Research Article

Evaluation of the Growth Performance of *Spirulina platensis* in Different Concentrations of Kosaric Medium (KM) and Papaya Skin Powder Medium (PSPM)

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ABSTRACT

Aim: The aim of the study was to evaluate the efficacy and cost-effectiveness of *Spirulina platensis* cultivation using different concentrations of Papaya Skin Powder Medium (PSPM) in comparison to Kosaric Medium (KM). The experiment sought to compare the outcomes of Spirulina cultivation under various concentrations of PSPM, with the objective of determining the feasibility of producing *S. platensis* at a reasonable cost. **Materials and Methods:** Each concentration was subjected to its respective treatment i.e., T_1 (15% PSPM), T_2 (20% PSPM), T_3 (25% PSPM) and T_4 (KM). Each treatment had three replications. The Papaya skin powder was added 15, 20 and 25% in 600 mL distilled water for each and kept in 1000 ml conical flask. The required amount of KM was produced in a 1000 ml conical flask containing 600 ml of distilled water. **Results:** The initial weight of *S. platensis* cells was 0.041 g/l, and they reached their maximum weight of 0.714 g/l in KM before decreasing to 0.498, 0.569, and 0.680 g/l in the 15%, 20%, and 25% PSPM, respectively. In comparison to other PSPM concentrations, the 25% concentration showed a considerably (p<0.01) faster growth rate. The proximate composition of Papaya Skin Powder was examined and it was found to have 21.19% protein, 8.45% lipid, 8.13% ash, 51.229 NFE, and 15.95% moisture. Temperature was measured to be between 24 and 31.8 °C, pH was 9.3 to 9.53, Dissolved Oxygen (DO) was 4.27 to 6.58 mg/l, voltage between a pH sensitive glass electrode (MVPH) was 128 to 149, and measurements of Total Dissolved Solid (TDS), electric conductivity, and hectopascal pressure unit (hpa%) were 1012 to 1476. Salinity was measured to be between 0.60 and 4.32. **Conclusion:** The growth rate of *S. platensis* cells exhibited a higher multiplication rate in KM as compared to various concentrations of PSPM. The rate of cell multiplication exhibited a significantly higher value (p<0.01) when exposed to a concentration of 25% PSPM in comparison to other concentrations. The 25% PSPM can be considered a financially feasible medium for large-scale cultivation.

Keywords: Growth performance, *Spirulina platensis*, Kosaric Medium (KM), Papaya Skin Powder Medium (PSPM).

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INTRODUCTION

Spirulina is a spiral-shaped, multicellular, filamentous blue-green algae that thrives in alkaline conditions in freshwater, brackishwater, and marine environments.[1] Spirulina is a planktonic, photosynthetic cyanobacterium that typically has a length of 300–500 μm. It has a rich history in the plant kingdom and fills a fascinating ecological and biological niche.[2] Any type of microalgae

can generate colors, proteins, and vitamins. According to,[3] Spirulina contains proteins, carbs, vitamins and minerals, carotene, and super antioxidants from several elements. According to[4] and,[5] Spirulina contains a high concentration of vitamins, lipids, essential fatty acids, linolenic acid, and 13.6% of carbs. Spirulina production and collection technology is indeed widely utilized for its unique properties as a microalgae in the field of nutrition. Spirulina growth is influenced by a number of variables, including temperature, light, and nutrient availability.[6] The composition and physiological status of phytoplankton are significantly influenced by external factors, particularly culture temperature.[7] According to,[8] the ideal temperature for Spirulina growth is between 30 and 35°C. Low temperatures inhibit the growth of Spirulina.

The other crucial element, pH, similarly controls the solubility of minerals and carbon dioxide in the medium, which either directly or indirectly affects the metabolism of algae.[9] According to,[10] Spirulina requires rather high pH levels between 9.5 and 9.8. Spirulina has been produced commercially in a number of nations across the world because of its great nutritional value, high protein content, and excellent source of food.[11] It is noted that, the rich nutritional profile and health-improving properties of Spirulina make it a widely prized food. Similar to this,[12] demonstrated that Spirulina has an edible source of vitamins, 12-14% carbohydrate, 55-75% protein, 6% minerals, and 5% fat. The results of research indicate that 1 kg of Spirulina spp. is equivalent to 1 kg of other vegetables.[12] Due to its high and good quality protein content, vitamin and essential fatty acid levels, super antioxidant pigments, antibacterial activity, and anticancer capabilities, the fast-growing and large-sized Spirulina has the potential to be used as a substitute protein source for cultured fish.[1]

