Published with Open Access at Journal BiNET
Vol. 29, Issue 01: 2433-2441
Journal of Bioscience and Agriculture Research
Journal Home: www.journalbinet.com/jbar-journal.html

Growth performance of *Spirulina platensis* in supernatant of digested rotten guava

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Article received: 10.06.2022; Revised: 22.07.2022; First published online: 30 August, 2022

ABSTRACT

This study was performed to evaluate the culture potentiality and growth performance of Spirulina (Spirulina platensis) in supernatant of three different concentrations of digested rotten guava (DRG) and Kosaric Medium (KM) as control. The study consisted of three different concentrations of rotten guavas, such as 20 (T_1), 40 (T_2) and 60 (T_3) were allowed to digest under aeration. After 34 days, three replications showed the reddish white-colored supernatant screened and taken in a 2.0 L conical flask. Then, Spirulina was inoculated to grow in these three digested rotten guava media (DRGM) with the addition of micronutrients and KM and 9.0 g/L $NaHCO_3$ for 14 days. The cell weight of Spirulina was attained at a maximum of 12.43±0.10 mg/L (dry wt. basis) in KM, followed by 0.818 ± 0.002, 0.815 ± 0.0014 and 0.809± 0.0011 mg/L in supernatant of 60, 20 and 40% DRGM, respectively on the 10th day of culture. A similar trend was also observed in the case of specific growth rates (based on cell weight and chlorophyll a) and total biomass (mg/L) of Spirulina. Cell weight of Spirulina had a highly significant ($P<0.01$) correlation with the chlorophyll a content ($r= 0.746$) and total biomass ($r= 0.742$) of Spirulina. The results on the growth performance showed that Spirulina grown in supernatant of 60% DRGM was significantly ($P<0.01$) higher (T_3) than that of Spirulina grown in supernatant of 20% (T_1) and 40% DRGM (T_2).

***Key Words**: Culture potentiality, Growth performance, Spirulina platensis, Digested Rotten Guava Media (DRGM)*

Cite Article: Islam, M. S., Habib, M. A. B., Rahman, M. H. and Arifuzzaman, M. (2022). Growth performance of *Spirulina platensis* in supernatant of digested rotten guava. Journal of Bioscience and Agriculture Research, 29(01), 2433-2441.
Crossref: https://doi.org/10.18801/jbar.290122.295

I. Introduction

Bangladesh has no fish feed industries as of the 1980s. In 1982, Bangladesh's Saudi-Bangla Feed Factory established the nation's first fish feed company. Bangladesh now has more than 500 fish feed businesses. Within the nation, sizable feed enterprises have grown to boost aquaculture productivity by using enough feed. The need for high-quality feed is rising every day due to the expansion of aquaculture practices. Fish development requires high-quality feed. Keep the feed conversion ratio

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(FCR) close to 1, highly dependent on quality feed. The right amount of protein in the feed is essential for the higher growth of different fish species. The utilization of net protein should be around 27 percent. However, fish and bone meals 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 grew naturally within the wild in saltwater, alkaline lakes, and natural springs. Spirulina is cultivated in artificial reservoirs worldwide and harvested for supplementary diets in some contexts. For centuries, civilizations have cultivated and cherished Spirulina, highly recommended for its health-improving benefits. The native people in Africa have used microalgae as a staple of their daily diet supplement because 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).

Spirulina could also be grown in agro-industrial wastes, rotten fruits and chicken wastes. Among fruits, huge quantities of spoils guava (rotten) are available in numerous markets within the country. Therefore, market is allowed to digest (aerobic & anaerobic) and supernatant is also used to expand 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 the growth and many of the factors are essential for the assemblage of Spirulina at a larger scale, of which nutrient availability and temperature are most significant. The filamentous cyanobacteria like Spirulina can produce a great quantity of biomass as considered the 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 cultivation. Therefore, 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 great interest due to its significant attributes of cellular contents like polyunsaturated fatty acids, carotenoids, vitamins, minerals, and other pigments that have outstanding antioxidant activity (Cohen and Vonshak, 1991; Bhat and Madyastha, 2001). 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 is comparable to 1000 kg of other vegetables due to its remarkable existence of alimentation (Kato, 1991).

