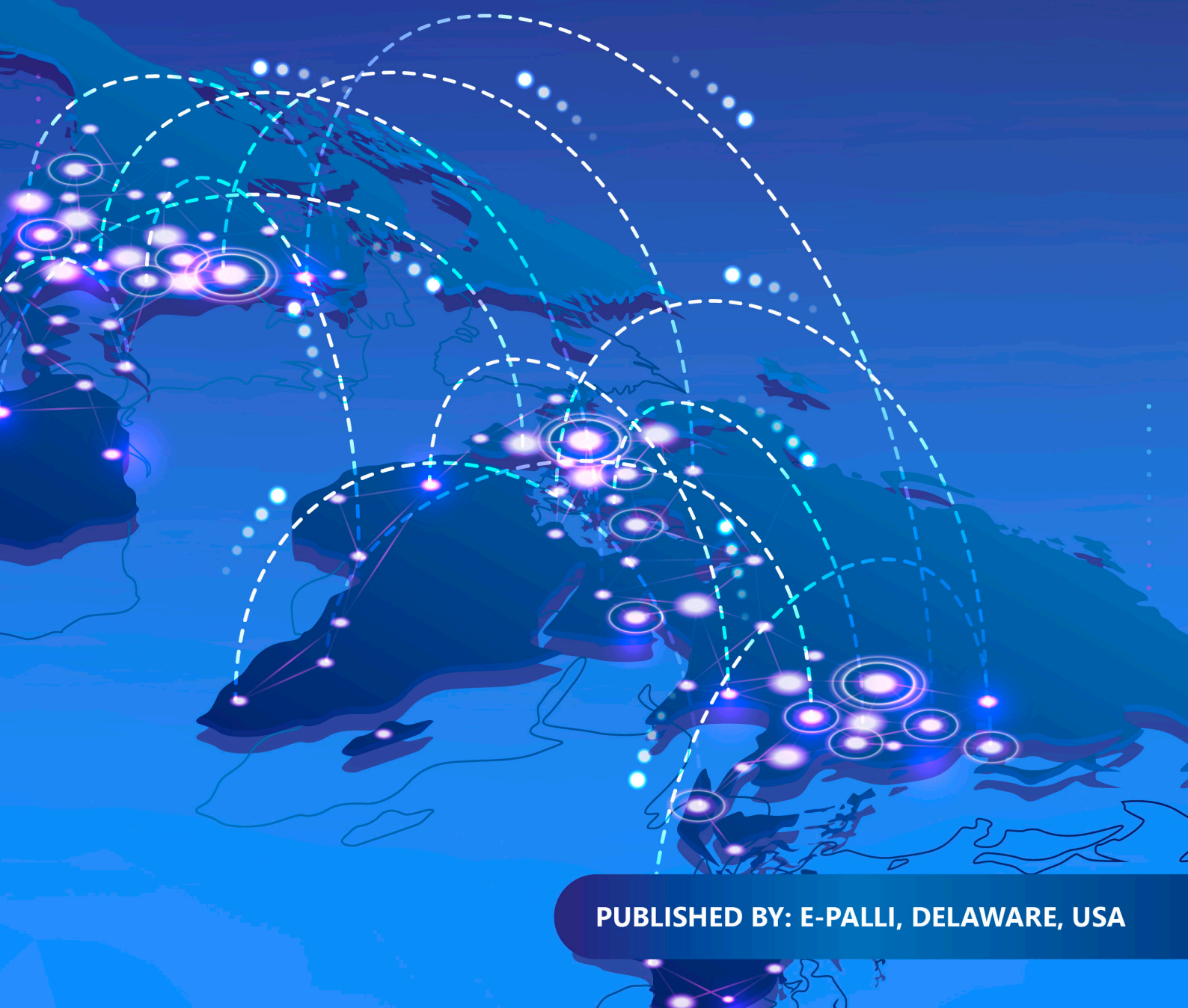
AMERICAN JOURNAL OF MULTIDISCIPLINARY RESEARCH AND INNOVATION (AJMRI)

ISSN: 2158-8155 (ONLINE), 2832-4854 (PRINT)

VOLUME 1 ISSUE 4 (2022)

Indexed in



PUBLISHED BY: E-PALLI, DELAWARE, USA

American Journal of Multidisciplinary Research and Innovation (AJMRI)

Volume 1 Issue 4, Year 2022
ISSN: 2158-8155 (Online), 2832-4854 (Print)
DOI: https://doi.org/10.54536/ajmri.v1i4.579
https://journals.e-palli.com/home/index.php/ajmri