The majority of the variable operational expenses in aquaculture fish farming are related to feed. Basically, the combination of protein as a feedstock determines the quality of the feed. The protein content of feed ingredients is the biggest cost contributor. The demand for high-quality feed has become a serious issue for aquaculture production as the industry has become more prevalent. Around the world, there is a growing need for sufficient feed. Numerous feed production enterprises have been established across the nation to fulfill the rising demand for feed and ensure a steady supply of high-quality feed, which is crucial for fish growth. It is crucial for the quality of the given feed ingredients to keep the Feed Conversion Ratio (FCR) close to 1.0. Because it promotes rapid growth and is essential for the maturation of the immune system, the feed for fish culture should include a significant amount of the necessary protein content.

Spirulina continues to have a high cost of manufacturing. Therefore, it is crucial that the commercial production of Spirulina can be made cost-effective without sacrificing production efficiency by the decrease of the input cost with affordable and easily available ingredients. The goal of the current study was to compare the growth performance of *Spirulina platensis* under various concentrations of of PSPM and KM.

MATERIALS AND METHODS

Inoculum collection and pure stock maintaining condition:

The *Spirulina platensis* strain (Figure 1) of cyanobacterium was obtained from BAU in Mymensingh and was kept alive in a 500 ml conical flask that had been sterilized and contained 100 ml of Kosaric Medium.[13]

Culture Media and Experimental Design

Spirulina platensis was cultured using two different media types: Kosaric Medium (KM) and Papaya Skin Powder Medium (PSPM). Three PSPM doses were employed as T_1, T_2, and T_3, each of which had three replications. To compare the growth performance of Spirulina in T_1, T_2, and T_3, KM was employed as T_4 with three replications.

Experimental Setup

An experimental setup (Figure 2) was kept for *S. platensis* cultivation from January to March 2022 in the Nutrition Division of Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh at room temperature 32°C, light intensity 12/12 hr light-dark photoperiod, and maintained 1500 lux to 3500 lux.[14] 12 conical flasks, each with a volume of 1000 ml, were used for dry heating in an oven at 70°C. During the day, the temperature and lux were measured.

Figure 1: Microscopic view of *Spirulina*.

Figure 2: Experimental setup.

Preparation of PSPM

The Papaya was procured from the neighborhood market in Mymensingh, and the peel was removed before being sun-dried for seven days and then kept in a oven for overnight. Papaya skin was dried completely before being ground up with an electric blender and then filtered through a 300 ml screen to produce extremely small particles. Papaya skin powder was added 15, 20 and 25% in 600 ml distilled water for each and kept in 1000 ml conical flask and mixed well. Then the flask transferred to the moist heat autoclave for sterilized at 120°C for 15 min and then cooled media was mixed well.

Preparation of KM

The several chemical components of stock solutions were dissolved in distilled water to prepare KM. The necessary quantity of each stock solution is retained in a conical flask with a 1000 ml capacity, 600 ml of distilled water, and is thoroughly mixed. The media was then well mixed in accordance with PSPM preparation after the flask had been sterilized in a moist heat autoclave at 120°C for 15 min.

Evaluation of Cell (g/L) Multiplication of *S. platensis*

By measuring optical density at a 1-day interval for each replication of each treatment, the biomass concentration was calculated.[15] Individual sample biomass was shown by optical density. By using a high-speed chilled Micro-centrifuge (Japan) to centrifuge 10 ml of samples from each treatment, the dry weight of *S. platensis* was calculated. In order to obtain dry biomass, the acquired samples were washed with distilled water, placed in an oven set at 105°C for 24 hr, and then cooled in a desiccator.[16] The following formula was used to compute *S. platensis* cell multiplication growths in response to various treatments:

Cell productivity (*Px*) according to.[17]

$$Px(\text{mg/L/day}) = (X_m - X_i / t_m),$$

Where:

X_i=initial cell concentration (mg/L),

X_m=maximum cell concentration (mg/L),

t_m=cultivation time related to maximum cell concentration (days).