Spirulina is created of between 55 and 70% protein (more than beef, chicken, and soybeans), contains all the essential non-essential amino acids, yet has 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 and Habib et al., 2008). Spirulina has been studied for single cell protein (SPC), vitamins, minerals, proteins and polyunsaturated fatty acids (γ-linolenic acid), therapeutic properties and antioxidant activity. The cost and composition of cultivation media are challenging for commercially viable production. The foremost convincing trials were conducted among populations that traditionally eat Spirulina for their supplementary diets. Its consumption is regular but reasonably low, 10-12 g/per/day (Cysewski, 1983). 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 for the expansion of Spirulina; to investigate growth parameters and proximate composition of Spirulina cultured in supernatant digested rotten guava, and to search out out the acceptable concentration of the medium for maximum growth of *Spirulina platensis*.

II. Materials and Methods

Study area

The study was carried out in the Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University at Live Food Aquaculture Laboratory, Mymensingh, Bangladesh.

Culture of Microalgae

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 (BAU), Mymensingh-2202, and Bangladesh. It was thought that the proximate composition of this media might be suitable for the growth of culture species.

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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 at Fish Nutrition Laboratory of Faculty of Fisheries, BAU, Mymensingh-2202, following standard methods (Horwitz, 1984).

Moisture: The sample was weighed in a previously pre-weighed small crucible in triplicates. The samples contained in the crucible were dried to moisture from it at 105°C for 24 hrs. After drying, the crucible samples were cooled with a desiccator's help. Then, cooling was done at room temperature and weighed in a sensitive balance for the measurement. Finally, the percentage of moisture in the sample was calculated using the following equation:

$$\%\text{moisture} = \frac{x-y}{x} \text{x } 100$$

Where, X = Wt. of sample before drying; Y = Wt. of sample after drying.

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

$$\%\ \text{Ash} = \frac{Wt.of\ ash\ with\ preweighed\ crucible - wt.of\ crucible}{wt.of\ dried\ sample}\ x\ 100$$

Culture and collection of Spirulina platensis

The stock of *Spirulina platensis* was collected from the live food culture laboratory, Department of aquaculture, BAU and twelve conical flasks (2L capacity) were used for the culture of Spirulina.

Pure stock maintenance of Spirulina platensis

The pure stock of *Spirulina platensis* was maintained in the laboratory in Kosaric medium (KM) (Modified after Zarrouk's, 1996). The growth performance of *Spirulina platensis* was recorded on every alternative day and was monitored under microscope to confirm its purity, following the guidelines given by Bold and Wynne (1978), Vymazal (1995) and Phang and Chu (1999).

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

For the culture of *Spirulina platensis* the Rotten guava medium and kosaric medium (KM) were prepared to maintain the proper procedures, whereas Kosaric medium (KM) was considered a control for the study. The preparation of culture media for *Spirulina platensis* along with the compositions of Rotten Guava Medium (RGM) and Kosaric medium (KM) presented in Table 01, Figure 01 and 02.

Table 01. The composition of Kosaric medium (Modified after Zarrouk's, 1996) for the culture of *Spirulina platensis*

Sl. No.	Chemicals/compounds	Concentration in stock solution g/l
1	$NaHCO_3$	9.0
2	K_2HPO_4	0.250
3	$NaNO_3$	1.250
4	K_2SO_4	0.50
5	NaCl	0.50
6	$MgSO_47H_2O$	0.10
7	$CaCl_2$	0.02
8	$FeSO_42H_2O$	0.005
9	A_5 micronutrient solution[a]	0.5ml/L
	a) A_5 micronutrient solution	G/L
	i) H_3BO_4	2.86
	ii) $MnCl_2.4H_2O$	1.81
	iii) $ZnSO_47H_2O$	0.22
	iv) $CuSO_4.7H_2O$	0.08
	v) MoO_3	0.01
	vi) $CoCl_2.6H_2O$	0.01