Potentiality of Digested Rotten Guava Medium (DRGM) in Replacement of Kosaric Medium (KM): Perspective of *Spirulina platensis* culture

Md. Hashibur Rahman[1*], Mohammad Ashraful Alam[2], Flura[2], Md. Saiful Islam[3], Md. Arifuzzamand[4], Md. Moniruzzaman[2], Al-Amin[1], Sharmin Sultana[1], Asma Jaman[1], Md. Abu Kawser Didar[2], Md. Mustafiz[3]

Article Information

Received: September 11, 2022

Accepted: September 29, 2022

Published: September 30, 2022

Keywords

Digested Rotten Guava Medium (DRGM), Spirulina Platensis, Culture Potentiality, Growth Performance

ABSTRACT

This study was conducted to evaluate the culture potentiality and growth performance of *Spirulina* (*Spirulina platensis*) in supernatant digested rotten guava (DRG) in replacement of Kosaric Medium (KM). The *Spirulina* was inoculated to grow in digested rotten guava media (DRGM) (treatments) with the addition of 9.0 g/L NaHCO3 and 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 (T_1), 20 (T_2) and 40% DRGM (T_3), respectively on the 10th day of culture. The cell weight of *spirulina* grown in these media had highly significant ($P<0.01$) correlation with the chlorophyll a content ($r = 0.746$) and total biomass ($r = 0.742$) of *spirulina*. The results showed that the growth performance of *spirulina* in supernatant of 60% DRGM was significantly ($P<0.01$) higher than that of *spirulina* grown in supernatant of 20% and 40% DRGM. 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 optimum level during the culture period. The results showed that, the different concentration of digested rotten guava (20%, 40%, 60%) has potential to increase the growth rate of *Spirulina*. Therefore, the DRG medium may be commercially used for mass culture of *Spirulina platensis*.

INTRODUCTION

With the aim of increased aquaculture production through applying adequate feed large numbers of feed industries are developed in the country. Due to increased aquaculture practice, demand of good quality feed is increasing day by day. Prime quality feed is essential for fish growth. Maintain feed conversion ratio (FCR) close to 1 is highly depends upon good feed. Feed should have adequate protein content which facilitates high growth. Net protein utilization should be around 27 percent. But fish meal and bone meal are not available in our country. So, we can find to alternative sources. We can use to alternative fish meal to *spirulina*. *Spirulina* is a "superfood" which is the most nutritious, rich in protein and concentrated whole food known to humankind. *Spirulina* has been so popular in the present world's context due to its high nutritional value. Due to its high and good quality protein, vitamins, essential fatty acids contents, antioxidant pigments, antimicrobial activity, and anticancer properties *Spirulina* which is a fast-growing cyanobacteria have been used as a possible alternative source of protein for cultured fish. As it is known that, the biomass of *Spirulina platensis* is nutritionally rich in protein it may be the better alternative to fish protein to reduce the cost of feed as 70% of the total operating costs belongs to the feed supplement in terms of whole culture period.

As food microalgae are used since about 2,000 years ago in China. For thousands of years although microalgae are mentioned because the source of nutrients (Borowitzka,1999), to develop microalgal biotechnology it began only within the middle of the past century (Spolaore *et al.*, 2006). Depending upon the source, *Spirulina* contains unusually high amounts of protein, between 55 and 70% by dry weight (Phang *et al.*, 2000). Though with reduced amounts of methionine, cystine, and lysine, as compared to plain proteins it is an entire protein containing all essential amino acids. It's however, superior to all or any standard plant protein, like that from legumes (Richmond, 2004). As a complementary dietary ingredient for fish, shrimp and poultry *Spirulina* has been used of feed and increasingly as a vitamin supplement and protein to aquafeeds (Muller, 2000).

Now-a-days, *Spirulina platensis* is gaining great interest for its cellular contents such as vitamins, minerals, polyunsaturated fatty acids, carotenoids and other pigments that have antioxidant activity (Cohen & Vonshak, 1991; Madhava & Bhat, 2000). According to the researchers, one kg of *Spirulina spp* is similar to 1000 kg of other vegetables (Kato, 1991). *Spirulina* is made of between 55 and 70% protein (more than beef, chicken, and soybeans), all the essential non-essential amino acids, as well 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). Its consumption is regular but at a fairly low level, 10-12 g/per/day, except pregnant women who eat considerably more (Cysewski, 1983).