Estimation of Physico-Chemical Characteristics

The physico-chemical parameters were measured by a Hanna multiparameter (Model: Hi 98194). These included pH, temperature, Dissolved Oxygen (DO), Total Dissolved Solids (TDS), Electric Conductivity (EC), Hectopascal Pressure Unit (hpa %), and salinity.

Proximate Composition Analysis

Spirulina was centrifuged at the end of the study and kept in the freezer at 80°C to be examined for its proximate composition. The Laboratory of Fish Nutrition and Feed Technology at BFRI, Mymensingh, carried out Standard Crude Protein Analysis.[18] and Crude Lipid Analysis.[19]

Statistical Analysis

The acquired data were subjected to a one-way ANOVA analysis to determine whether or not there is a significant difference ($p<0.05$) between the treatment means.

RESULTS

Estimation of Cell (g/L) Multiplication of *S. platensis*

The cells of *S. platensis* multiplied at different concentrations/treatments, such as T_1 (15%), T_2 (20%) and T_3 (25%) of PSPM was 0.041±0.008 to 0.498±0.042 g/L; 0.041±0.008 to 0.569±0.034 g/L; 0.041±0.008 to 0.680±0.019 g/L, respectively and in KM (T_4) cell multiplication was found 0.041±0.008 to 0.714±0.015 g/L. Both media types and various PSPM concentrations showed variation in the increase of cell multiplication. The development of cell multiplication of *S. platensis* at various concentration of PSPM and KM concentrations was shown in Figure 3 and Table 1. In comparison to different PSPM concentrations, the growth of *S. Platensis* cells multiplied more quickly in KM. The growth cell multiplication rate was significantly higher ($p<0.01$) at 25% PSPM concentration compared to other concentrations. In response to various

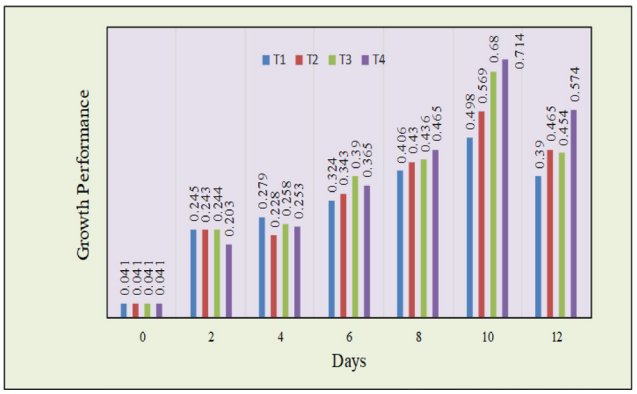

Figure 3: Growth performance of *S. platensis*.

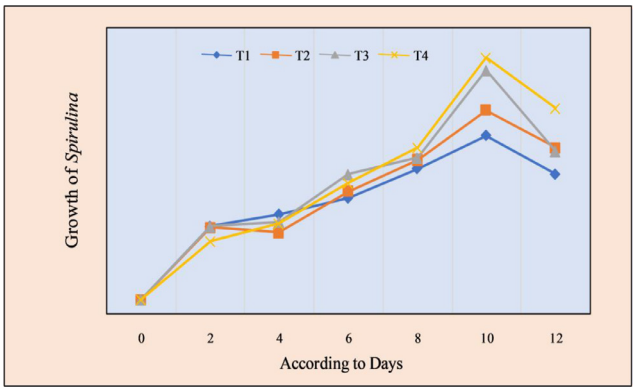

Figure 4: Growth Performance Curve of *S. platensis*.

Table 1: Mean (S.E.) of cell multiplication (g/L) of *S. platensis* in Papaya Skin Powder Medium (PSPM) and Kosaric Medium (KM).

Sampling day	T_1 (15% PSPM)	T_2 (20% PSPM)	T_3 (25% PSPM)	T_4 (KM)
0	0.041±0.008	0.041±0.008	0.041±0.008	0.041±0.008
2	0.245±0.038	0.243±0.042	0.244±0.021	0.203±0.013
4	0.280±0.032	0.228±0.041	0.258±0.029	0.253±0.019
6	0.324±0.046	0.343±0.032	0.390±0.017	0.365±0.021
8	0.406±0.031	0.430±0.035	0.436±0.034	0.465±0.013
10	0.498±0.042	0.569±0.034	0.680±0.019	0.714±0.015
12	0.390±0.035	0.465±0.043	0.454±0.017	0.574±0.019

Table 2: Physico-chemical parameters of culture media.

