International Journal of Fisheries and Aquatic Research
www.fishjournals.com
ISSN: 2456-7248
Received: 05-07-2022, Accepted: 22-07-2022, Published: 08-08-2022
Volume 7, Issue 2, 2022, Page No. 21-29

Assessment of the physico-chemical properties and specific growth rate (SGR) of *Spirulina platensis* in lab scale cultivation

Md Saiful Islam[1], Md Ahsan Bin Habib[1], Md Hashibur Rahman[2*], Md Arifuzzaman[3], Sagorika Smriti[4], Al-Amin[2], Sharmin Sultana Mukti[2]

[1] Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh
[2] Bangladesh Fisheries Research Institute, Headquarters, Mymensingh, Bangladesh
[3] Bangladesh Fisheries Research Institute, Shrimp Research Station, Bagerhat, Bangladesh
[4] WorldFish Bangladesh and South Asia Office, Dhaka, Bangladesh

Abstract
An experiment was conducted to evaluate the Specific Growth Rate (SGR) and affiliated physico-chemical attributes of *Spirulina platensis* in supernatant of three different concentrations of digested rotten guava (DRG), and Kosaric Medium (KM) as control. Three different concentrations such as 20, 40 and 60 rotten guavas were allowed to digest under aerated condition. After 34 days, the reddish white colored supernatant was screened and taken in 2.0 L conical flask with three replications. Then, Spirulina was inoculated to grow in these three digested rotten guava media (DRGM) (treatments) with the addition of 9.0 g/L $NaHCO_3$, micronutrients and KM for a period of 14 days. The cell weight of Spirulina was attained a maximum of 12.43±0.20 mg/L (dry wt. basis) in KM followed by 0.818 ± 0.003, 0.815 ± 0.0015 and 0.809± 0.0012 mg/L in supernatant of 60, 20 and 40% DRGM, respectively. The percentage of crude protein (53.35 ± 0.32%) of Spirulina grown in supernatant of 40% DRGM significantly ($p < 0.05$) lower than that of cultured in KM (58.36 ± 0.32%). But crude lipids (10.15 ± 0.14%) of Spirulina cultured in supernatant of 60% DRGM was significantly ($P < 0.05$) and almost two times higher than that of Spirulina grown in KM (6.30 ± 0.22%). It means that for the production of high lipid content in Spirulina, supernatant of DRGM may be used. The physico-chemical parameters *viz*. light intensity (2748 to 2768 lux/m^2/s), temperature (19.0 to 22.2°C), pH (8.1 to 10.6), alkalinity (1522 to 2698 mg/L), nitrate-N (1.25 to 3.64 mg/L) and phosphate-P (11.30 to 55.40 mg/L) were within the optimum level during the culture period. So, mass culture of Spirulina may be practiced in supernatant of 60% DRGM.

Keywords: specific growth performance (SGR*), Spirulina platensis,* digested rotten guava media (DRGM), kosaric medium (KM)

Introduction

For hundreds of years, civilizations over decades cultivated and cherished spirulina highly recommended for its health-improving benefits. The native people in Africa have used the microalgae as a staple of their daily diet supplement on account of its concentrated nutritional aspects. It grows well in supernatant of various digested agro-industrial wastes available in Bangladesh and thus imparted for the commercial culture to inflict the nutritional requirements of the country (Satter, 2017) [18].

Now-a-days, Bangladesh has over 500 fish feed industries. To increase aquaculture production by applying adequate feed, large numbers of feed industries are developed within the country. Because of the increased aquaculture practice, demand for excellent quality feed is increasing daily. High-quality feed is essential for fish growth. Maintain feed conversion ratio (FCR) near 1 extremely dependent upon good feed. Feed should have adequate protein content which facilitates the higher growth of fish species. The utilization of net protein should be around 27 percent. Among fruits, huge quantity of spoils guava (rotten) is available in numerous markets within the country. Therefore, market is allowed to digest (aerobic & anaerobic) and supernatant is also used for the expansion of Spirulina. This inexpensive low-cost medium was also accustomed to producing Spirulina which may significantly contribute to the fisheries sector to obtain the sustainable production of fish. It takes inorganic nutrients for their growth and many of the factors are important for the assemblage of spirulina at a larger scale, of which nutrient availability and temperature are most significant. The filamentous cyanobacteria like spirulina are able to produce a great quantity of biomass as considered as most compatible microorganisms for the employment of waste and wastewaters. Also, these wastes reduce the price of nutrient medium and act as a source of low-cost nutrient medium for the cultivation.

However, the fish and bone meal aren't available in our country. So, we intended to provide alternative sources of fish meals to Spirulina. Spirulina could be a “superfood” which is highly nutritious and rich in protein. It is a vibrant history that occupied an intriguing biological niche and grow naturally within the wild in saltwater, freshwater alkaline lakes and natural springs. Spirulina is additionally cultivated in man-made reservoirs around

the world and harvested for the maintaining of supplementary diets in some context. The cell of Spirulina usually contains distinguished amount of protein (50-70%), 10-12% carbohydrate, 6% fat, 7% minerals and noted with a notable quantity of vitamins. According to the research findings, the nutritional value of 1 kg of Spirulina comparable to 1000 kg of other vegetables due to its remarkable existent of alimentation (Kato, 1991) [12]. The commercial production of Spirulina is often made cost effective by reducing the input cost with cheap and readily available materials. Now-a-days, Spirulina is acquiring a great interest due to its significant attributes of cellular contents like polyunsaturated fatty acids, carotenoids, vitamins, minerals, and other pigments that have an outstanding antioxidant activity (Cohen and Vonshak, 1991; Bhat and Madyastha, 2000; Madhava *et al*., 2000) [4, 1, 13].

