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Cell Weight, Chlorophyll a Content and Total Biomass Production of *Spirulina platensis*: Impact of Culture Media and Affiliated Correlation

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ABSTRACT

This experiment was inquired to apprehend the associated relationship among the cell weight, chlorophyll a and total biomass production of *Spirulina platensis* grown in different concentration of digested rotten guava medium (DRGM) and kosaric medium (KM). This study was carried out to disclose the culture potentiality of different substrates and their impact on production through the assessment of their consequential correlation. The completely randomized design (CRD) was taken into consideration to accomplish the experiment in laboratory condition. The maximum cell weight was assumed in KM (12.41 ± 0.23) followed by 0.816 ± 0.0017, 0.813 ± 0.0013 and 0.807± 0.0011 mg/L in supernatant of 60%, 20% and 40% DRGM, respectively on the 10th day of culture. The correlation ($P< 0.01$) with the chlorophyll a content ($r = 0.745$) and total biomass ($r = 0.741$) revealed significant association and strong correlation with the obtained cell weight in these culture media. In kosaric medium, the growth performance was significantly higher ($P<0.01$) than that of grown in the different concentration of DRGM (60%, 40% and 20%). The content of chlorophyll a grown in KM (10.54 ± 0.33 mg/L) was significantly higher ($P< 0.01$) in comparison of 60% (0.863 ± 0.0018), 40% (0.769 ± 0.0015) and 20% DRGM (0.771 ± 0.13). The highest biomass of *S. platensis* was acquired in KM (705.52 ± 2.59 mg/L) and varied significantly ($P < 0.05$) than that of cultured in supernatants of 20% (67.76 ± 0.42), 40% (51.45 ± 0.26) and 60% (57.74 ± 0.26) DRGM. The culture techniques in DRG medium may dwindle the cost of production in comparison with KM and might be considered as a media for *Spirulina platensis* cultivation.

INTRODUCTION

Due to increased aquaculture practice, the demand for good quality feed is increasing throughout the world. The application of adequate feed, large numbers of feed industries are developed in the country and the supply of quality feed is essential for fish growth. Maintaining feed conversion ratio (FCR) close to 1 is highly dependent upon the quality of the supplied feed ingredients. Feed should exist adequate protein content which facilitates high growth and crucial for the development of the immune response. Net protein utilization should be approximately 27-28%. But unfortunately, fish meal and bone meal are not available in Bangladesh hence alternative sources are needed to replenish the omission. *Spirulina* can be considered as an alternative to fish meal due to its high nutritional value. It is a blue-green algae (BGA) that grows in both freshwater and saltwater. It is rich in protein and concentrated whole food known to humankind for their diet.

It occupies an intriguing biological and ecological niche and consists of vibrant history in the plant kingdom. It is a blue-green microalga which is spiral-shaped and grows naturally in an aquatic environment. It gives the water greenish hue and shows deep blue-green color. This blue green alga is also cultivated around the world and harvested in man-made reservoirs. For centuries, the civilizations are highly cherished for *Spirulina* due to its health-improving benefits and high nutritional profile. It usually grows well in supernatants of different digested agro-industrial wastes which is easily available in Bangladesh (Satter, 2017).

By reducing the input cost with cheap and readily available materials, the commercial production of *Spirulina* can be made cost effective without sacrificing the production efficiency. They are microscopic, very small and usually measured 300-500 micrometer in length. It contains 55-75% protein, 12-14% carbohydrate, 5% fat, 6% minerals and an edible source of vitamins. According to research findings, 1000 Kg of other vegetables is similar to 1 Kg of *Spirulina* spp. In terms of nutritional aspects (Kato, 1991). For the production of *Spirulina* at large scale, the most important and considerable factors are nutrient availability, light and temperature (Becker, 1984).

Some other filamentous cyanobacteria and *Spirulina* are found to be most compatible microorganisms for the utilization of waste and wastewater to produce large quantities of biomass and reduces the cost of nutrient medium and act as a source of cheap nutrient medium for the cultivation. Several cultivation methods like open ponds, tubular photobioreactors, inclined glass panels

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have been tried. Cost and composition of cultivation media along with growth rate of the algae were challenging factors for commercially viable production. The most convincing trials are of course those conducted among populations which traditionally eat this BGA. The study was performed to compare the cell weight, chlorophyll a and total biomass of *Spirulina platensis* grown different substrates (DRGM and KM) to find out the suitable concentration of the medium for maximum production.