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Figure 01. Collected rotten guava

Figure.02. Dry rotten guava

To decompose the 5.0 L glass bottle 50 g/L rotten guava was allowed under aerobic conditions for 34 days (Figure 03). Three concentrations of digested rotten guava at the rate of 20%, 40% and 60% were prepared from the dilution of light reddish white colored supernatant from bottle (Figure 04). Then the three different concentrations with each replication were kept in 1.0 L flask (Figure 05). The composition and various concentration of RGM and KM are shown in Table 02 and Table 03, respectively.

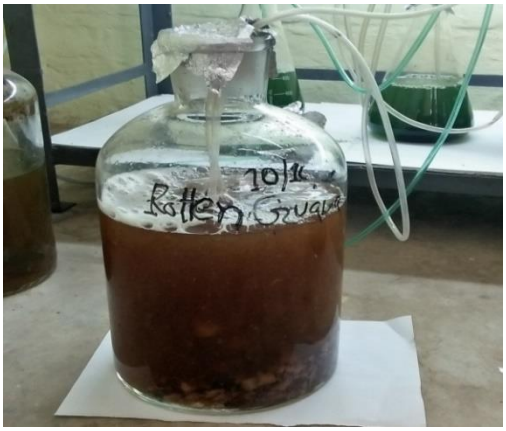

Figure 03. After 34 days of digestion

Figure 04. Culture of *Spirulina platensis* in supernatant of DRGM and KM in 1.0 L flasks on initial day of culture into iron made rack.

To prepare rotten guava medium, 5 L volumetric flask was filtered with plankton net after (10.10.18 to 14.11.2018) 34 days with maintaining the digestion and continuous aeration (Figure 06). Then 0.8 g (0.2 g/L) urea was added and the medium was sterilized at 115^{o}C for 15 minutes and mixed well by high pressure bumping water autoclave. The media were kept three days after autoclaving to confirm the purity of the stock before culture of microalgae. The amount (Table 02) of ingredients from no. 1 to 8 was weighed and taken in a 1.0 L conical flask to prepare Kosaric medium. Then 0.5 ml micronutrient solution was pipetted in the flask to make the volume 1.0 L with the addition of distilled water. The autoclaving, mixing and cooling were carried out pursuing the procedure to prepare the digested rotten guava media.

Experimental design of *Spirulina platensis* culture

The RGM and KM were used to culture *Spirulina platensis.* The *Spirulina platensis* was collected and inoculated from the pure stock culture. Experimental design is shown in (Table 02).

Table 02. Experimental design for *Spirulina platensis* culture

Types of medium	Treatments	Replications	Amounts of rotten guava (%)	Duration of culture (days)
Supernatant of DRGM	1	3 (101, 102 and 103)	20	14
	2	3 (201, 202 and 203)	40	
	3	3 (301, 302 and 303)	60	
Kosaric Medium(KM)	4	3 (KM-1, KM-2 and KM-3)	-	14

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Figure 05. Culture of *Spirulina platensis* in supernatant of DRGM and KM in 1.0 L flasks on 14th day of culture into iron made rack.

Figure 06. Culture of *Spirulina platensis* in supernatant of DRGM in 5.0 L flasks of culture for total biomass estimation and analysis of proximate composition.

Culture of Spirulina in digested rotten guava media (DRGM) and Kosaric medium (KM)

In this study, with three replications, the different concentrations of DRGM (20%, 40% and 60%) and KM as control were used to grow *S. platensis* in a 1.0 L volumetric flask. To produce a culture containing 10% spirulina suspension, Spirulina was inoculated into each culture flask (Optical density at 620 nm = 0.20) (Habib, 1997). For getting the required density 20 ml of spirulina suspension is usually required. All the flasks were kept under fluorescent lights (TFC, FL-40 SD/38-day light, Taiwan). An electric aquarium aerator (SB-348A) was used for the continuous aeration. To record dry cell weight, chlorophyll content of Spirulina seven sub-samplings (15ml vial) were carried out on every alternative day from each flask and recorded the findings. All the glassware was sterilized at 70°C overnight with dry heat during the experimental period.