Spirulina has been studied for single cell protein (SPC), vitamins, minerals, proteins and polyunsaturated fatty acids (γ-linolenic acid), therapeutic properties, antioxidant activity. The cost and composition of cultivation media are challenging for commercially viable production. Spirulina is created of between 55 and 70% protein (more than beef, chicken, and soybeans), contains all the essential non-essential amino acids, yet as high levels of iron; beta carotene; minerals and multivitamins, including vitamin B12; and phycocyanin, a pigment protein antioxidant complex found only in blue-green microalgae (Habib *et al.*, 2003 & Habib *et al.*, 2008) [8, 10].

The foremost convincing trials were conducted among populations which traditionally eat Spirulina for their supplementary diets. Its consumption is regular but reasonably low, 10-12 g/per/day (Cysewski, 1983) [5]. The culture and growth performance of Spirulina in supernatant of digested rotten guava was conducted to estimate the various physico-chemical parameters of culture media and to investigate the specific growth rate (SGR) of *Spirulina platensis* to identify the potential aspects of lab scale cultivation and the way forward with the prospects of commercial culture.

Materials and Method

Study Area

The study carried out in Live Food Aquaculture Laboratory, Department Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh-2202, and Bangladesh.

Collection of Rotten Guava

The rotten guava was selected as medium for *Spirulina platensis* culture. It was collected from Kamal Ronjit market (K.R.) of Bangladesh Agricultural University, Mymensingh-2202, and Bangladesh. It was thought that the proximate composition of this media might be suitable for the growth of culture species.

Analysis of proximate composition of rotten guava (RG)

Before media preparation, the proximate composition of rotten guava was analyzed to know its nutritional status. The analysis was performed in Fish Nutrition Laboratory, Department Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh-2202, and Bangladesh, following standard methods (Horwitz, 1984).

Crude protein

Kjeldhal Auto 1030 Analyzer was used for determination of crude protein content of samples. A sample of 0.5g and a blank were digested in the digestion tube. 10 ml of concentrated sulphuric acid (H_2SO_4), 2.0 ml of H_2O_2 and one Kjeldhal tablet were added in the tubes and mixed gently by electric mixer. Then, the digestion tubes were set in digestion chamber fixing at 420°C for 45 minutes. The digestion the tubes were allowed to cool and 75 ml of distilled water was added in each tube. 50 ml of 40% NaOH was added before titration. After titration with 1% boric acid and 0.2 N HCl the reading for the samples and blank were recorded. The readings were calculated the following formula:

$$\text{Percentage of nitrogen} = \frac{\text{Milliequivalent wt.of N} \times \text{ml of titrant} \times \text{strength of HCl}}{\text{Samples wt. (g)}} \times 100$$

For animal, % Protein = % Nitrogen x 6.25; and
For plant, % Protein = % Nitrogen x 5.85

Crude lipid

Lipid content was estimated by solvent extraction of lipid using Soxlet apparatus. 2.0 g dried samples were taken into extraction thimbles to place into the extraction unit along with the weighed extraction cups having 50 ml of solvent as acetone. Extraction of first 15 minutes was in the boiling position and the cups were released and dried in the oven for 30 minutes. The percentage of crude lipid was determined using the following equation:

$$\% \text{ Lipid} = \frac{\text{Weight of cup with lipid} - \text{initial weight of cup}}{\text{weight of sample (g)}} \times 100$$

Ash

The pre-weighed crucible containing dried sample from the moisture determination was pre-ashed. The samples were kept into a muffle furnace at 550°C for 6.0 hrs. The crucible containing ash was cooled in a desiccator. The percentage of ash was determined by using the following:

$$\% \text{ Ash} = \frac{\text{Weight of ash with preweighed crucible} - \text{weight of crucible}}{\text{weight of dried sample}} \times 100$$

NFE

NFE were calculated the following formula:
Nitrogen Free Extract = 100 - (Moisture + Crude protein + Crude lipids + Ash).

Analysis of physico-chemical properties of rotten guava (RG)

Physico-chemical properties of digested rotten guava were analyzed using different chemicals and equipment's. These properties such as pH, total suspended solids, total dissolved solids, dissolved oxygen, total alkalinity, nitrate-N (NO_3-N) and phosphate-P (PO_4-P) of digested rotten guava were analyzed in the laboratories of Live Food Culture, Nutrition and Water Quality of the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh-2202, and Bangladesh.
All of these properties were analyzed using the procedures which are as follows:

pH

pH of digested samples of liquid rice starch was determined using pH meter (Model HI 98129, HANNA).