MATERIALS AND METHODS

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 *S. 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.

Experimental Design of *Spirulina Platensis* Culture

Three types media viz., Rotten guava (RG) were used to culture *Spirulina platensis*. Inoculum of *Spirulina* was collected from the pure stock culture. Experimental design is shown in (Table 1 and Table-2).

Table 1: Experimental design for *Spirulina platensis* culture

Types of medium	Treatments	Replications	Amounts rotten guava (%)	Duration of culture (days)
Supernatant of DRGM	1	3	20	14
	2		40	
	3		60	
Kosaric Medium (KM)	4		-	

Table 2: Collection of Sample in 15 ml of plastic vial with every alternate day

Day	Date	KM (ml)	Treatment-1 (Replication, vial ml) (20%)	Treatment-2 (Replication, vial ml (40%)	Treatment-3 (Replication, vial ml (60%)
		Cell weight (mg)	Cell weight (mg)	Cell weight (mg)	Cell weight (mg)
2	24/11/2018	0.797	0.794	0.741	0.753
4	26/11/2018	0.785	0.751	0.753	0.051
6	28/11/2018	0.833	0.791	0.762	0.802
8	30/11/2018	0.844	0.812	0.805	0.796
10	02/12/2018	0.823	0.814	0.807	0.818
12	04/12/2018	0.823	0.811	0.803	0.813
14	06/12/2018	0.810	0.804	0.807	0.803

Estimation of cell weight (dry weight) of *Spirulina platensis* (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:

$$\text{Dry weight (mg/L)}, \; W = \frac{FFW - IFW}{\text{Amount of sample taken for filtration (ml)}} \times 100$$

Where,

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

Estimation of chlorophyll a of *S. platensis* (Clesceri *et al.*, 1989)

The samples of *S. platensis* were collected in different times and chlorophyll a content of *S. platensis* was estimated. Ten ml of *S. platensis* sample were filtered with an electric filtration unit using filter papers (Sartorius filter paper of 0.45 µm mesh size and 47 mm). These filtered samples together with filter paper was taken into test tubes, ground with glass rod and finally mixed with 10 ml of 100% redistilled acetone. To inhibit the contact of light each of the test tubes was wrapped with foil papers. The wrapped test tubes were kept into a refrigerator (LMS Laboratory Refrigerator) over night. Then the refrigerated samples were homogenized for 2 minutes

followed by centrifugation at 4000 rpm for 10 minutes. After centrifugation, the supernatant was isolated and taken for chlorophyll a determination. Optical densities of the samples were determined at 664 nm, 647 nm 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)

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

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

Preparation of media (KM and DRGM)
Compositions of Rotten Guava Medium (RGM) were prepared for culture of *S. platensis*. 50 g/L rotten guava was allowed to decompose in 5.0 L glass bottle for 34 days under aerobic condition in the Live Food Culture laboratory, Department of Aquaculture, BAU, Mymensingh. 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 with three replications were taken in 1.0 L flask. For the preparation of rotten guava medium, digested and continuous aeration 5 litter volumetric flask was filtered with plankton net after (10.10.18 to 14.11.2018) 34 days left. Then 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 115oC 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. Mixing, autoclaving and cooling were carried out pursuing the procedure used during the preparation of digested rotten guava media.

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 1% level of probability (Zar, 1984).