Table 03. 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 (g)	Cell weight (g)	Cell weight (g)	Cell weight (g)
2	24/11/2018	KM-2, 15 0.797 g	102, 15 0.795 g	202, 15 0.748 g	302, 15 0.751 g
4	26/11/2018	KM-1, 15 0.789 g	101, 15 0.750 g	201, 15 0.751 g	301, 15 0.053 g
6	28/11/2018	KM-3, 15 0.830 g	103, 15 0.792 g	203, 15 0.762 g	303, 15 0.804 g
8	30/11/2018	KM-2, 15 0.844 g	102, 15 0.810 g	202, 15 0.807 g	302, 15 0.797 g
10	02/12/2018	KM-1, 15 0.823 g	101, 15 0.815 g	201, 15 0.809 g	301, 15 0.818 g
12	04/12/2018	KM-3, 15 0.821 g	103, 15 0.810 g	203, 15 0.805	303, 15 0.810 g
14	06/12/2018	KM-2, 15 0.811 g	102, 15 0.805 g	202, 15 0.801 g	302, 15 0.805 g

Cell weight estimation (dry weight) of Spirulina (Clesceri et al., 1989)

A Sartorius filter paper of mesh size 0.45 µm and diameter 47 mm was used to filter the sample containing 15 ml spirulina suspension. The filter papers were dried in an oven for 24 hrs at 70°C and weighed before filtration. To remove insoluble salts, the filtered samples were washed three times. Then the filter papers were settled in a glass petri dish and kept in the oven at 70°C overnight. The petri dish was put into desiccator for 20 minutes for cooling and then the filter papers were weighed. By using the following equation, the dry weight of algae on the filter paper was measured:

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

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

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Total biomass of spirulina (S. platensis)
By using the following formula, total biomass was calculated, which was given by Vonshak and Richmond (1988):

Total biomass = Chlorophyll a x 67

Analysis of proximate composition Spirulina
On 10th day of Spirulina culture, the best optical density was found. In that day, Spirulina was collected in petridish, filtered, and kept in an oven at 40°C for overnight for the drying purpose. Then the *S. platensis* was analyzed in the Fish Nutrition Laboratory to know the proximate composition by following the standard methods (Horwitz, 1984).

Statistical analysis
Analysis of variance (ANOVA) of chlorophyll a of *S. platensis* and mean cell weight and cultured in different media (treatments) were done to assess whether any significant difference among the treatment mean was done by Ducan's Multiple Range Test (DMRT) at 1% level of probability (Zar, 1984).

III. Results

Cell weight of Spirulina
The cell weight was observed to be increased from initial day (first day) up to 10th day (0.815 ± 0.0014 g/L) of culture of 20% digested rotten guava (DRG) and then decreased up to 14th day (0.723 ± 0.0013 g/L) of experiment. The highest cell weight of 0.809 ± 0.0011g/L was found when grown in 40% DRG. The cell weight consecutively increased from initial day (first day) (0.0023 ± 0 g/L) up to 10th day (0.818 ± 0.0012g/L) of culture in 60% DRG, and then found to dwindle the production up to 14th day (0.725 ± 0.0011g/L) of experiment. Highest cell weight of 12.43 ± 0.21g/L was obtained in Kosaric medium on 10th day and then decreased up to (3.44 ± 0.021) on 14th day of the experiment (Figure 07).

