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Research Article

Use of liquid rice starch as a source of carbon for growth of *Spirulina platensis*

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ABSTRACT

A study was conducted to examine the culture and growth performance of *S. platensis* in three different concentrations (25%, 50% and 100%) of digested liquid rice starch media (DLRSM) with 0.2 g/L urea and Kosaric medium (KM) as control for a period of three months from March to May 2018. Each experiment was done in triplicates for a period of 14 days. The growth rate of *S. platensis* was found to vary with different concentrations of the medium. The cell weight of *S. platensis* was attained to highest 12.42 mg/L (dry weight) in KM followed by 11.26 mg/L, 8.35 mg/L and 9.11 mg/L in 25%, 50% and 100% of DLRSM, respectively on the 10^{th} day of culture period. Similar trend also observed in case of chlorophyll a content of *S. platensis.* The proximate composition of rice starch was analyzed. The percentage of moisture, protein, lipid, ash, crude fiber and nitrogen free extract (NFE) were95.1, 0.2616, 0.1055, 0.1097, 0.1468 and 3.9669, respectively on the basis of moisture (%) and 4.59, 5.70, 2.30, 2.39, 3.20 and 86.41, respectively on the basis of dry matter (%). The results on the growth performance showed that the growth of *S. platensis* was significantly ($P<0.01$) higher when grown in 25% concentration of DLRSM than other concentrations in 50% and 100% of DLRSM. The physico-chemical parameters viz. light intensity (2685 to 2773 lux/m^2/s), temperature (29.6 to 31.5°C), pH (8.3 to 9.9), alkalinity (1150 to 3032 mg/L), Nitrate-N (1.32 to 6.30 mg/L) and phosphate-P (9.75 to 61.50 mg/L) were within optimum level during the culture period.

INTRODUCTION

Rice starch is one of the most available nutrients which easily founded in Bangladesh. Liquid rice starch is produced about billion liters in a year. This waste is easily available all over the country in all the time. Therefore, this inexpensive waste material may be used to produce *S. platensis* culture which can contribute significantly for the development of fisheries and fish production. For centuries, civilizations of the world over have cultivated and cherished *Spirulina* for its health-improving benefits (Habib, 1998). The Aztecs harvested the microalgae from Lake Texcoco in Mexico. The native people in Chad, Africa, have used the microalgae as a staple of their daily diet because of its concentrated nutritional value and prolific growth in the pure saltwater lakes of the region. *Spirulina* is most commonly found in natural lakes having high pH value i.e. 8 to 10 all over the earth. *Spirulina* has been consumed from a very long past time in many parts of the world as a food supplement for human as well as animals in various forms like healthy drink, tablets and powder etc. because of its alimentary value (Ruiz *et al*., 2003). Now-a-days, *S. 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 and Vonshak, 1991; Bhat and Madyastha, 2000; Madhava *et al*., 2000).

Microalgae play an important role in the oxygen as well as carbon dioxide balance in the water. It also acts as an ideal waste remover in nature (Redalije *et al.,* 1989). It acts not only on agro-industrial but also animal wastes as well by converting them into food materials. *Spirulina* spp. is multicellular, blue green algae. They are very small a microscopic and 300-500 micrometer in length. However, according to the researchers, one kg of *Spirulina* spp. is similar to 1000 kg of other vegetables (Kato, 1991). *Spirulina* contains 50-70% protein, 10-12% carbohydrate, 6% fat, 7% minerals and a lot of vitamins. *Spirulina* is made

of between 55 - 70 % protein (more than beef, chicken and soybeans), all the essential and 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 this blue-green microalgae. These nutrients are lacking in most diet.

The microalgae *S. platensis* are filamentous, helicoidally and also cosmopolitan in nature. They are usually very small and microscopic and 300 to 500 micro meter. It flourishes very well in alkaline, saline waters where the pH (9-11) too high for most of the species to thrive in. Starch is the principal storage polysaccharides produced by green plants. Starch consists of two D-glucose polymers: amylose and amylopectin. Amylose has a linear structure with α-1, 4-linked glucan, while amylopectin is a highly branched molecule characterized by α -1, 4-linked and α -1, 6 branching links (Perez and Bertoft, 2010). In food industries it performs various functions as thickener, binder, disrupting agent, stabilizer, texture modifier, gelling and bulking agent, useful in the preservation of canned and frozen foods, in the formulation of syrups, essences 1423 and beverages, in confectionery and bakery, snacks and marshmallows (Burrell and Copeland, 2003).