With the event of aquaculture, the requirements of bulk

[1] Bangladesh Fisheries Research Institute, Headquarters, Mymensingh, Bangladesh
[2] Bangladesh Fisheries Research Institute, Riverine Station, Chandpur, Bangladesh
[3] Department of Aquaculture, Bangladesh Agricultural University, Bangladesh
[4] Bangladesh Fisheries Research Institute, Shrimp Research Station, Bangladesh
[*] Corresponding author's email: hasibkhan94bfri@gmail.com

feed materials and substituents like soybean flour, organic and other resources are constantly rising and costs are increasing every year. Therefore, the research for new sources of raw materials has been a crucial attention. As a replacement of feed material microalgae has many advantages which make it become superior to other source cyanobacteria species (source of protein, fatty acids, vitamins, etc.). Thus, the benefits of microalgae appear increasingly in aquaculture industry. Therefore, the utilization of microalgae as feed additives is more broadly getting emphasized to use in aquaculture. As a critical think about promoting normal growth and sustaining fish health in aquaculture operations proper nutrition has been recognized (Illman *et al*., 2000). Diets particularly animal and plant-based may be a factor that has significantly contributed to the huge expansion of fish farming.

The S. platensis were cultured in various concentrations of digested rotten guava medium, the ultimate goal for producing a cheap alternative source of protein which has not yet been achieved and which cost of production is still higher than that of conventional and non-conventional sources of protein. So, it's needed to indicate a culture media for S. platensis to spare the high cost of inorganic media. So, attempt has been made to find out any inexpensive organic media containing high amount of protein for *Spirulina* culture. Therefore, the present work has been undertaken to study the growth performance of S. platensis in various concentrations of DRGM in replacement of KM.

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.

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, 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). (Table 1).

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 (NO3-N) and phosphate-P (PO4-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).

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 photometer (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 photometer (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).

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

Rotten guava medium and kosaric medium (KM) were prepared for culture of *Spirulina platensis*. Simultaneously, Kosaric medium (KM) was prepared for S. platensis culture as a control. Compositions of Rotten Guava Medium (RGM) and Kosaric medium (KM) were prepared for culture of *Spirulina 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 were taken in 1.0 L flask with three replications. Different concentration and composition of rotten guava medium and kosaric medium are shown in the table 2.

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 (Table 2). 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.

Table 1: Composition of Kosaric medium (Modified after Zarrouk's, 1996) for *Spirulina platensis* culture

Sl. No.	Chemicals/compounds	Concentration in stock solution g/l
1	$NaHCO_3$	9.0
2	$K2HPO_4$	0.250
3	$NaNO_3$	1.250
4	$K2SO_4$	0.50
5	NaCl	0.50
6	$MgSO_47H_2O$	0.10
7	$CaCl_2$	0.02
8	$FeSO_42H_2O$	0.005
9	A5 micronutrient solutiona	0.5ml/L
	a) A5 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

For the preparation of Kosaric medium, the above-mentioned amount (Table 1) of ingredients from no. 1 to 8 was 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 2). Four treatments, three from supernatant of DRGM for their different concentrations (20%, 40% and 60%) and one KM as control each with three replications were used to grow microalgae, S. platensis in 1.0 L volumetric flask. *Spirulina* was inoculated into each culture flask to

Table 2: Experimental design for *Spirulina platensis* culture using supernatant of three different concentrations of digested rotten guava (DRG)

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		-	14

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

produce a culture containing 10% *spirulina* suspension (Optical density at 620 nm = 0.20) (Habib, 1998). Twenty ml of *spirulina* suspension needed for getting the required density. All the flasks were kept under fluorescent lights (TFC, FL-40 SD/38-day light, Taiwan) in light: dark (12h: 12h) conditions in live food culture laboratory. These culture flasks were continuously aerated using electric aquarium aerator (SB-348A). Seven sub-samplings (15ml vial) (Table-5) was carried out at every alternative day from each flask to record dry cell weight and chlorophyll a content of *spirulina*, and properties of culture media. All the glassware used in the experiment was sterilized with dry heat at 70°C overnight.

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 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.

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).

RESULTS

Proximate composition of DRGM

Moisture of *Spirulina* grown in the supernatant of three different digested rotten guava (DRG) and Kosaric medium was varied from 9.44 to 9.55 %. The moisture content (%) was suitable for the preservation of the samples for future analysis.

There was no significant variation among the crude protein of *Spirulina* grown in the supernatant of three different DRG (Table 3). The percentage of crude protein of *Spirulina* was 53.25±0.32, 53.35±0.34 and 53.28±0.32% when grown in the supernatant of 20, 40 and 60% DRG media, respectively.