Total suspended solids (TSS) and total dissolved solids (TDS)

50 mg digested rotten guava (RG) was filtered through pre-weighed filter paper of mesh size 0.45 µm. The filtered water was taken in pre-weighed crucible and then filter paper and crucible with water were put in oven at 105°C overnight. Then the dried filter paper and crucible were taken out from the oven after switched off, put in the desiccator and kept for at least 15 minutes for cooling. The TSS was calculated using the following equation:

1. TSS (mg/l) = (Final weight of filter paper - initial weight of filter paper)/Volume of DRG; and
2. TDS (mg/l) = (Final weight of crucible - initial weight of crucible)/Volume of DRG

Alkalinity

10 ml of DRG (due to high concentration) was taken in the 20 ml plastic bottle and then mixed with 1 drop Bromophenol blue. The colour of the solution turned into blue and then titrated with acid solution (HI 3811-0 reagent) until the colour became yellow (end point). The total amount of titrant was recorded and total alkalinity was recorded by following formula:
Alkalinity (mg/l) = Total amount of titrant (ml) x 300

Nitrate-N (Available N)

10 ml of filtered (Sartorius filter paper, 0.45 µm) digested rotten guava was taken in the cuvette and mixed with Nitrate HR reagent. It was then agitated to mix thoroughly for 1.0 minute and put in the machine (LR Phosphate, Model HI 93713, HANNA). The machine was on and data was read after 4.0 minutes at 660 nm.

Phosphate-P (Available P)

10 ml of filtered (Sartorius filter paper, 0.45 µm) DRG was taken in the cuvette and mixed with Phosphate HR reagent. It was then agitated for at least 30 seconds to mix thoroughly and put in the machine (LR Nitrate, Model HI 93713, HANNA). The machine was on and data was read after 2.0 minutes at 880 nm.

Culture and collection of Spirulina platensis

Spirulina platensis was collected from the stock in the live food culture laboratory, Department of aquaculture, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh-2202, and Bangladesh. Twelve conical flasks (2 L capacity) were used for the culture of spirulina.

Maintenance of pure stock culture of Spirulina platensis

Pure stock culture of *Spirulina platensis* was maintained in the laboratory in Kosaric medium (KM) (Modified after Zarrouk's, 1996). Growth of *Spirulina platensis* were observed at every alternative day and was checked under microscope to confirm its purity following some keys given by Bold and Wynne (1978), Vymazal (1995) and Phang and Chu (1999) [21, 15].

Preparation of digested rotten guava media (DRGM) and Kosaric medium (KM)

50 g/L rotten guava was allowed to decompose in 5.0 L glass bottle for 34 days under aerobic condition and then a light reddish white colored supernatant from bottle was diluted and made three concentrations at the rate of 20%, 40% and 60% digested rotten guava. Then the supernatant of three different concentrations were taken in 1.0 L flask with three replications.

Then 5 litter volumetric flask was filtered with plankton and the filtered rotten guava was diluted and added 0.8 g (0.2 g/L) urea according to the above direction with three replications using distilled water. Then the medium was mixed well and sterilized at 115°C for 15 minutes by high pressure bumping water autoclave. After autoclaving, the media were kept 3 days to be sure about any contamination free before culture of micro algae.
For the preparation of Kosaric medium, the ingredients were weighed and took in a 1.0 L conical flask. Then 0.5 ml micronutrient solution was pipetted in the flask and distilled water was added to make the volume 1.0 L. Mixing, autoclaving and cooling were carried out pursuing the procedure used during the preparation of digested rotten guava media.

Experimental design of Spirulina platensis culture
Three types media *viz*., Rotten guava (RG) and Kosaric medium (KM) were used to culture *Spirulina platensis.* Inoculum *Spirulina platensis* was collected from the pure stock culture. Experimental design is shown in (Table 4).

Estimation of cell weight (dry weight) of spirulina (Clesceri *et al*., 1989)
Sample containing 15 ml spirulina suspension was filtered through a Sartorius filter paper of mesh size 0.45 µm and diameter 47 mm. The filter papers were dried in an oven for 24 hrs. overnight at 70°C and weighed prior to filtration. The filtered samples were washed three times to remove insoluble salts. After that the filter papers were put in a glass petri dish and kept in the oven at 70°C overnight. For cooling, petri dish was put into desiccator for 20 minutes and then filter papers were weighed. The dry weight of algae on the filter paper was measured using the following equation:

Dry weight (mg/L),

$$W = \frac{FFW - IFW}{\text{Amount of sample taken for filtration (ml)}} \times 100$$

Where,
W = Cell dry weight in mg/L;
FFW = Final filter paper weight in g; and
IFW = Initial filter paper weight in g.

Total biomass of spirulina (S. platensis)
Total biomass was calculated using the following formula given by Vonshak and Richmond (1988):
Total biomass = Chlorophyll a x 67

Specific growth rate (µ/day) of cultured Spirulina on the basis of dry weight

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

Where,
X_1 = Dry weight of biomass concentration of the end of selected time interval;
X_2 = Dry weight biomass concentration at beginning of selected time interval;
And t_1-t_2 =Elapsed time between selected time in the day.

Specific growth rate (µ/day) of cultured Spirulina on the basis of chlorophyll a

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

Where, X_1 = Chlorophyll a at the end of selected time interval;
X_2 = Chlorophyll a at the beginning of selected time interval;
And t_1-t_2 = Elapsed time between selected time in the day.

Specific growth rate (µ/day) of cultured Spirulina on the basis of total biomass

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

Where, X_1 = Total biomass at the end of selected time interval;
X_2 = Total biomass at the beginning of selected time interval; and
t_1-t_2 = Elapsed time between selected time in the day.