The cell walls of *Spirulina* consist of polysaccharide which has a digestibility of 86% and can be easily absorbed by human body. Feeding on *Spirulina* helps to improve disease resistance of high valued fish resulting in an increase in their survival rate for 15 to 30%. Therefore, to accelerate the development of aquaculture industry, it is important to culture *Spirulina* can be easily understood. The ultimate goal of this experiment is to develop low cost media for large scale production of *Spirulina.*

The present study was conducted to observe the culture and growth performance of *S. platensis* in digested liquid rice starch media to evaluate the culture and growth performance of *S. platensis* in digested liquid rice starch media (DLRSM); and to analyze the proximate composition of *S. platensis* that grown in DLRSM.

MATERIALS AND METHODS

Study Area

The experiment was conducted in Live Food Culture Laboratory, Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh for a period of three months from March to May, 2018.

Culture of Microalgae

Selection and collection of liquid rice starch

The liquid rice starch was selected as media for *S. platensis culture*. The liquid rice starch was collected into 250ml conical flask from Shahed Shamsul Haque Hall (SSHH) dining of Bangladesh Agricultural University, Mymensingh-2202.

Analysis of proximate composition of liquid rice Starch

The proximate composition of any media means moisture, ash, protein, lipid, crude fiber, and carbohydrate. The media was liquid and the main chemical elements of LRS were moisture, ash, protein, lipid, crude fiber, carbohydrate and nitrogen free extract (NFE) were analyzed in triplicates following the standard methods (AOAC, 2016; Rahman *et al*., 2015; Bhuiyan *et al*., 2018; Yeasmin *et al*., 2018). All of these were analyzed by using equipment's in the laboratory of the Fish Nutrition, Faculty of Fisheries, Bangladesh Agricultural University; Mymensingh. (Table 1).

Moisture

The moisture content of samples are determined by the methods of AOAC (2016); Rahman *et al*. (2015); Bhuiyan *et al*. (2018); Yeasmin *et al*. (2018) (Table 1).

Crude protein

The crude protein content of samples are determined by the methods of AOAC (2016); Rahman *et al*. (2015); Bhuiyan *et al*. (2018); Yeasmin *et al*. (2018) (Table 1).

Crude lipid

The crude lipid content of samples are determined by the methods of AOAC (2016); Rahman *et al*. (2015); Bhuiyan *et al*. (2018); Yeasmin *et al*. (2018) (Table 1).

Ash

The ash content of samples are determined by the methods of AOAC (2016); Rahman *et al*. (2015); Bhuiyan *et al*. (2018); Yeasmin *et al*. (2018) (Table 1).

Table 1. Proximate composition of liquid rice starch

Name of the component (RS)	Moisture basis (%)	Dry matter basis (%)
Moisture	95.41	4.59
Protein	0.2616	5.70
Lipid	0.1055	2.30
Ash	0.1097	2.39
Crude fiber	0.1468	3.20
Carbohydrate	3.9669	86.413

Analysis of physico-chemical properties of liquid rice starch

Physico-chemical 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 poultry waste were analyzed in the laboratories of Live Food Culture, Nutrition and Water Quality of the Faculty of Fisheries, BAU, Mymensingh.

pH

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

Total suspended solids and total dissolved solids

This was analyzed by using the following method methods and procedure (HP Module, 1999).

Alkalinity

This was analyzed by using the following method - (APHA, 1976) and using alkalinity test kit (Thermo Fisher Scientific Company, 2008).

Nitrate-N (Available N)

This was analyzed by using Nitrite Nitrogen test kit (LR Phosphate, Model HI 93713, HANNA) through standard methods. Colorimetric Method (American Public Health Association, 1998), and in EPA method 354.1 (EMSL-Ci, 2003) to determine nitrite ion in waters as well as Nitrite nitrogen the following method - (APHA, 1976).

Phosphate-P (Available P)

This was analyzed by using Molybdenum blue method and with the help of phosphorus meter (LR Nitrate, Model HI 93713, HANNA) (WTW, 2003).