Crude lipids (%) of *spirulina* cultured in supernatant of 20% (10.10± 0.16%), 40% (10.14 ± 0.17%) and 60% (10.15 ± 0.14%) DRG varied significantly ($P < 0.05$) from that of *spirulina* grown in the Kosaric medium (6.30 ± 0.22%) (Table 3). Ash (%) of *Spirulina* grown in Kosaric medium (13.10 ± 0.12%) had significant ($P<0.05$) difference from that of *spirulina* cultured in supernatant of 20% (10.22 ± 0.13%) 40% (10.16 ± 0.17%) and 60% (10.33 ± 0.21%) digested rotten guava. Nitrogen free extract (%) of *spirulina* cultured in the supernatant of 20% (16.28 ± 0.32%), 40% (16.10 ± 0.18%) and 60% (15.99 ± 0.22%) digested rotten guava (DRG) varied significantly ($P<0.05$) from that of *Spirulina* grown in Kosaric medium (12.02 ± 0.28%) (Table 3). Very small amount of crude fibre (%) was found in *spirulina* grown in the supernatant of three different digested rotten guava (DRG), and Kosaric medium (Table 3).

Table 3: Experimental design for *Spirulina platensis* culture using supernatant of three different concentrations of digested rotten guava (DRG)

Treatments	T1 (20% DRG)	T2 (40% DRG)	T3 (60% DRG)	T4 (KM)
Moisture	9.44 ± 0.05	9.55 ± 0.04	9.53 ± 0.05	9.50 ± 0.06
Crude Protein	53.25 ± 0.32^b	53.35 ± 0.34^b	53.28 ± 0.32^b	58.36 ± 0.32^a
Crude Lipids	10.10 ± 0.16a	10.14 ± 0.17^a	10.15 ± 0.14^a	6.30 ± 0.22^b
Ash	10.22 ± 0.13^b	10.16 ± 0.17^b	10.33 ± 0.21^b	13.10 ± 0.12^a
NFE*	16.28 ± 0.32^a	16.10 ± 0.18^a	15.99 ± 0.22^a	12.02 ± 0.28^b
Crude Fibre	0.70 ± 0.04	0.69 ± 0.03	0.71 ± 0.04	0.71 ± 0.03

**NFE (Nitrogen Free Extract) = 100 - (Moisture + Crude protein + Crude lipids + Ash). Figures in common letters in the same row do not differ significantly at 5% level of probability.*

Physico-chemical properties of different media of supernatant of digested rotten guava contained *spirulina* (*Spirulina platensis*) culture

Light intensity

Light intensity was varied slightly in different days in all the four culture media contained *spirulina* (Fig.1). However, light intensity (lux/m^2/s) was varied from 2750 ± 21 lux/m^2/s on first day to 2768 ± 25 lux/m^2/s on 8th day of culture with slight variation in other days when *spirulina* grown in supernatant of 20% digested rotten guava (DRG). It was varied from 2750 ± 28 lux/m^2/s on first day to 2765 ± 31 lux/m^2/s on 10th day of experiment when *spirulina* cultured in supernatant of 40% DRG. Similarly, it was observed 2750± 28 lux/m^2/s on the first day and 2765 ± 31 lux/m^2/s on 10th day of experiment when *spirulina* grown in supernatant of 60% DRG. Light intensity was found to be 2750 ± 28 lux /m^2/s on first

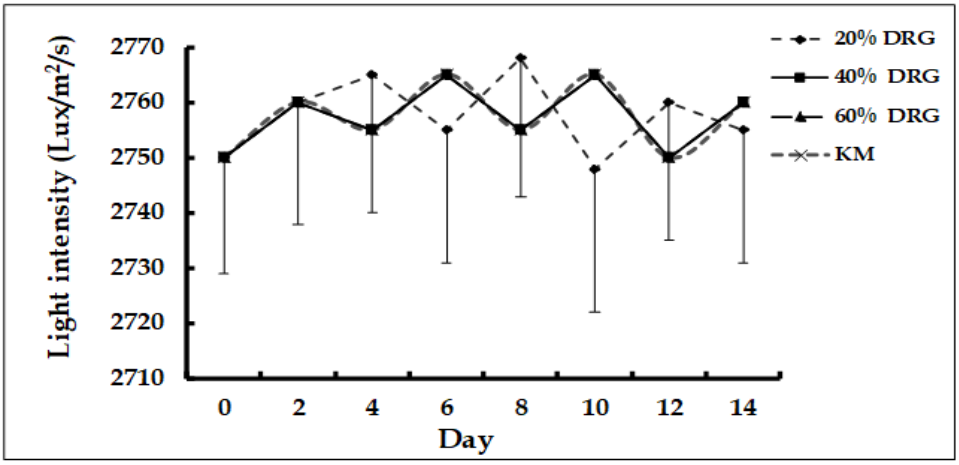


Figure 1: Mean values of light intensity (Lux/m2/s) during culture of *Spirulina platensis* in supernatant of three different digested rotten guava, and Kosaric medium. Vertical bars represent standard errors.