RESULTS

Chlorophyll a of *Spirulina*
Chlorophyll a of *Spirulina* was found higher on 10th day of culture than other days of culture in supernatant of all the media (Figure1). Chlorophyll a of *Spirulina* increased from first day (0.00158 ± 0 mg/L) up to 10th day (0.771 ± 0.0013 mg/L) of culture in 20% digested rotten guava media (DRGM) and then decreased up to 14th day (0.710 ± 0.0012 mg/L) of experiment. However, chlorophyll a of *Spirulina* cultured in supernatant of 40% DRG was 0.769 ± 0.0015 mg/L on 10th day and then decreased up to 14th day (last day) of culture. Chlorophyll a of *Spirulina* grown in supernatant of 60% DRG was 0.863 ± 0.18 mg/L on 10th day from first day (0.0016 ± 0) and then decreased up to 14th day (last day) of experiment, where the highest chlorophyll a of *Spirulina* cultured in Kosaric medium was 10.54 ± 0.33 mg/L on 10th day from first day (0.0015 ± 0) and decreased up to 14th day (last day) (4.17 ± 0.11) of experiment (Figure 1).

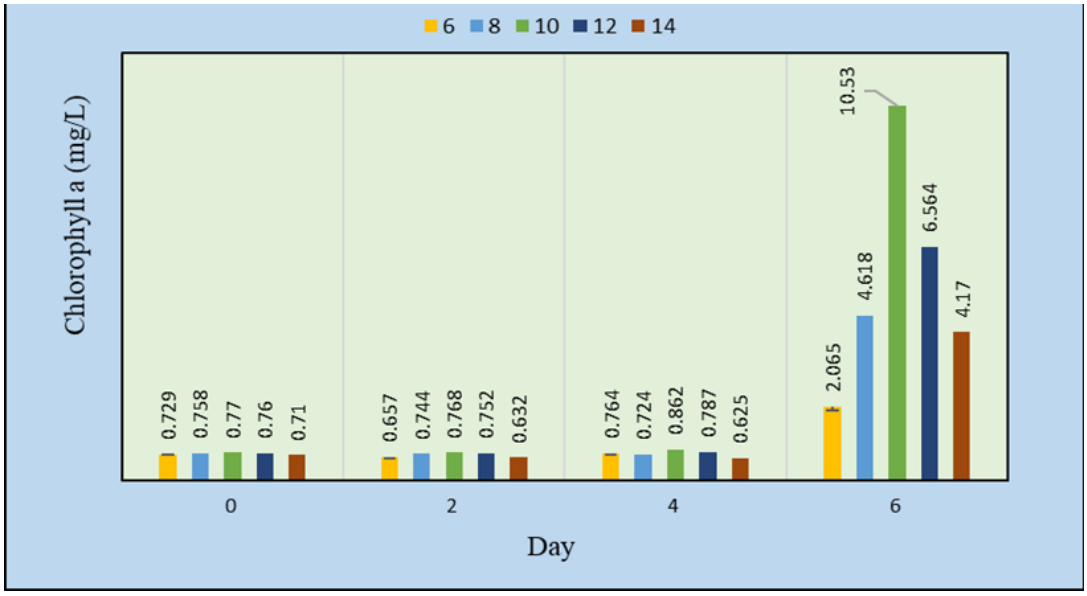

Figure 1: Mean values of chlorophyll a (mg/L) of *S. platensis*

Total biomass of *S. platensis*

Total biomass (mg/L) of *S. platensis* grown in all the media was found to be higher on 10th day of culture than other days of experiment (Figure 2). Total biomass was increased from first day (0.106 ± 0.003) up to 10th day (67.76 ± 0.42 mg/L) in the culture of 20% digested rotten guava media (DRGM) and then decreased up to 14th day (47.57 ± 0.42 mg/L) of experiment. The highest total biomass grown in the culture of 40% DRGM was recorded 51.46 ± 0.28 mg/L on 10th day of culture and then decreased up to 14th day (42.35 ± 0.19 mg/L) during the experiment. Total biomass cultured in 60% DRGM was increased from first day (0.107 ± 0.04 mg/L) up to 10th day (57.75 ± 0.20 mg/L) and then decreased up to 14th day (41.88 ± 0.14 mg/L) of experiment. The highest total biomass was obtained in Kosaric medium (705.51 ± 2.53 mg/L) on 10th day and then decreased up to 14th day (279.39 ± 1.33 mg/L) during experiment (Figure 2).