Statistical Analysis

Analysis of variance (ANOVA) of mean cell weight and chlorophyll a of *S. platensis* cultured in different media (treatments) were done and to find whether any significant among treatment mean was done by Ducan's Multiple Range Test (DMRT) at 5% level of probability (Zar, 1984) [22].

Results

Physico-chemical properties of different media

Nitrate-N (NO_3-N)

Nitrate N (Available N) of three supernatant of digested rotten guava and Kosaric medium was recorded. It was decreased from 2.86 ± 0.12 mg/L (first day) to 1.25 ± 0.15 mg/L (10th day) of experiment and then increased up to 14th day of experiment when spirulina cultured in supernatant of 20% digested rotten guava (DRG) (Fig.1). The trend of nitrate-N was found to decrease from first day (2.86 ± 0.12 mg/L) to 10th day (1.25 ± 0.15 mg/L) of culture and then increased up to 14th day of experiment when spirulina grown in supernatant of 40% DRG (Fig.1). It was found that nitrate-N (1.40± 0.15 mg/L) was recorded on first day of experiment which was decreased up to 10th day (1.20 ± 0.15 mg/L) in media contained spirulina and then decreased up to 14th day of culture in supernatant of 60%.

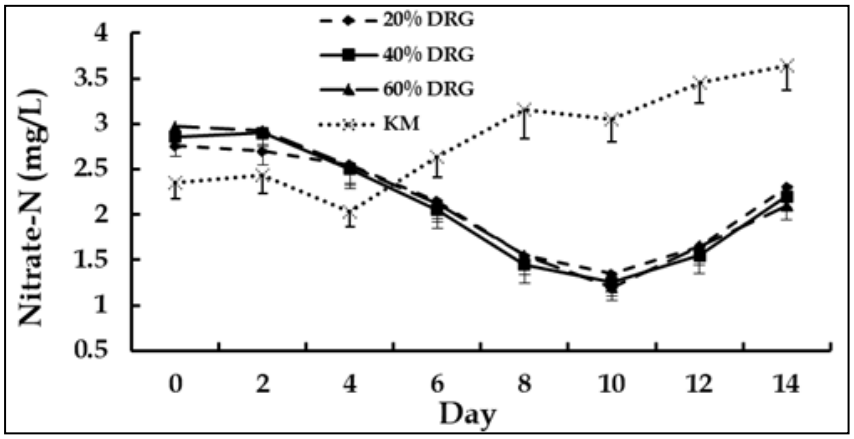

Fig 1: Mean values of nitrate-N (mg/L). Vertical bars represent standard errors

DRG. There was no definite trend of fluctuation of nitrate-N when spirulina was culture in Kosaric medium where it was found lowest (1.30 ± 0.16 g/L) on 4th day of culture and highest on last day (3.64 ± 0.27 mg/L) of culture (Fig.1).

Phosphate-P (PO_4-P)

Phosphate-P (Available P) was high in amount in the media in first day (32.12 ± 2.78 g/L) of experiment and gradually decreased in amount up to 10th day (11.30 ± 1.22 g/L) of culture of 20% digested rotten guava (DRG) contained Spirulina, and again increased from 12th day of culture. Similar trend was followed in the cases of culture in supernatant of 40 and 60% DRG, an Kosaric medium (Fig.2). It was found to decrease from first day (42.32 ± 2.40 g/L) of experiment up to 10th day (16.60 ± 1.16 g/L) but increased from 12th to14th day of experiment in the culture of 40% DRG contained spirulina. Similarly, it was decreased from first day (55.40 ± 5.50 g/L) of experiment up to 10th day (20.35 ± 1.50 g/L) of culture and then increased up to 14th day of experiment in the culture of 60% DRG (Fig.2). It was found to record highest nitrate-N in Kosaric medium on first day (32.51 ± 2.46 g/L) of culture which was decreased up to 10th day (11.52 ± 0.80 g/L) and then increased up to 14 th day of experiment (Fig.2).

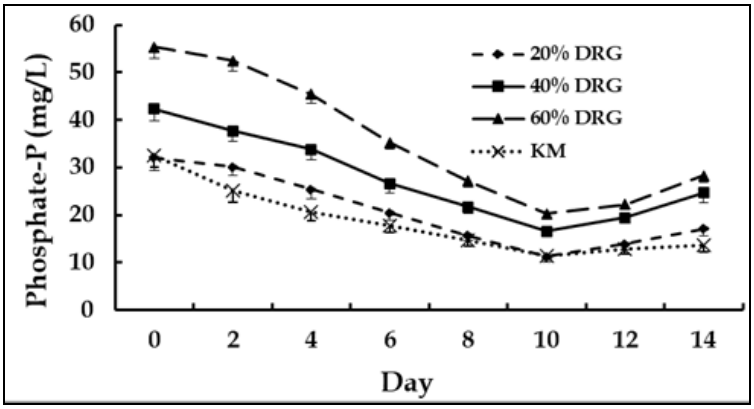

Fig 2: Mean values of phosphate-P (mg/L). Vertical bars represent standard errors

Optical density

Optical density (OD) of media contained spirulina was found to increased up to 10th day of culture in all the media of digested rotten guava (DRG), and Kosaric medium and then decreased up to 14th day of experiment (Fig.3). However, highest OD of 20% DRG culture contained spirulina was 0.631 ± 0.0023, where highest OD of 40% DRG culture contained spirulina was found 0.704 ± 0.0015. The OD of supernatant of 60% DRG contained spirulina was 0.725 ± 0.0012. The highest optical density of Kosaric medium contained spirulina was 2.63 ± 0.20 (Fig.3).