day when *spirulina* grown in Kosaric medium and 2765 ± 16 lux /m²/s on 10th day of experiment (Fig.1).

pH

The pH values were above 8.1±0.10 in all the media which were highly alkaline suitable for *spirulina* culture. The trends of fluctuation pH are shown in Fig.2. During the 14 days experiment, it was increased from 8.10±0.10 on first day to 10.60±0.30 on 10th day of experiment when *spirulina* cultured in supernatant of 20% digested rotten guava (DRG) and then it was decreased to 8.30± 0.20 on last day (14th day) of experiment. It was found 8.10±0.10 on the first day which was increased to 10.70 ±0.30 on 10th day of experiment when *spirulina* grown in supernatant of 40% DRG and then decreased up to 14th day (8.40±0.20) of experiment. Similar trend of fluctuation of pH observed when *spirulina* cultured in supernatant of 60% DRG (Fig.2). Similarly, it was found 8.60±0.36 on first day of experiment and the increased up to 10th day (10.60±0.30) of experiment, and then

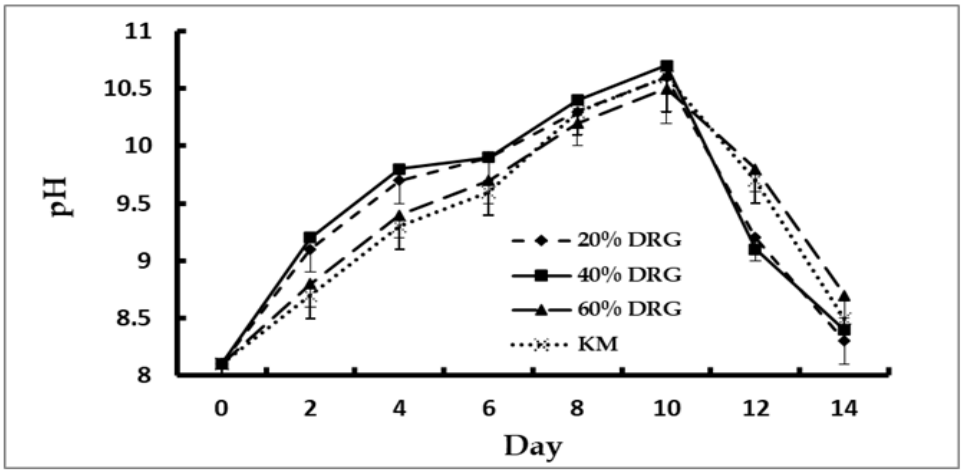


Figure 2: Mean values of pH of culture during *Spirulina platensis* in Supernatant of three different digested rotten guava media, and Kosaric medium. Vertical bars represent standard errors

decreased up to the last day (14th day) of experiment when *spirulina* grown in Kosaric medium (Fig.2).

Alkalinity

Total alkalinity of all the media contained *spirulina* was found high during the experiment (Fig.3). However, it was found lowest (2244±95 mg/L) on first day and highest (2675±143 mg/L) on 10th day of experiment and then gradually decreased (2130±175 mg/L) up to 14th day (last day) of experiment with little fluctuations when *spirulina* cultured in supernatant of 20% digested rotten guava (DRG). Total alkalinity was recorded 2262±92 mg/L on first day of experiment and increased up to 10th day (2698±123 mg/L) with decreased values up to 14th day (2244 ± 100 mg/L) of experiment when *spirulina* cultured in supernatant of 40% DRG. It was found around 2294±92 mg/L on first day which was increased up to 2695±123 mg/L to 10th day of experiment, and then decreased up to 14th day (2274±100 mg/L) of experiment when *spirulina* grown in supernatant of 60% DRG.

The concentrations of total alkalinity was found 2324±180 mg/L on first day and then increased up to 6th day (3048±260 mg/L) of experiment and then decreased up to 14th day (1522±120 mg/L) of experiment (Fig.3).