Culture and collection of S. platensis

Microalgae, *S. platensis was* collected from the imported stock of Dr. Md. Ahsan Bin Habib, Professor, Department of aquaculture, Bangladesh Agricultural University, Mymensingh-2202.

Maintenance of pure stock culture of S. platensis

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

Preparation of digested liquid rice starch media (DLRSM) and Kosaric medium (KM)

50 ml/L dry liquid rice starch was allowed to decompose in 5.0 L glass bottle for 32 days under aerobic condition in the Live Food Culture laboratory, Department of Aquaculture, BAU, Mymensingh. Then an off white colored supernatant from bottle was diluted and made three concentrations at the rate of 25, 50 and 100% digested liquid rice starch. Then the supernatant of three different concentrations were taken in 1.0 L flask with three replications. Simultaneously, Kosaric medium (KM) was prepared for *S. platensis* culture as a control. Compositions of Rice Starch Medium (RSM) and Kosaric Medium (KM) were prepared for culture of *S. platensis*. Different concentration and composition of rice starch medium and kosaric medium are shown in the table 2 and 3 respectively.

Table 2: Concentration of digested liquid rice starch medium

Rice starch ingredients	Concentration or dilution of DLRSM (%)
Digested liquid rice starch medium (DLRSM)	25
Digested liquid rice starch medium (DLRSM)	50
Digested liquid rice starch medium (DLRSM)	100

For the preparation of liquid rice starch medium, digested and continuous aeration 5 liter volumetric flask added 4 liter distilled water with 250 ml rice starch was filtered with plankton net after (date: 18.02.18 to 22.03.2018) 32 days left. Then the filtered rice starch 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^{o}Cfor 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 3. Composition of Kosaric medium (Modified after Zarrouk's, 1966) for *S. platensis* culture

Chemicals/compounds	Concentration in stock solution g/l
$NaHCO_3$	9.0
K_2HPO_4	0.250
$NaNO_3$	1.250
K_2SO_4	0.50
NaCl	0.50
$MgSO_47H_2O$	0.10
$CaCl_2$	0.02
$FeSO_42H_2O$	0.005
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

For the preparation of Kosaric medium, the above mentioned amount (Table 3) of ingredients from no. 1 to 8 was weighted by the help of electric balance and took in a 1.0 L conical flask. Then 0.5ml micronutrient solution was pipetted in the flask and distilled water was added to make the volume 1.0LMixing, autoclaving and cooling were carried out pursing the procedure used during the preparation of DLRSM.

Experimental design of S. platensis culture

Three types media viz., Rice starch media (RSM) and Kosaric medium (KM) were used to culture *S. platensis* (Table 4).

Table 4: Experimental design

Types of medium	Treatments	Replications	Amounts liquid rice starch (%)	Duration of culture (days)
Supernatant of DLRSM	T_1	3 (101, 102 and 103)	25	14
	T_2	3 (201, 202 and 203)	50	
	T_3	3 (301, 302 and 303)	100	
Kosaric Medium(KM)	T_4	3 (KM-1, KM-2 and KM-3)	-	14

Culture of S. platensis in supernatant of digested liquid rice starch media (DLRSM) and Kosaric Medium

Four treatments, three from supernatant of DLRSM for their different concentrations (25%, 50% and 100%) 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 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 was 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^{o}C overnight.

Table 5: Collection of Sample in 15 ml of plastic vial with every alternate day

Day	Date	KM (ml)	Treatment-1 (Replication, vial ml)	Treatment-2 (Replication, vial ml	Treatment-3 (Replication, vial ml
2	05/04/2018	KM-1, 15	101, 15	201, 15	301, 15
4	07/04/2018	KM-2, 15	102, 15	202, 15	302, 15
6	09/04/2018	KM-3, 15	103, 15	203, 15	303, 15
8	11/04/2018	KM-1, 15	101, 15	201, 15	301, 15
10**	13/04/2018	KM-2, 15	102, 15	202, 15	302, 15
12	15/04/2018	KM-3, 15	103, 15	203, 15	303, 15
14	17/04/2018	KM-1, 15	101, 15	201, 15	301, 15

**[KM] and 1 no. are same grow and high growth at 10th days

Estimation of cell weight (dry weight) of Spirulina

The cell weight of *Spirulina* was determined by the method of Clesceri *et al*. (1989).