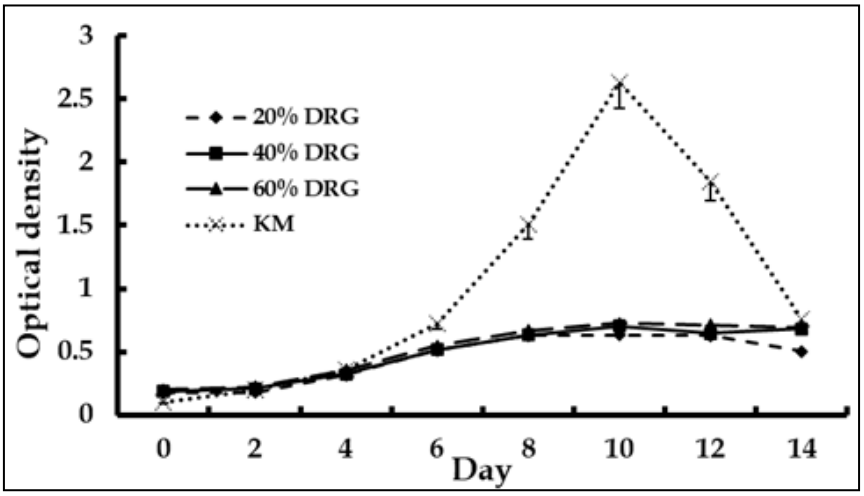

Fig 3: Mean values of optical density (Vertical bars represent standard errors)

Total biomass of Spirulina

Total biomass (mg/L) of spirulina cultured in Kosaric medium (705.51 ± 2.53) was significantly ($P < 0.05$) higher than that of *Spirulina* grown in supernatant of 20% (67.77 ± 0.43), 60% (57.75 ± 0.20) and 40% (51.46 ± 0.28) (Table 1). There was no significant difference found among the total biomass of spirulina cultured in supernatant of 29, 40 and 60% DRG.

Table 1: Comparison of cell weight, chlorophyll a and total biomass of *Spirulina platensis*

Parameters	T1 (20% DRG)	T2 (40% DRG)	T3 (60% DRG)	T4 (KM)
Optical density	0.631 ± 0.002^{b}	0.704 ± 0.0015^{b}	0.725 ± 0.0012^{b}	2.63 ± 0.20^{a}
Cell weight (mg/L)	0.815 ± 0.0015^{b}	0.809 ± 0.0012^{b}	0.818 ± 0.0013^{b}	12.43 ± 0.20^{a}
Chlorophyll a (mg/L)	0.770 ± 0.14^{b}	0.768 ± 0.0012^{b}	0.862 ± 0.0012^{b}	10.53 ± 0.32^{a}
Total biomass (mg/L) *	67.77 ± 0.43^{b}	51.46 ± 0.28^{c}	57.75 ± 0.20^{bc}	705.51 ± 2.53^{a}

*Total biomass = Chlorophyll a x 67 (Vonshak and Richmond, 1988). Figures in common letters do not differ significantly at 5% level of probability.

Correlation among the growth parameters of spirulina

Cell weight of spirulina (*Spirulina platensis*) had highly significant ($P < 0.01$) direct correlation with chlorophyll a ($r = 0.746$) of spirulina grown in the supernatant of different digested rotten guava, and Kosaric medium during the study (Fig. 4). Similarly, total biomass of *S. platensis* was highly ($P < 0.01$) and directly correlated with chlorophyll a ($r = 0.795$) of spirulina cultured in the supernatant of various digested rotten guava, and Kosaric medium (Fig. 5). Again, total biomass of spirulina was found to be highly ($P < 0.01$) and directly correlated with the cell weight ($r = 0.742$) of spirulina grown in the supernatant of different digested rotten guava, and Kosaric medium (Fig. 6).

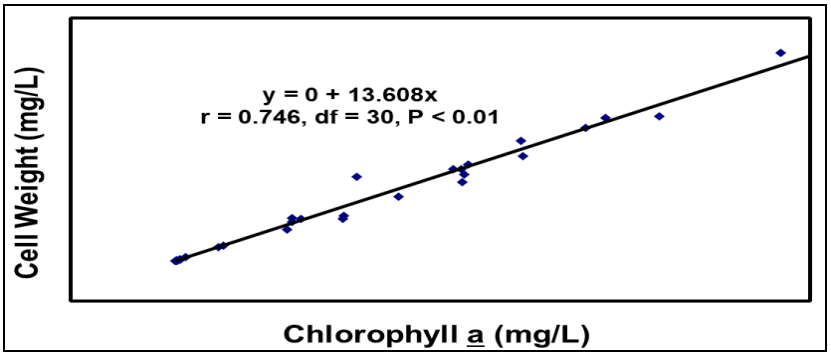

Fig 4: Correlation coefficient (r) of cell weight (mg/L) of *Spirulina platensis* with chlorophyll a (mg/L)