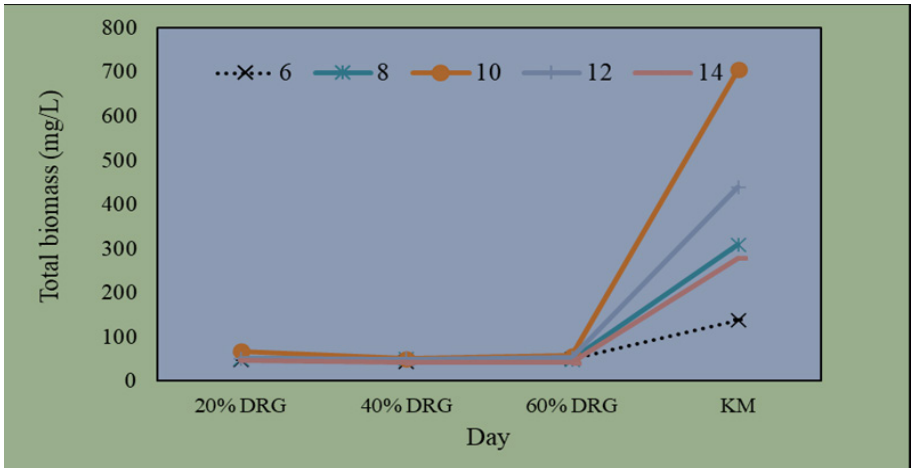

Figure 2: Mean values of total biomass (mg/L) of *S. platensis*

Comparison of growth parameters of *S. platensis*

The chlorophyll a (10.53 ± 0.32 mg/L) of *Spirulina* grown in 60% DRG (0.862 ± 0.0012) was significantly (P < 0.01) higher than that of cultured in 40% (0.768 ± 0.0012) and 20% DRG (0.770 ± 0.14). There was no significant difference among the Chlorophyll a grown in supernatant of 20, 40 and 60% DRG during the study (Table-3).

Table 3: Comparison of cell weight, chlorophyll a and total biomass of *S. platensis*

Parameters	T1 (20% DRG)	T2 (40% DRG)	T3 (60% DRG)	T4 (KM)
Cell weight (mg/L)	0.813 ± 0.0013^{b}	0.807 ± 0.0011^{b}	0.816 ± 0.0017^{b}	12.41 ± 0.23^{a}
Chlorophyll a mg/L)	0.771 ± 0.13^{b}	0.769 ± 0.0015^{b}	0.863 ± 0.0018^{b}	10.54 ± 0.33^{a}
Total biomass (mg/L) *	67.76 ± 0.42^{b}	51.45 ± 0.26^{c}	57.74 ± 0.26^{bc}	705.52 ± 2.59^{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 *S. platensis*

Cell weight of *S. platensis*) had highly significant (P< 0.01) direct correlation with chlorophyll a (r = 0.746) which grown in the supernatant of different digested rotten guava, and Kosaric medium during the study (Figure3). Similarly, total biomass was highly (P< 0.01) and directly correlated with chlorophyll a (r = 0.795) that cultured in the supernatant of various digested rotten guava, and Kosaric medium (Figure4). Again, total biomass was found to be highly (P< 0.01) and directly correlated with the cell weight (r = 0.742) grown in the supernatant of different digested rotten guava, and Kosaric medium (Figure5).

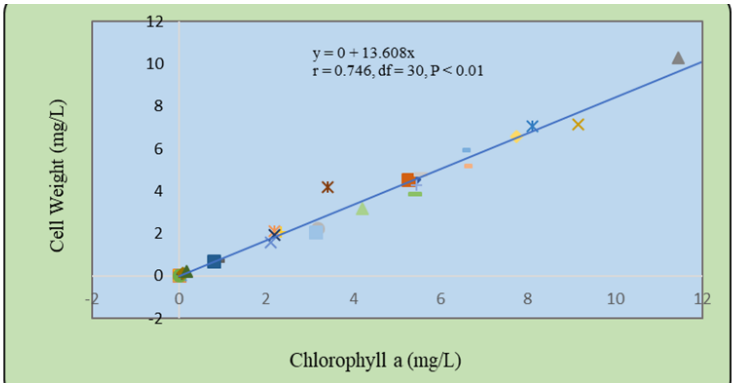

Figure 3: Correlation coefficient (r) of cell weight (mg/L) of *S. platensis* with chlorophyll a (mg/L)