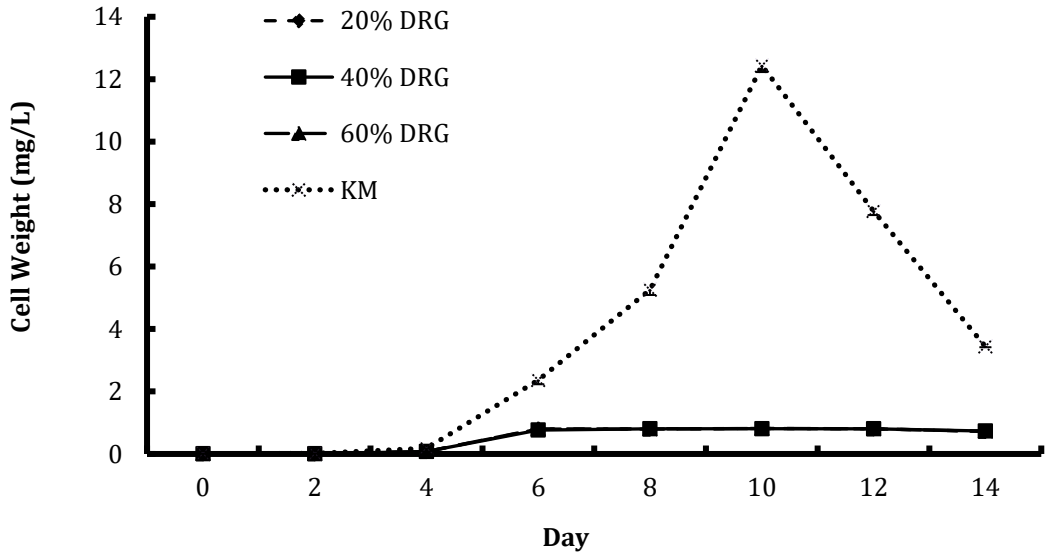

Figure 07. Mean values of cell weight (mg/L) in DRGM and KM.

Chlorophyll a of spirulina
Chlorophyll a in 20% digested rotten guava media (DRGM) was found to be increased up to (0.770 ± 0.0012 g/L) and then dwindled up to (0.710 ± 0.0012 g/L. However, chlorophyll a in supernatant of 40% DRG was 0.768 ± 0.0012 g/L. 7.365 ± 0.20 g/L in 60% of DRG was. While, the highest chlorophyll a in Kosaric medium was 10.53 ± 0.32 g/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 08).

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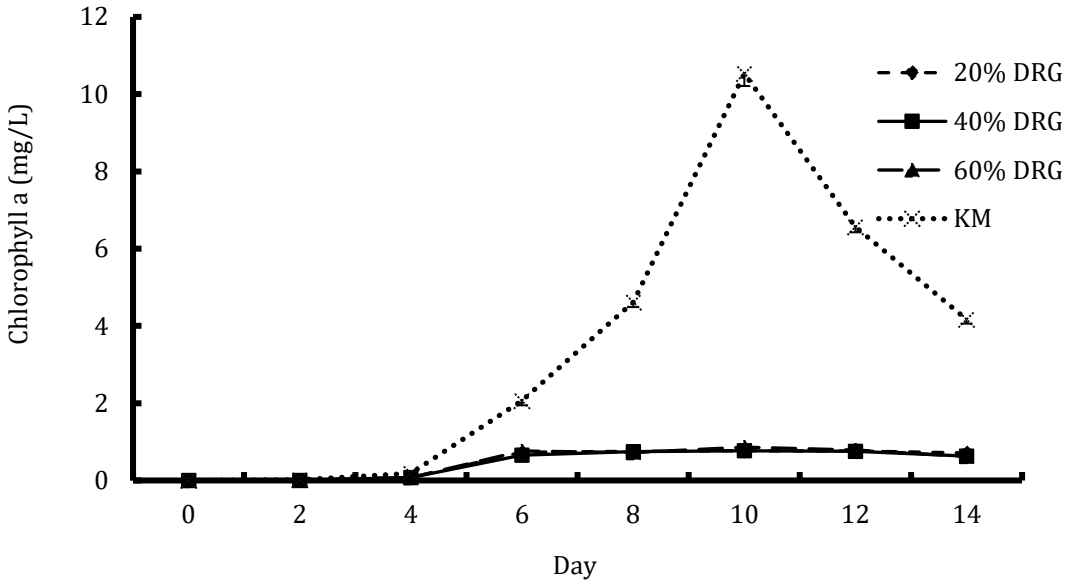