Estimation of chlorophyll a of Spirulina

The chlorophyll a of *Spirulina* was determined by the method of Clesceri *et al*. (1989).

Total biomass of Spirulina (S. platensis)

Total biomass was calculated using the following formula given by Vonshak and Richmond (1988).

Total biomass = Chlorophyll a × 67

Specific growth rate (SGR) on the basis of dry weight, chlorophyll a content and total biomass of *Spirulina* (Clesceri *et al.,* 1989)

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

SGR (μ/day) = In $(X_1-X_2)/t_1-t_2$

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 (μ/day) = In $(X_1-X_2)/t_1-t_2$

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 (μ/day) = In $(X_1-X_2)/t_1-t_2$

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.

Analysis of Physico-chemical parameters of culture media

Physical parameters

Temperature

Water temperature (^{o}C) of the culture media was measured during the time of sampling day by a Celsius thermometer.

Light intensity

Light intensity (lux/m^2/s) was measured during sampling day by using a lux-meter [digital instrument, Lutron (LX-101)].

Chemical parameters

pH, Alkalinity, Available N and Available P

The chemical parameters such as pH, Total solids (TSS+TDS), alkalinity, nitrate-N, phosphate-P and Total N of the culture media were recorded following the procedures given by Clesceri *et al*., (1989) in the laboratory.

Analysis of proximate composition Spirulina

The chemical parameters such as moisture, crude protein, crude lipid, ash, crude fibre and NFE of the culture media were recorded following the procedures given by AOAC (2016); Rahman *et al*. (2015); Bhuiyan *et al*. (2018); Yeasmin *et al*. (2018) and Clesceri *et al*. (1989) in the laboratory.

Statistical analysis

Data of cell weight, chlorophyll a, total biomass, and specific growth rates in respect to dry cell weight, chlorophyll a, total biomass and proximate composition of *Spirulina* in respect to four treatments were analyzed following one ways Analysis of Variance (ANOVA) and their significant differences using Turkey's test followed

Duncan's New Multiple Range (DNMR) test at 5% level of probability (Zar, 1984).

RESULTS

Physico-chemical characteristics of Liquid Rice Starch

Color, odour and structure

The color of the liquid rice starch was off white color with good smell (odour). The structure was almost semi-liquid (Table 6).

Temperature (°C)

Temperature of liquid rice starch was little bid higher than normal ambient temperature. It was ranged from 28.50 to 28.90ºC (Table 6).

Total solid (TSS + TDS)

Total solid is the addition of total suspended solids and total dissolved solids of liquid (rice starch) which was ranged from 1542-1620 mg/L (Table 6).

pH

pH of liquid rice starch was ranged from 6.80 to 6.95 which was alkaline in nature (Table 6).

Alkalinity

Alkalinity of digested liquid rice starch was quite high and ranged from 201 to 225 mg/L (Table 6).

Nitrate-N (NO_3-N)

Nitrate-N (Available N) of digested liquid rice starch was ranged from 1.20 to 1.35 mg/L (Table 6).

Phosphate-P (PO_4-P)

Phosphate-P (Available P) of the digested liquid rice starch was high and varied from 1.70 to 2.10 mg/L (Table 6)

Table 6: Characteristics of liquid rice starch just after collection

Characteristics of liquid rice starch (LRS)	Comments
Colour	Off white
Odour	Good
Structure	Semi-liquid
Temperature	28.50-28.90ºC
pH	6.80-6.95
Total solids (TSS + TDS)	1542-1620 mg/L
Alkalinity	201-225 mg/L
Total N	2.05-2.12 mg/L
Available N (NO_3-N)	1.20-1.35 mg/L
Available P (PO_4-P)	1.70-2.10 mg/L

Physico-chemical properties of supernatant of Digested Liquid Rice Starch (DLRS)

Temperature

Temperature of supernatant of digested liquid rice starch (DLRS) used to culture *Spirulina* was varied from 27.40 to 27.70ºC (Table 7).

pH

pH of supernatant of DRT used for *Spirulina* culture was found to range from7.10 to 7.20 (Table 7).

Total solid (TSS + TDS)

Total solid (TSS + TDS) of supernatant of DLRS used to culture *Spirulina* was reduced due to decomposition which was ranged from 135 to 177 mg/L (Table 7).