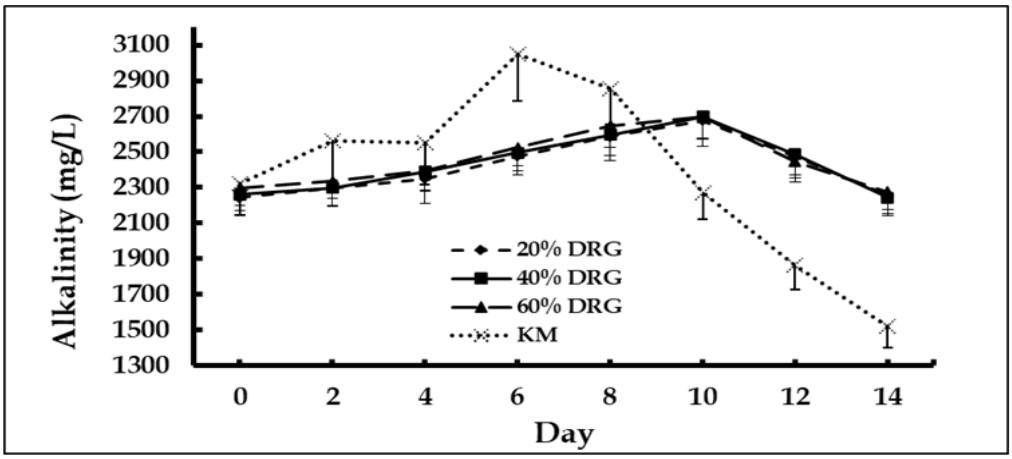


Figure 3: Mean values of alkalinity (mg/L) during culture of *Spirulina platensis* in supernatant of three different digested rotten guava, media, and Kosaric medium. Vertical bars represent standard errors

Nitrate-N (NO3-N)

Nitrate N (Available N) of three supernatant of digested rotten guava and Kosaric medium was recorded (Fig.4). 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). 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. 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.4). DRG. There was no definite trend of

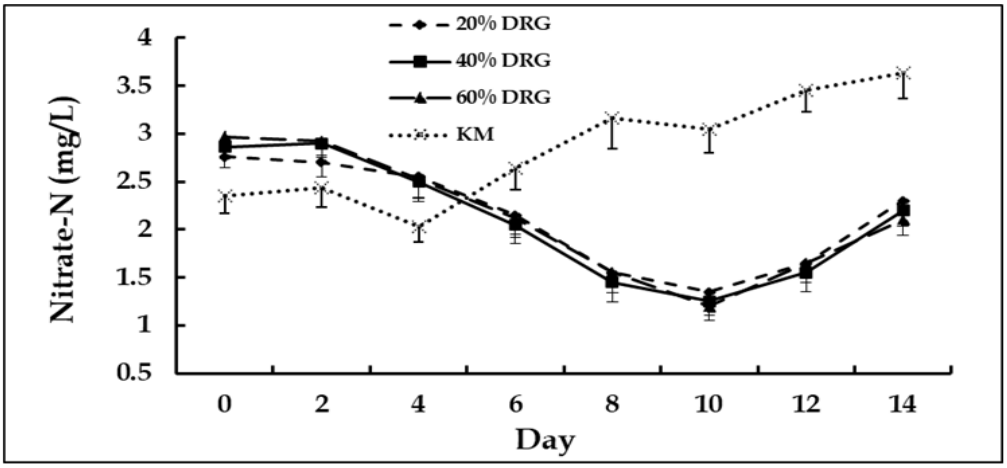


Figure 4: Mean values of nitrate-N (mg/L) during culture of *Spirulina platensis* in supernatant of three different digested rotten guava, media, and Kosaric medium. Vertical bars represent standard errors.

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.4).

Cell weight of *spirulina*

Cell weight (mg/L) of *spirulina* cultured in all the media was found higher on 10th day of culture than other days (Fig.5). Cell weight of *spirulina* increased from initial day (first day) up to 10th day (0.815±0.0015 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 *spirulina* was found to be 0.809± 0.0012g/L when grown in 40% DRG (Appendix 6, Fig. 8). Cell weight of *spirulina* increased from initial day (first day) (0.0023±0 g/L) up to 10th day (0.818±0.0013g/L) of culture in 60% DRG, and then decreased up to 14th day (0.725±0.0011g/L) of experiment. Highest cell weight of Kosaric medium contained *spirulina* was 12.43 ±0.21g/L on 10th day and then decreased up to 14th day (3.44±0.021) of experiment (Fig.5).