Figure 08. Mean values of chlorophyll a (mg/L) of *Spirulina platensis*

Total biomass of Spirulina

Total biomass of Spirulina was found to be increased from first day (0.106 ± 0.003) up to 10th day (67.77 ± 0.44 g/L) in the culture of 20% digested rotten guava media (DRGM) and then decreased up to 14th day (47.57 ± 0.42 g/L). The total biomass of Spirulina in 40% DRGM was observed and grown up to 51.46 ± 0.28 g/L, 57.75 ± 0.20 g/L in the 60% DRGM (Figure 09). The highest total biomass was recorded in Kosaric medium (705.51 ± 2.53 g/L).

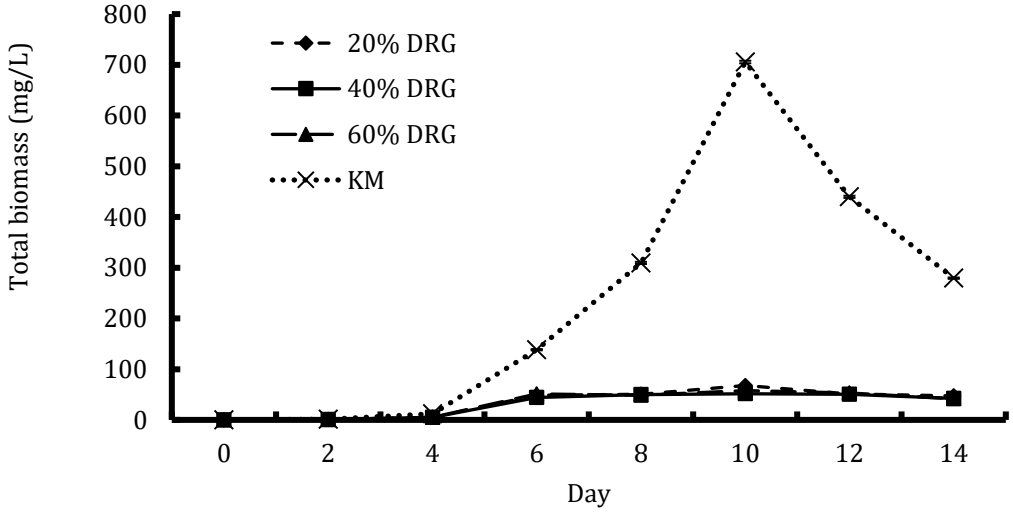

Figure 09. Mean values of total biomass (mg/L) of *Spirulina platensis*

IV. Discussion

In this experiment, the growth performance in the supernatant of 60% DRGM was better than 20% and 40% supernatant of DRGM. This variation might be appeared due to the dissimilation in the nutrient concentrations and composition of the different media. In this study, *Spirulina platensis* showed the highest growth performance in KM other than DRGM. It might take place due to the propitious availability and suitable nutritional requirements for the growth of the species. Habib and Kohinoor (2018) found that the supernatant of 45% digested poultry waste resulted in more satisfactory growth than other concentrations indicating significant outcomes in the present findings. On the other hand, 40% DRGM showed lower growth performance concerning the supernatant of 20% and 60% DRGM. It could happen due to the lower nitrogen and phosphate concentration of the nutrients media. The concentration of 60% DRGM performed better results in terms of growth performance because of the availability of suitable amount of required nutrient contents.