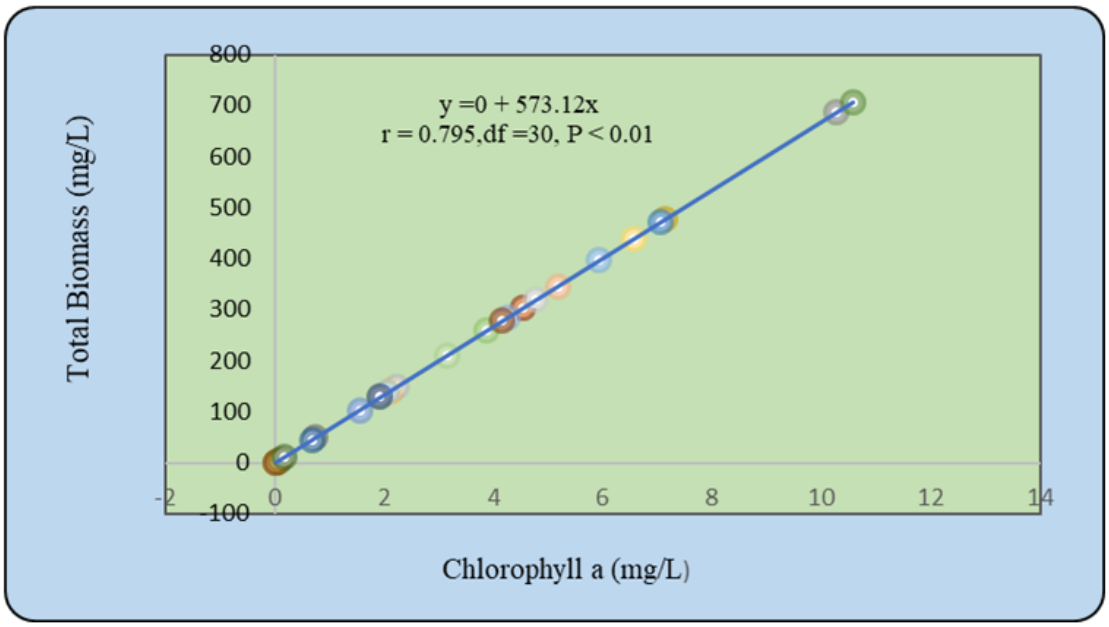

Figure 4: Correlation coefficient (r) of total biomass (mg/L) of *S. platensis* with chlorophyll a (mg/L)

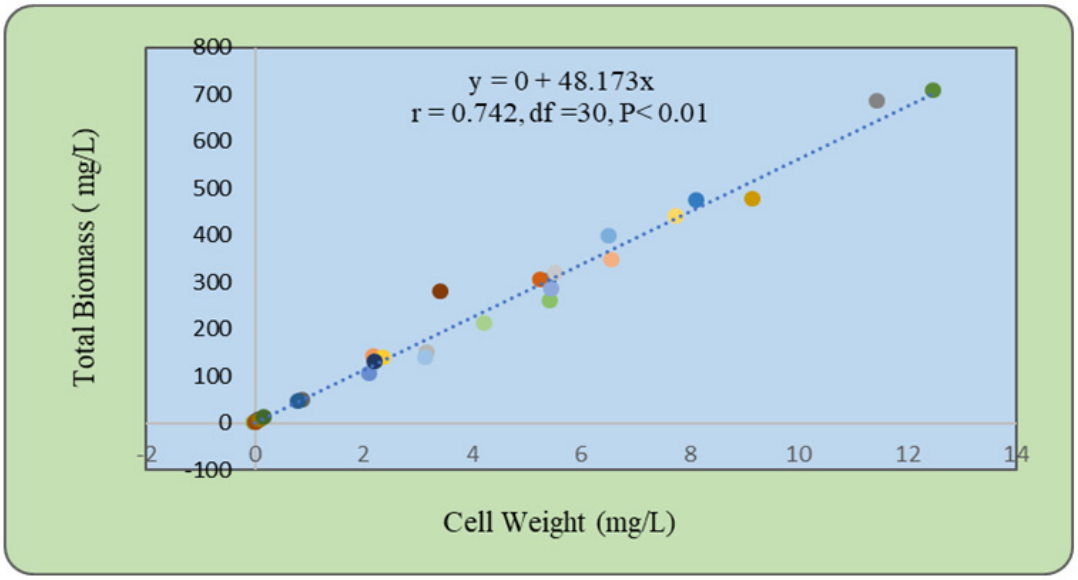

Figure 5: Correlation coefficient (r) of total biomass (mg/L) of *S. platensis* with cell weight (mg/L)

