5 Mean of nitrate-nitrogen (mg/L) of different concentrations of papaya skin powder medium (PSPM) and kosaric medium (KM) contained *Spirulina platensis*

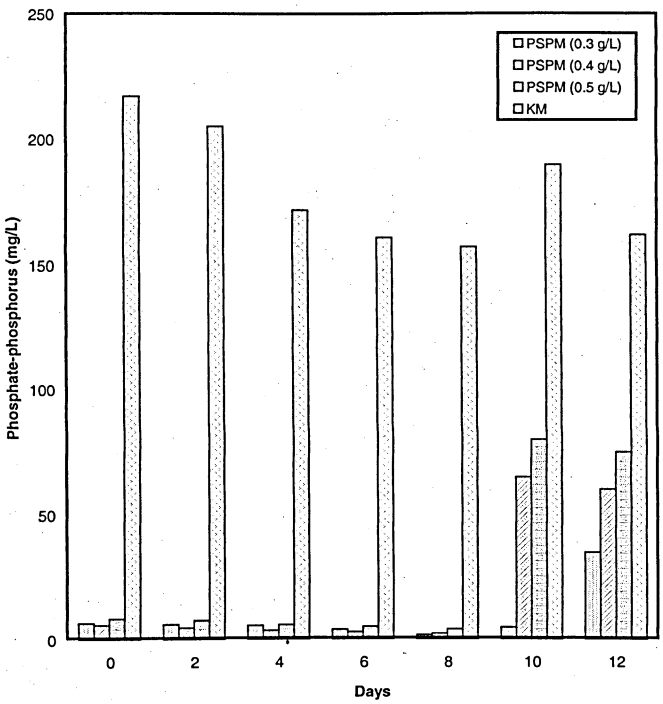

Fig. 6 Mean of phosphate-phosphorus (mg/L) of different concentrations of papaya skin powder medium (PSPM) and kosaric medium (KM) contained *Spirulina platensis*

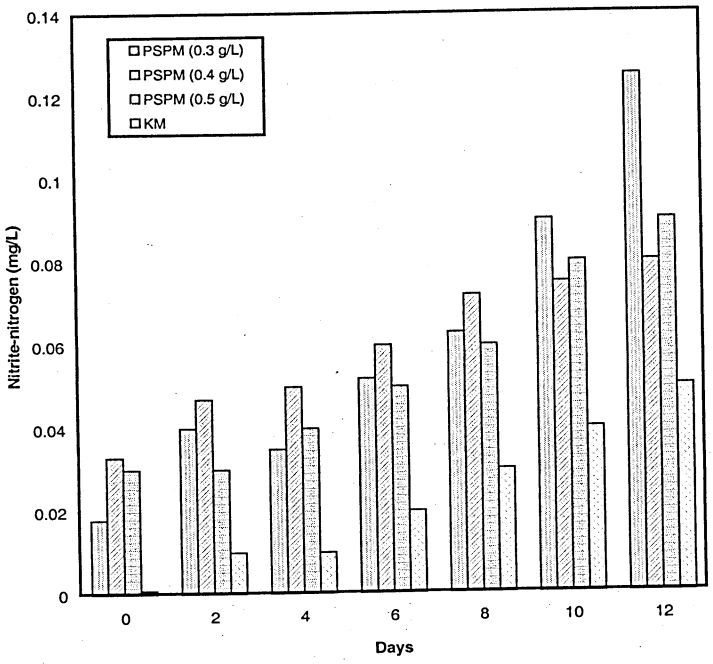

Fig. 7 Mean of nitrite-nitrogen (mg/L) of different concentrations of papaya skin powder medium (PSPM) and kosaric medium (KM) contained *Spirulina platensis*

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