Parameters	Treatments			
	T_1 (15% PSPM)	T_2 (20% PSPM)	T_3 (25% PSPM)	T_4 (KM)
pH	9.30±0.05	9.44±0.02	9.42±0.01	9.42±0.13
Temperature	24.00±0.08	29.39±0.37	28.47±0.40	28.16±0.84
DO	4.27±0.10	5.86±0.22	6.47±0.35	6.58±2.03
MVPH	128.70±4.62	143.18±11.59	149.47±0.43	147.13 ±5.75
TDS	1070.67±72.60	1101.00±90.80	1026.00±103.11	3875.00±1554.52
EC	1501.17±146.37	2218.00±208.88	2011.33±193.41	2576.12±515.89
hpa%	1012.00±0.50	1014.33±1.04	1018.17±1.26	1017.33±2.08
Salinity	0.60±0.08	0.88±0.32	0.64±0.20	4.32±1.86

treatments, cell multiplication increased during the course of the study period (Figure 4).

Estimation of Physico-Chemical Characteristics

A significant physical factor in the growth and spread of microalgae is light intensity. In PSPM and KM, *S. platensis* showed best performance at light intensities of 2250 and 2500 lux/m^2/s, respectively. The physico-chemical parameters, including pH, temperature, Dissolved Oxygen (DO), Total Dissolved Solids (TDS), Electric Conductivity (EC), Hectopascal Pressure Unit (hpa%), Salinity, and measuring the Voltage between a pH-sensitive Glass Electrode (MVPH), were recorded (Table 2 and Figure 5). Dissolved oxygen is one of the fundamentally essential chemical components for the healthy development of microalgae. During the experiment, it was discovered that the Dissolved Oxygen concentration ranged from 4.27 to 6.58 mg/L. The pH range of the Spirulina during the cultivation phase was determined to be 9.30 to 9.53, and the maximum cell weight was found at pH values of 9.42 and 9.44 in PSPM and KM, respectively. Temperatures

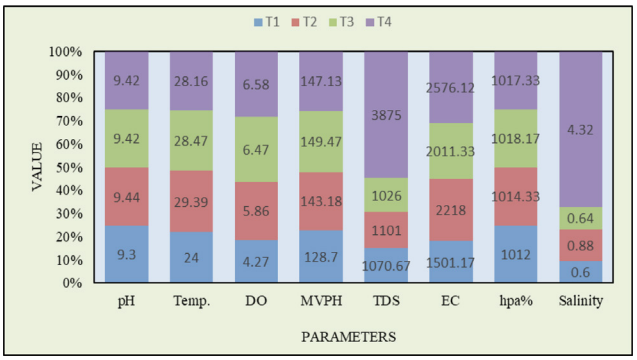

Figure 5: Physico-chemical parameters of the culture media.

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

Proximate composition	Percentage (%)
Moisture	15.95
Protein	21.19
Lipid	8.45
Ash	8.13
Nitrogen Free Extract (NFE)	51.29

during the development phase were largely similar. At 28.16°C, the highest cell growth at 25% PSPM concentration was 0.689 g/L and 0.714 g/L in KM.

At the end of the experiment, the proximate composition of the powdered Papaya skin were examined. The corresponding percentages for protein, fat, ash, nitrogen-free extract, and moisture were 21.19%, 8.45%, 8.13%, 51.29%, and 15.95% (Table 3).

Effect of Aeration on Various Nitrogen Sources

The Spirulina species performs better with maximal dry weight when aeration is used. The highest amount of Chlorophyll was seen during aeration when using an aquarium pump. As a result, aeration increases the Chlorophyll content of different Spirulina species. In order to ensure that the Spirulina filaments receive adequate lighting, aeration strengthened the culture media and distributed them evenly throughout the production system. Additionally, it facilitated the efficient distribution of oxygen concentrations and eliminates some inhibitory substances, such as CO_2. Therefore, aeration is necessary to maintain regular nutrient distribution and to get rid of surplus oxygen. The air pump was used to provide continual aeration in order to meet the need of Spirulina for CO_2 as well as to prevent the algae from settling to the bottom of the flask. It should be noted that, temperature stratification and cell settling can be avoided by making sure the continuous mixing of the culture media. It is also necessary for the growth of *S. platensis*. Inadequate aeration reduces the generation of biomass and the effectiveness of energy utilization.