DISCUSSION

The cell weight of *Spirulina platensis* in supernatant of digested rotten guava were found 0.0023 to 0.815 mg/L in 20% digested rotten guava media (DRGM), 0.0024 to 0.809 mg/L in 40% DRGM, 0.0023 to 0.818 mg/L in 60% DRGM and 0.0023 to 12.43 mg/L in KM. The growth performance of *Spirulina platensis* in supernatant of 60% DRGM was found better than 20% and 40%

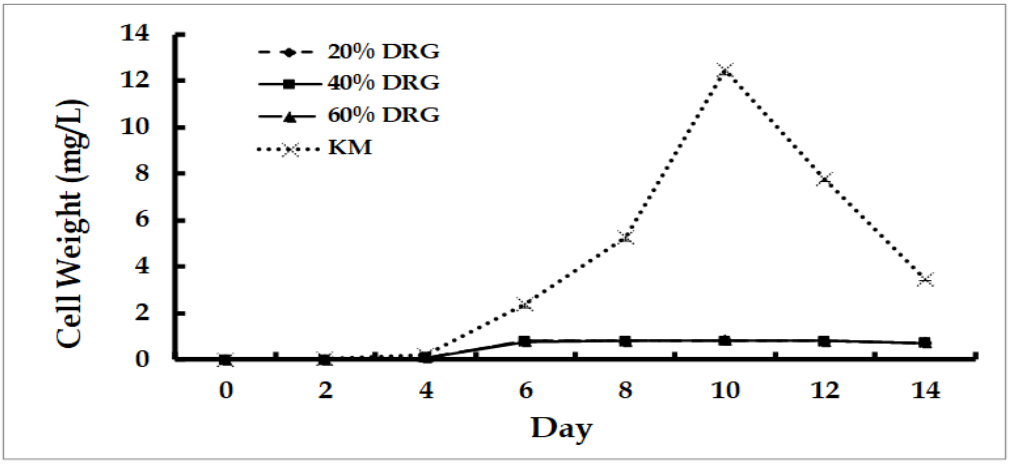


Figure 5: Mean values of cell weight (mg/L) of *Spirulina platensis* grown in supernatant of three different digested rotten guava, and Kosaric medium. Vertical bars represent standard errors.

DRGM. This variation might be due to the differences in nutrient concentrations and composition of varied media. In controlled KM *Spirulina platensis* showed the highest growth performance. It may be happened due to suitability and availability of the nutrients for the growth of the species. Habib and Kohinoor (2018) found that supernatant of 45% digested poultry waste gave very good growth of *spirulina* than other lower concentrations. In the present study, 40% DRGM showed lower growth performance of *Spirulina platensis* in relation to 20 and 60% DRGM. This might be due to lower nitrogen and phosphate concentration. The concentration of 60% DRGM revealed better growth performance as the required level of nutrient content was availed. The comparative study of growth performance of *Spirulina platensis* in different concentration of the media indicates higher dilution followed lower concentration of nutrients and lower growth performance.

During the present study, *spirulina* grew well in kosaric medium, 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 *Spirulina platensis*, the exponential phase was found up to 10th day from the beginning and then the cell weight declined i.e. stationary phase started. The physical properties such as light intensity, aeration and temperature played a significant role to the whole culture system. During the culture system the climate condition was more or less suitable and less suitable and favorable for the growth of S. platensis. Satter (2017) 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) 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 physicochemical properties viz. temperature (30.06 ^{o}C), 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) 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.

Zarrouk (1996) conducted an experiment the pioneer in detailed study on the response of *Spirulina platensis* to light. In his simple experiment, he reached a conclusion that the highest growth of *Spirulina platensis* was saturated at the level of 2500-3000 lux/m^2/s. The highest growth of *Spirulina platensis* in the present study was found at light intensity of 2710 lux/m^2/s and 2740 lux/m^2/s at 5g/L concentration of the media and KM on the 10th day of culture. This variation might be due to difference in space and difference of light source.

In the present study, supernatant of digested rotten guava was used as a media of three concentrations for the culture of *Spirulina platensis*. 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), Satter (2017). The availability of phosphate-phosphorus has been considered very important in cultured media of plankton production. The range of light intensities was 2748 ton2768 lux/m^2/s during culture period. The ranges of temperature were 19.0 to 22.2°C during the culture period. The highest temperature was recorded 22.2°C in the KM on the initial day of culture. 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

In this experiment, the growth performance of *Spirulina* was observed in different concentrations of digested rotten guava (DRG) and Kosaric Medium (KM). 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 results showed that the growth performance of *spirulina* in supernatant of 60% DRGM was significantly ($P<0.01$) higher than that of *spirulina* grown in the supernatant of 20% and 60% DRGM. It indicates that, the different concentration of digested rotten guava (20%, 40%, 60%) has potential to increase the growth rate of *Spirulina*. This medium may be used commercially and economically after screening and certain period of aeration for mass culture of *Spirulina platensis*, as the collection and preparation of these organic media required little cost, less labor and is available throughout Bangladesh. However, it might be suggested that more research and insights is needed to account cost-benefit analysis for evaluating the grow-out potential of *Spirulina* in lab-based cultivation.