Alkalinity

Alkalinity of supernatant of DLRS used to culture *Spirulina* was high which was ranged from 150 to 170 mg/L (Table 7).

Nitrate N (NO_3-N)

Nitrate N (Available N) of supernatant of DLRS used for *Spirulina* culture was also high in amount and varied from 1.40 to 1.70 mg/L (Table 7).

Phosphate P (PO_4-P)

Phosphate P (Available P) of supernatant of DLRS used to culture *Spirulina* was very high and found to vary from 1.50 to 1.60 mg/L (Table 7).

Total N

Total N of supernatant of DLRS used for *Spirulina* culture was found also high in amount and ranged from 1.40 to 1.70 mg/L (Table 7).

Table 7: Physico-chemical properties of supernatant of digested liquid rice starch after 32 days digestion in aerobic condition

Characteristics of liquid rice starch	Comments
Temperature	27.40-27.70°C
pH	7.10-7.20
Total solid (TSS + TDS)	135-177 mg/L
Alkalinity	150-170 mg/L
Total N	1.40-1.70 mg/L
Available N (NO_3-N)	1.10-1.30 mg/L
Available P (PO_4-P)	1.50-1.60 mg/L

Proximate composition of rice starch on moisture and dry basis

Moisture

It was measured from rice starch which ranged from 95.41% (moisture basis) and 4.59% (dry basis) (Table 8).

Crude protein

Crude protein of rice starch was varied from 0.2616% (moisture basis) and 5.70% (dry basis) (Table 8) which quite in percent.

Crude lipid

Crude protein was very low in amount in digested rice starch and ranged from 0.1055% (moisture basis) and 2.30% (dry basis) (Table 8).

Ash

Ash of rice starch was ranged from 0.1097% (moisture basis) and 2.39% (dry basis) (Table 8).

Crude fibre

Crude fibre in rice starch was not high in amount but ranged from 0.1468% (moisture basis) and 3.20% (dry basis) (Table 8).

Nitrogen free extracts (NFE)

The NFE of rice starch was high in amount and varied from3.9669% (moisture basis) and 81.92% (dry basis) (Table 8).

Table 8: Proximate composition (%) of rice starch on the basis of moisture and dry weight.

Composition	Moisture basis (%)	Dry basis (%)
Moisture	95.41	4.59
Crude protein	0.2616	5.70
Crude lipids	0.1055	2.30
Ash	0.1097	2.39
Crude fiber	0.1468	3.20
NFE*	3.9669	81.92

*NFE (Nitrogen Free Extract) = 100 - (Moisture + Crude protein + Crude lipids + Ash).

Physico-chemical properties of different Spirulina (S. platensis) media

Light intensity

Light intensity (lux/m^2/s) was varied from 2715± 30 on first day to 2765 ± 27 lux/m^2/s on the last day with slight variation T_1. It was varied from 2715 ± 25 on first day to 2740 ± 25 lux /m^2/s on the last day in T_2. Similarly, it was observed 2685± 27 on the first day and 2738 ± 29 on the last day (14th day) in T_3. Light intensity was found to be 2705 ± 15 lux /m^2/s on 1stday and 2773 ± 21 lux /m^2/s on the last day (14th day) in T_4 (Fig. 1).

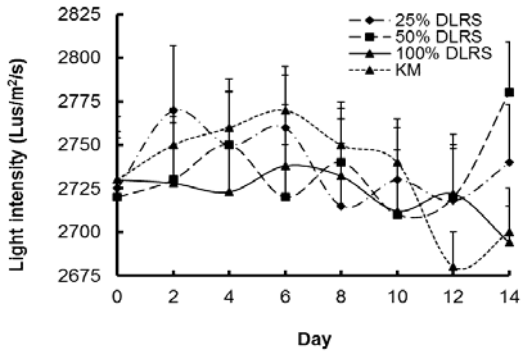

Fig. 1: Mean values of light intensity (Lux/m^2/s) during culture of *S. platensis*

Temperature

The temperature round in T_1 was found 30.3 ± 0.31°C (lowest) on the first day to 31.1 ± 0.37°C at the end (14th day) with slight rise on 2nd, 10th and 12th day of experiment. It was also followed; the similar trend of fluctuation from first 30.2 ± 0.25°C lowest to 31.1 ± 0.32°C last day in T_2 and It was found temperature variation from 29.6 ± 0.28°C lowest to 31.2 ± 0.45°C in T_3. But, it was recorded 30.3 ± 0.30°C lowest on the first day of experiment to 31.5 ± 0.38°C at the end of experiment in T_4 (Fig. 2).