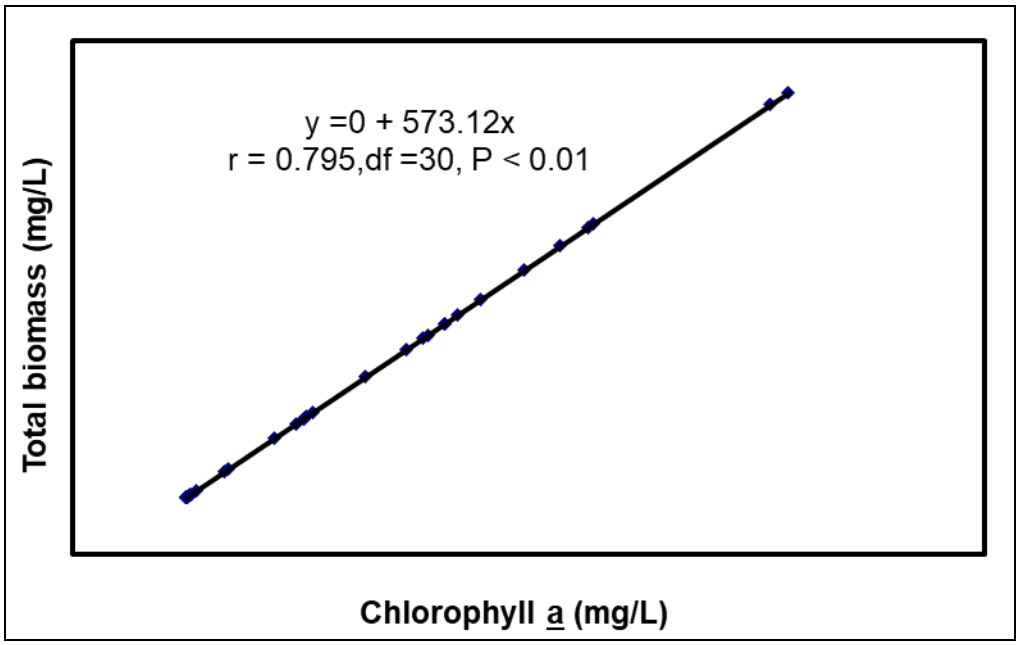

Fig 5: Correlation coefficient (r) of total biomass (mg/L) of *Spirulina platensis* with chlorophyll a (mg/L

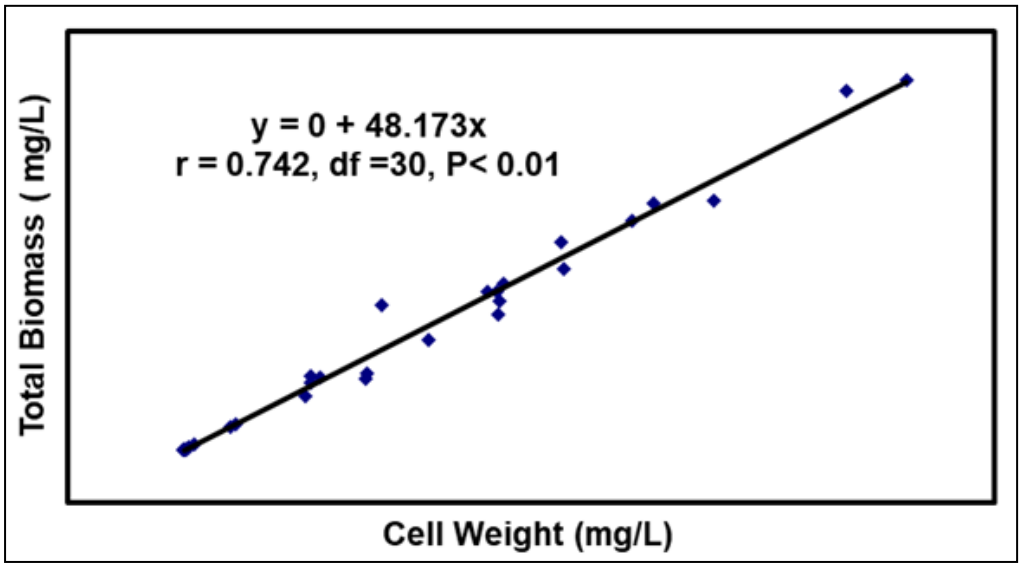

Fig 6: Correlation coefficient (r) of total biomass (mg/L) of *Spirulina platensis* with cell weight (mg/L)

Specific growth rates (SGR) of spirulina (Spirulina platensis)

Specific growth rate (SGR) in respect to cell weight of spirulina grown in Kosaric medium was significantly ($P < 0.05$) higher than that of spirulina cultured in the supernatant of 20, 40 and 60% digested rotten guava (DRG) (Table 2). There was no significant ($P > 0.05$) difference among the SGRs of cell weight of *Spirulina* grown in supernatant of 20, 40 and 60 DRG. The SGR in respect to Chlorophyll a of spirulina cultured in Kosaric medium was significantly ($P < 0.05$) varied from that of spirulina grown in the supernatant of 20, 40 and 60% DRG (Table 2). There was no significant ($P > 0.05$) difference when spirulina grown in supernatant of 20, 40 and 60% DRG. The SGR in respect to total biomass of spirulina cultured in Kosaric medium was significantly ($P < 0.05$) varied from that of spirulina grown in the supernatant of 20, 40 and 60% DRG (Table 2). There was no significant ($P < 0.05$) difference recorded among the SGRs on the basis of total biomass of *S. platensis* grown in the supernatant of 20, 40 and 60% DRG.