DISCUSSION

The cells of *S. Platensis* underwent multiplication under various treatments like T_1 (15%), T_2 (20%) and T_3 (25%) of PSPM and in KM (T_4). Both types of medium and different concentrations of PSPM exhibited variability in the rate of cell multiplication. The development of cells exhibited variation across different concentrations and media, as reported by.[20] The authors additionally proposed that the observed variation may be attributed to the manipulation of media concentrations and nutritional contents. When comparing various concentrations of PSPM, it was shown that the growth rate of *S. platensis* cells was significantly higher in KM. The presence of favourable environmental conditions, including pH, temperature, oxygen levels, and nutrient availability, may contribute to the observed differences in PSPM concentrations compared to other parameters. A significant physical factor in the growth and spread of microalgae is light intensity. At rates of 25–30 klux/m^2/s, *S. platensis* growth slowed down.[13] This variance might have occurred because different substrates contain different species strains and nutritional profiles.[21] also conducted an experiment at light intensities ranging from 1500 to 2500 lux, while,[22] noted that Spirulina cells must be able to control their photosynthetic efficiency by varying the nutritional medium content. During the experiment, it was discovered that the Dissolved Oxygen concentration ranged from 4.27 to 6.58 mg/L. The variation in dissolved oxygen could be caused by a change in the rate of photosynthesis occurring in the culture media. Higher oxygen content, according to,[23] and,[24] reduces the pigment's development rate during bleaching.

One of the most important chemical factors in growing Spirulina is the pH of the culture medium. A pH of higher than 9.5 must always be maintained for Spirulina cultures to remain free of contamination from other algae. It was discovered that the Spirulina was in good health, that the filament had not changed shape, and that the color was acceptable when compared to KM. Similar to this, an investigation by[3] using various media showed that the Spirulina culture was developed healthily and that the morphology of *S. platensis* filament also kept its color and shape.

Temperature is the most important structural component for the development of all biological things. According to,[25] the ideal temperature for the strain of

Spirulina photosynthesis was found to be between 28°C and 29.39°C, which is essentially equal to the present study. On the other hand,[21] reported that the growth performance of Spirulina was better at 30°C compared to 25°C and 35°C and also mentioned below 25°C and 35°C, minimal growth was observed. The corresponding percentages for protein, fat, ash, nitrogen-free extract, and moisture were 21.19%, 8.45%, 8.13%, 51.29%, and 15.95%. The results of this investigation were comparable to those of[26] and.[27]

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PSPM: Papaya Skin Powder Medium; **KM:** Kosaric Medium; **NFE:** Nitrogen Free Extract; **DO:** Dissolved Oxygen; **MVPH:** pH Sensitive Glass Electrode; **TDS:** Total Dissolved Solid; **HPa:** Hectopascal Pressure.

SUMMARY

The cells *S. platensis* multiplied at different concentrations/treatments [T_1 (15%), T_2 (20%), and T_3 (25%) of PSP medium and KM (T_4)]. Both media types and various PSPM concentrations showed variation in the increase of cell multiplication. In comparison to different PSPM concentrations, the growth of *S. platensis* cells multiplied more quickly in KM. The growth cell multiplication rate was substantially higher ($p<0.01$) at 25% PSPM concentration compared to other concentrations. The current study examines the growth and multiplication of Spirulina in Kosaric Medium and various concentrations of PSPM. However, it shows the best growth traits when grown on Kosaric Medium (0.714g/L). The growth rate at 25% of the different Papaya Skin Powder Medium concentrations was considerably ($p<0.01$) greater. To get accurate information about cell multiplication, Spirulina cultivation in a lab has some limitations. However, the outcomes should support the development of spirulina for commercial use, and 25% PSPM should be regarded as a financially viable medium for extensive growing.

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