Conflict of Interest

There is no conflict of interest.

REFERENCES

Bhat, V.B., & Madyastha, K.M. (2000). Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: protection against oxidative damage to DNA. *Biochemical and Biophysical Research Communications, 285*(2), 262–266.

Borowitzka, M.A. (1999). Commercial production of microalgae: ponds, tanks, tubes and fermenters. *J. Biotechnol., 70*, 313–321.

Clesceri LS, Greenberg AE and Trussell RR (1989). 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. 10-203.

Cohen, Z., & Vonshak, A. (1991). Fatty acid composition of *Spirulina* and *Spirulina*-like cyanobacteria in relation to their chemotaxonomy. *Phytochemistry, 30*, 205.

Cysewski, (1983). Hawaiian *Spirulina*: Superfood for Super Health 73-4460 Queen Kaahumanu Highway, Suite 102, Kailua-Kona, HI 96740 USA.

Dineshkumar R, Narendran R & Sampathkumar P (2016). Cultivation of *Spirulina platensis* in different selective media. *Indian Journal of Marine Science, 45*(12), 1749-1754.

Habib MAB (1998). Culture of selected microalgae in rubber and palm oil effluents and their use in the production of enriched rotifers. Doctoral Thesis, University of Putra. Malaysia. pp. 532.

Habib MAB & Kohinoor AHNM (2018). 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. chapter-2, 66-70.

Habib, M.A.B., Parvin, M., Huntington, T.C., & Hasan, M.R. (2008). *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. 33 pp.

Habib MAB, Yusoff FM, Phang SM & Mohamed S (1997). Nutritional values of chironomid larvae grown in palm oil mill effluent and algal culture. *Aquaculture, 158,* 195-205.

Habib, M.A.B., Yusoff, F.M., Phang, S.M., & Mohamed, S. (2003). Growth and nutritional values of Moina micrura fed on Chlorella vulgaris grown in digested palm oil mill effluent. *Asian Fisheries Science, 16*(1-2), 107-119.

Horwitz W (1984). *Official Methods of Analysis of the Association of Official Analytical Chemists.* 14th Edition. Association of Official Analytical Chemists, Washington DC. USA. pp. 1018.

Illman, A.M., Scragg, A.H., and & Shales, S.W. (2000). Increase in Chlorella strains calorific values when grown in low nitrogen medium. *Enzyme Microb. Technol., 27*, 631–635.

Kato, T. (1991). Chemistry of microalgae and their application to food. *Food Chemistry, 8*, 30-35.

Madhava, K.M., & Bhat, V.B. (2000). Scavenging of peroxy-nitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: protection against oxidative damage to DNA. *Biochemical and Biophysical Research Communications, 13285*(2), 262–266.

Manigandan M (2014). Mass cultivation and determination of biochemical composition of *Spirulina platensis* in three different media. *International journal of Pharmacology and Bio Science, 5*(3) 847-854.

Muller-Feuga, A. (2000). The role of microalgae in aquaculture: situation and trends. *J. Appl. Phycol., 12*, 527–534.

Phang, S.M., Miah, M.S., Chu, W.L., & Hashim, M. (2000). *Spirulina* culture in digested sago starch factory waste water. *J. Appl. Phycol., 12*, 395–400.

Richmond, A. (2004). *Handbook of microalgal culture: biotechnology and applied phycology*. Blackwell Science, Oxford.

Satter A (2017). 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. pp. 143.

Sharker MGU (2002). 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. pp. 58.

Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. *J. Biosci. Bioeng., 101*, 87–96.

Sukumaran P, Nulib R, Halimmon N, Simoh S, Omar H & Ismail A (2018). Formulation of cost-effective medium using urea as a nitrogen source for Arthrospira platensis cultivation under real environment. *Annual Research and Review in Biology. 22*(2) 1-12.

Zar JH (1984). Biostatistics. Prentice-Hall Inc., Englewood Cliffs, New Jersey, USA. pp. 718.

Zarrouk C (1996). 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. pp. 412.thesis, University of Paris, France. pp. 412.