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In this study, the digested organic medium like rotten guava was identical to the consequence of Dineshkumar et al. (2016) and Sukumaran et al. (2018). During the study, the exponential phase was gradually upward up to 10th day from the beginning and then the cell weight consecutively declined. The physical properties such as temperature, light intensity and aeration enacted an emergent role in the culture unit. The climate condition was found inclinable and suitable for the growth of *S. platensis*. Satter (2017) recorded the cell weight of 4.0 g/L in *S. platensis,* which was significantly ($P<0.05$) higher in digested poultry waste than in other used media. Similarly, Sharker (2002) experimented with various concentrations (0.3, 0.4 and 0.5 g/L) of papaya skin powder medium (PSPM) and in Kosaric medium (KM) for three months. In the concentrations of PSPM, the initial cell weight was found 0.0004 g/L with a maximum weight of 0.720 g/L. He also observed the resembling trend in the chlorophyll a content and the growth rate was significantly ($P<0.01$) higher in the concentration of 0.3 g/L PSPM than in other concentrations of PSPM. Sukumaran et al. (2018) recorded satisfactory growth in different nutrient media. Manigandan (2014) found better growth performance in synthetic medium followed by fertilizer medium and seawater for the experiment.

The chlorophyll a content was attained high in KM (10.53 mg/L) followed by 0.862 mg/L in 60% supernatant of DRGM. These experimental findings were more or less similar to the findings of Phang et al. (2000), Habib et al. (2003) and Satter (2017). In addition, Dineshkumar et al. (2016) studied *Spirulina platensis* also grew well in natural mediums such as Conway medium and BGII medium. In the present study, three different concentrations of digested rotten guava were used as a media for the culture of *Spirulina platensis.* The supernatant of 60% digested rotten guava showed maximum optical density compared to KM, which is similar to the findings of Habib et al. (1997), Habib et al. (2003) and Satter (2017). Furthermore, from the above discussion, the growth performance of Spirulina in supernatant of 60% DRGM was found to be better than the supernatant of 20% and 40% DRGM.

IV. Conclusion

In this experiment, the growth performance of Spirulina was observed in different concentrations of digested rotten guava (DRG) and Kosaric Medium (KM). The maximum cell weight was attained of 12.43±0.10 mg/L (dry wt. basis) in KM followed by 0.818 ± 0.002, 0.815 ± 0.0014 and 0.809± 0.0011 mg/L in supernatant of 60%, 20% and 40% DRGM, respectively. The results showed that the growth performance of Spirulina was significantly ($P<0.01$) higher in supernatant of 60% DRGM than that of 20% and 40%. The experimental data implied that the cultivation of Spirulina showed better yield and maximum growth in T_3 than T_1 and T_2 among different supernatant of DRGM. It indicates that the different concentration of digested rotten guava (20%, 40%, 60%) has the potential to aggrandize Spirulina's growth rate. 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 must be performed to evaluate the grow-out potential of Spirulina in lab-based cultivation.

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HOW TO CITE THIS ARTICLE?

Crossref: https://doi.org/10.18801/jbar.290122.295

MLA
Islam, M. S. et al. "The growth performance of *Spirulina Platensis* in supernatant of digested rotten guava". Journal of Bioscience and Agriculture Research, 29(01), (2022): 2433-2441.

APA
Islam, M. S., Habib, M. A. B., Rahman, M. H. and Arifuzzaman, M. (2022). The growth performance of *Spirulina Platensis* in supernatant of digested rotten guava. *Journal of Bioscience and Agriculture Research*, 29(01), 2433-2441.

Chicago
Islam, M. S., Habib, M. A. B., Rahman, M. H. and Arifuzzaman, M. "The growth performance of *Spirulina Platensis* in supernatant of digested rotten guava". Journal of Bioscience and Agriculture Research, 29(01), (2022): 2433-2441.

Harvard
Islam, M. S., Habib, M. A. B., Rahman, M. H. and Arifuzzaman, M. 2022. The growth performance of *Spirulina Platensis* in supernatant of digested rotten guava. Journal of Bioscience and Agriculture Research, 29(01), pp. 2433-2441.

Vancouver
Islam, MS, Habib, MAB, Rahman, MH and Arifuzzaman, M. The growth performance of *Spirulina Platensis* in supernatant of digested rotten guava. Journal of Bioscience and Agriculture Research, 2022 August, 29(01): 2433-2441.

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