Table 2: Specific growth rates (SGRs) on the basis of cell weight, chlorophyll a and total biomass of *Spirulina platensis*

Parameters	T1 (20% DRG)	T2 (40% DRG)	T3 (60% DRG)	T4 (KM)
SGR of cell weight	0.21 ± 0.010^{b}	0.22 ± 0.009^{b}	0.21 ± 0.011^{b}	0.31 ± 0.014^{a}
SGR of Chlorophyll a	0.22 ± 0.004^{b}	0.21 ± 0.003^{b}	0.23 ± 0.004^{b}	0.29 ± 0.011^{a}
SGR of total biomass	0.54 ± 0.012^{b}	0.52 ± 0.012^{b}	0.53 ± 0.013^{b}	0.81 ± 0.021^{a}

Figures in common letters in the same row do not differ significantly at 5% level of probability.

Discussion

The growth performance of *Spirulina platensis* in supernatant of 60% DRGM was found better than 20% and 40% DRGM. Habib and Kohinoor (2018) [7] found that supernatant of 45% digested poultry waste gave very good growth of spirulina than other concentrations which the significantly with present findings.

Satter (2017) [18] recorded the cell weight and chlorophyll a content of *S. platensis* was significant ($P<0.05$) higher in 4.0 g/L digested poultry waste than other media where light intensity, aeration and temperature played significant role to the culture system. Similarly, Sharker (2002) [17] conducted an experiment on the culture of *Spirulina platensis* 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 carried out for a period of 12 days. The growth rate of *Spirulina platensis* was found to vary in different media. The result indicated that the growth rate of *Spirulina platensis* was significantly ($P<0.01$) higher in 0.3 g/L concentration of PSPM than other concentrations PSPM.

The physicochemical properties *viz*. temperature (30.06 ºC), light intensity 2110 (lux/m^2/s), dissolved oxygen (4.84 mg/L), pH (12.08), nitrate-nitrogen (3.29 mg/L), phosphate-phosphorus (1.97 mg/L) and nitrate-N (0.6 mg/L) were observed. During this study lower dilution content higher nutrient which was the same result in the present findings.

Sukumaran *et al.*, (2018) [19] recorded good growth of spirulina (*Arthrospira platensis)* in different nutrient media. Where Manigandan (2014) found better growth of *Spirulina platensis* in synthetic medium followed by fertilizer medium and then sea water. In the present study, the chlorophyll a content of inoculated *Spirulina platensis* was 0.0015 mg/L which attained a high content of 10.53 mg/L which cultured in KM and 0.862 mg/L in 60% DRGM at the 10th day of culture. These findings are not more or less similar with the findings of Phang *et al.,* (2000) [16], Habib *et al.* (2003) [10] and Satter (2017) [18]. This might be due to lower nitrogen and phosphate concentration of the nutrients in the media. Dineshkumar *et al.,* (2016) [6] studied that *Spirulina platensis* grew well in natural medium such as Conway medium, zoarrouic medium (kosaric medium) and BGII medium.

The supernatant of 60% digested rotten guava showed maximum optical density on the 10th day of culture comparing with KM which has the similarity 6th with the findings of Habib *et al.* (1997, 2003) [9, 10], Satter (2017) [18]. The availability of phosphate-phosphorus has been considered very important in cultured media of plankton production. The phosphate-phosphorus was found higher on initial culture and minimum on the 10th day in respectively of 20%, 40% & 60% DRGM. Meanwhile, the optical density was minimum in initial 10th day and maximum on the 10th day in respectively of 20, 40 & 60% DRGM.

The Physical and chemical characteristics of the culture media *viz*., light intensity, temperature, pH, dissolved oxygen, cell weight and chlorophyll a were determined at every alternative day. During the culture period light intensity and temperature were more or less similar. The maximum pH was 10.7 recorded in supernatant of 40% digested rotten guava contained *Spirulina platensis* on the 10th day of culture and minimum pH was 8.1 recorded in supernatant of 20% digested rotten guava contained *Spirulina platensis* on the initial day of the culture.

The experiment shown the growth performance of *Spirulina platensis* was varied from different concentration of the media and KM. The initial cell weight was 0.0023mg/L which attained a maximum cell weight 12.43 mg/L in Kosaric medium and 0.818 mg /L in 60% DRGM, 0.809mg/L in 40% DRGM and 0.815mg/L in 0% DRGM on the 10th day of the culture period. Similarly, the chlorophyll a content of inoculated *S. platensis* was 0.0015mg/L which attained the highest content of 10.53mg/L in KM, and 0.862 in 60% DRGM, 0.768mg/L in 40% DRGM, 0.770 mg/L in 20% DRGM on the 10th day of culture period. A decreasing trend of cell weight was observed from 12th day of culture.