DISCUSSION

During the present study, digested organic medium like rotten guava which has the similarity with the findings of Dineshkumar *et al*., (2016) and Sukumaran *et al*., (2018). During culture of *S. platensis*, the exponential phase was found up to 10th day from the beginning and then the cell weight declined i.e., stationary phase started. Satter (2017) recorded the cell weight and chlorophyll a content of *S. platensis* was highly significant (P<0.05) in 4.0 g/L where light intensity, aeration and temperature played significant role to the culture system. The cell weight of *S. platensis* in KM attained 4.17 to 12.41 followed by supernatant of digested rotten guava medium (0.0023 to 0.813 mg/L in 20% DRGM, 0.0024 to 0.807 mg/L in 40% DRGM, 0.0023 to 0.816 mg/L in 60% DRGM). The growth performance of *S. platensis* in supernatant of 60% DRGM was found better than 20% and 40% DRGM. This variation might be due to the differences in nutrient concentrations and composition of varied media. This might be due to lower nitrogen and phosphate concentration of the nutrients in the media. The concentration of 60% DRGM which were better for the growth of *S. platensis* because of the nutrient content. Similarly, Sharker (2002) conducted an experiment on the culture of *S. 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. In the present study, the chlorophyll a content of inoculated *S. platensis* was 0.0015 mg/L which attained a high content of 0.863 mg/L in 60% DRGM at the 10th day of culture and highest content obtained in KM (10.54 mg/L). These findings are not more or less similar with

the findings of Phang *et al.* (2000), Habib *et al.* (2003), Khan ANMAI, Habib MAB, Khan *et al.*, (2018) and Satter (2017). This might be due to lower nitrogen and phosphate concentration of the nutrients in the media. Dineshkumar *et al.* (2016) studied that *S. platensis* grew well in natural medium (Conway medium,kosaric medium and BGII medium).

In the present study, supernatant of digested rotten guava was used as a media of three concentrations for the culture of *S. platensis*. From the above discussion, the growth performance of *S. platensis* in supernatant of 60% DRGM was found better than 20% and 40% DRGM and maximum produced perceived from the Kosaric Medium (KM).

The experiment revealed the growth performance of *S. platensis* where the initial cell weight was 0.0023mg/L which attained a maximum cell weight of 0.816 mg /L in 60% DRGM, 0.807mg/L in 40% DRGM and 0.813 mg/L in 20% 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 in KM (10.54 mg/L) followed by 0.863 mg/L in 60% DRGM, 0.769mg/L in 40% DRGM, 0.771 mg/L in 20% DRGM on the 10th day of culture period. This study finds out a suitable concentration of digested rotten guava medium (DRGM) as an organic nutrient medium for culture and growth of *S. platensis*.

CONCLUSION

In this experiment, the cell weight of *Spirulina* (*Spirulina platensis*) had significant ($P < 0.01$) correlation with chlorophyll a ($r = 0.746$) grown in the supernatant of different digested rotten guava, and Kosaric medium. Similarly, total biomass of *S. platensis* was highly ($P < 0.01$) and directly correlated with chlorophyll a ($r = 0.795$). Again, total biomass of *Spirulina* was found to be highly ($P < 0.01$) and directly correlated with the cell weight ($r = 0.742$). The chlorophyll a in KM (10.53 ± 0.32 mg/L) was significantly ($P < 0.01$) higher than that of *Spirulina* cultured in in 60% (0.863 ± 0.0012) 40% (0.769 ± 0.0012) and 20% DRG (0.771 ± 0.14). However, the production of *S. platensis* in Kosaric medium is expensive than DRG Medium. Therefore, DRG medium may be used commercially 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.

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