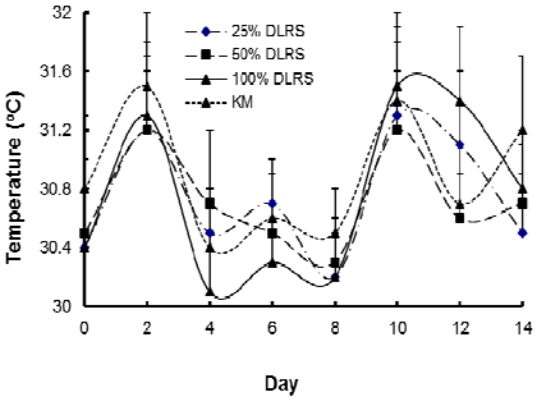

Fig. 2: Mean values of temperature (°C) during culture of *S. platensis*

pH

During the 14 days experiment, it was increased from 8.3 ± 0.21 on first day to 9.9 ± 0.23 on 10th day in T_1 and then it was decreased to 8.50 ± 0.21 on last day (14th day) of experiment. It was found 8.3 ± 0.21 on the first day which was increased to 9.3 ± 0.30 on 14th day of experiment T_2 and then decreased and increased on 14th day and again increased on the last day (14th day) of experiment. It was found 8.3 ± 0.21 on the first day which was increased to 9.8 ± 0.31 on 14th day in T_3 and then decreased and increased on 14th day and again increased on the last day (14th day) of experiment. Similar trend of fluctuation of pH observed in T_4 (Fig. 3).

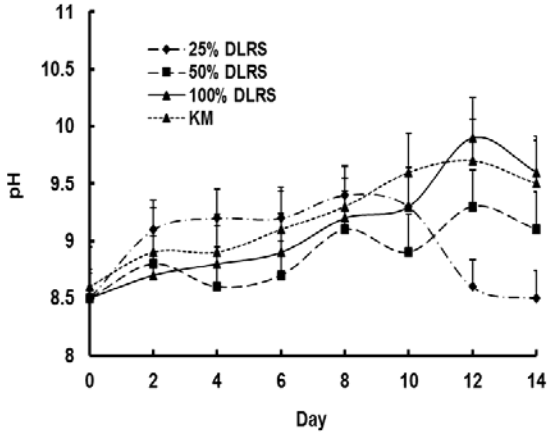

Fig. 3: Mean values of pH of culture during *S. platensis*

Alkalinity

It was found highest (2288 ± 112 mg/L) on 1st day of experiment and then gradually decreased (1150 ± 215 mg/L) up to 14th day (8th day) in T_1. Total alkalinity was recorded 1825 ± 163 mg/L on 1st day of experiment and increased up to 10th day (2010 ± 184 mg/L) with decreased value on 6th day (1725 ± 154 mg/L), and then decreased on 12th day and again increased on 14th day in T_2. It was found almost around 2125 ± 170 mg/L from first day to 4th day of experiment, decreased on 6th day (1575 ± 156 mg/L) and again increased on 8th day (2536 ± 230 mg/L) of experiment, and then decreased up to 14th day in T_3 (Fig. 4).

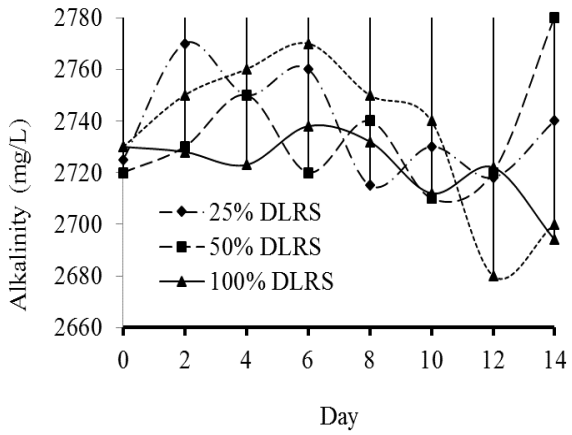

Fig. 4: Mean values of alkalinity (mg/L) during culture of *S. platensis*

Nitrate N (NO_3-N)