Conclusion

The percentage of crude protein (53.35 ± 0.32%) of Spirulina grown in supernatant of 40% DRGM significantly ($p < 0.05$) lower than that of spirulina cultured in KM (58.36 ± 0.32$^{\%}$). But crude lipids (10.15 ± 0.14%) of spirulina cultured in supernatant of 60% DRGM was significantly ($P < 0.05$) and almost two times higher than that of spirulina grown in KM (6.30 ± 0.22%). Specific growth rate (SGR) in respect to cell weight of spirulina grown in Kosaric medium was significantly ($P <0.05$) higher than that of spirulina cultured in the supernatant of 20, 40 and 60% digested rotten guava (DRG). The SGR in respect to Chlorophyll a of spirulina cultured in Kosaric medium was significantly ($P < 0.05$) varied from that of spirulina grown in the supernatant of 20, 40 and 60% DRG. This medium may be used commercially and economically after screening for mass culture of *Spirulina platensis*, as the collection and preparation of these organic media require little cost, less labour and is available throughout Bangladesh. However, it might be suggested that more research and cost-benefit analysis have to be performed to evaluate the grow-out potential of spirulina in lab-based cultivation.

References

1. Bhat VB, Madyastha KM. Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: protection against oxidative damage to DNA. Biochemical and Biophysical Research Communications,2000:13:285(2):262-266.
2. Bold HC, Wynne MJ. Introduction to the Algae. Structure and Reproduction. Englewood Cliffs. New Jersey., 1978, 706.
3. Clesceri LS, Greenberg AE, Trussell RR. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works. Association and Water Pollution Control Federation. 17th Edn., 1015 Washington D.C., USA., 1989, 10-203.

4. Cohen Z, Vonshak A. Fatty acid composition of *Spirulina* and *Spirulina*-like cyanobacteria in relation to their chemotaxonomy. Phytochemistry,1991:30**:**205.
5. Cysewski PhD. Hawaiian Spirulina: Superfood for Super Health 73-4460 Queen Kaahumanu Highway, Suite 102, Kailua-Kona, HI 96740 USA or email info@cyanotech.com., 1983.
6. Dineshkumar R, Narendran R, Sampathkumar P. Cultivation of *Spirulina platensis* in different selective media. Indian Journal of Marine Science,2016:45:(12):1749-1754.
7. Habib MAB, Kohinoor AHNM. Culture and production of house fry larvae and spirulina using poultry waste and their use as food for catfish post-larvae. Report on Advanced Research, Ministry of Education, Govt. of People Republic of Bangladesh., 2018, 66-70.
8. Habib MAB, Parvin M, Huntington TC, Hasan MR. Global Review on Culture, Production and Use of Spirulina as Food for Humans and Feed for Domestic Animals and Fish. In: TC Huntington (Editor), Report No. GF FIRID. RA2IP02000600. Food and Agriculture Organization (FAO) of United Nations, Rome, Italy., 2008, 33.
9. Habib MAB, Yusoff FM, Phang SM, Mohamed S. Nutritional values of chironomid larvae grown in palm oil mill effluent and algal culture. Aquaculture,1997:158:195-205.
10. Habib MAB, Yusoff FM, Phang SM, Mohamed S. Growth and nutritional values of *Moina micrura* fed on *Chlorella vulgaris* grown in digested palm oil mill effluent. Asian Fisheries Science.,2003:16(1-2):107-119.
11. Horwitz W (Editor). Official Methods of Analysis of the Association of Official Analytical Chemists. 14th Edition. Association of Official Analytical Chemists, Washington DC. USA., 1984, 1018.
12. Kato T. Chemistry of microalgae and their application to food. Food Chemistry,1991:8:30-35.
13. Madhava KM, Bhat VB. Scavenging of peroxy-nitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: protection against oxidative damage to DNA. Biochemical and Biophysical Research Communications,2000:13:285(2):262-266.
14. Manigandan M. Mass cultivation and determination of biochemical composition of *Spirulina platensis* in three different media. International journal of Pharmacology and
15. Phang SM, Chu WL. University of Malaya Algae Culture Collection (UMACC). Catalogue of Strain. Institute of Postgraduate Studies and Research, University of Malaya, Kualalumpur, Malaysia., 1999, 77.
16. Phang SM, Miah MS, Chu WL, Hashim H. Spirulina culture in digested sago starch factory waste water. Journal of Applied Phycology,2000:12**:**395-400.
17. Sharker MGU. Study of the culture of *Spirulina platensis* in various concentrations using papaya skin powder medium. MS. thesis submitted to the Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh-2202., 2002, 58.
18. Satter A. Culture and production of housefly larva and Spirulina using poultry waste, and their use as food for catfish post-larvae, PhD Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh., 2017, 143.
19. Sukumaran P, Nulib R, Halimmon N, Simoh S, Omar H, Ismail A. Formulation of cost-effective medium using urea as a nitrogen source for *Arthrospira platensis* cultivation under real environment. Annual Research and Review in Biology.,2018:22(2):1-12.
20. Vonshak A, Richmond A. Mass production of the blue green alga *Spirulina*: an overview. Biomass,1988:15:233-247.
21. Vymazal J. Algae and Element Cycling in Wetlands. CRC Press, Inc., Boca Raton, Florida, USA., 1995, 689.
22. Zar JH. Biostatistics. Prentice-Hall Inc., Englewood Cliffs, New Jersey, USA. 1984, 718.
23. Zarrouk C. Contribution a l'etude d'une cyanobacterie: influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima* (Setchell et Gardner) Geitler. PhD thesis, University of Paris, France., 1996, 412.