It was positively increased from 1.90 ± 0.18 mg/L (first day) to 3.40 ± 0.32 mg/L (6th day) of experiment and then decreased up to 14th day in T_1. The trend of nitrate-N was found to decrease from first day (2.06 ± 0.15 mg/L) to 8th day (1.32 ± 0.44 mg/L) of culture and then increased up to 14th day in T_2. Lowest amount of nitrate-N (1.44 ± 0.17 mg/L) was recorded and highest amount of nitrate-N (6.30 ± 0.50 mg/L) was found in T_3 on 14th day of culture. It was found lowest (1.32 ± 0.44 mg/L) in the T_2 on 8th day of culture and highest value was recorded in T_4 on 14th day of culture (Fig. 5).

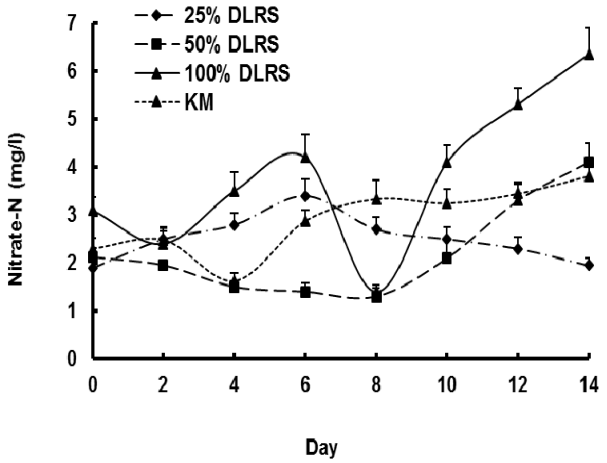

Fig. 5: Mean values of Nitrate-N (mg/L) during culture of *S. platensis*

Phosphate-P (PO_4-P)

Phosphate-P was high in amount in the media in first day of experiment and gradually decreased in amount up to 8th day in T_1, and again increased from 10th day up to 14th day of culture. But it was found to decrease from first day (46.30 ± 4.30mg/L) of experiment to 10th day (15.40 ± 1.26 g/L) but increased from 12th to 14th day of experiment in T_2. Similar trend was sharply followed in T_3 and T_4 (Fig. 6).

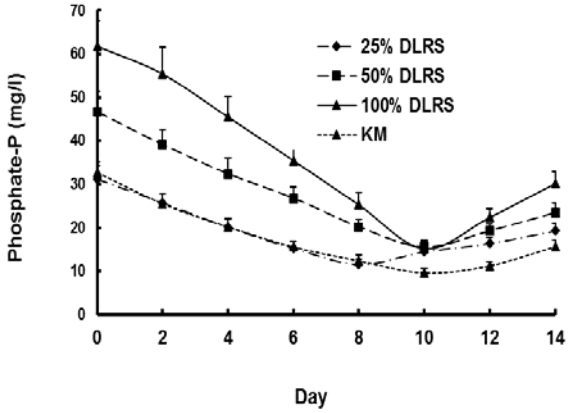

Fig. 6: Mean values of Phosphate-P (mg/L) during culture of *S. platensis*

Growth parameters of Spirulina

Optical density of media contained Spirulina

Optical density (OD) of media contained *Spirulina* was found to increased up to 10th day of culture of all the media of digested liquid rice starch media (DLRSM) and Kosaric medium and then decreased up to 14th day of experiment (Fig. 7).

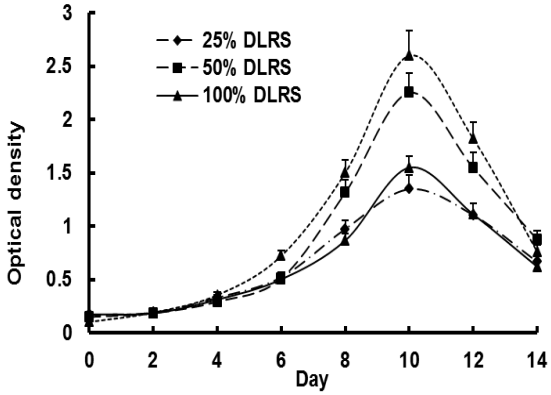

Fig. 7: Mean values of optical density of media contained *S. platensis*

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. 8).

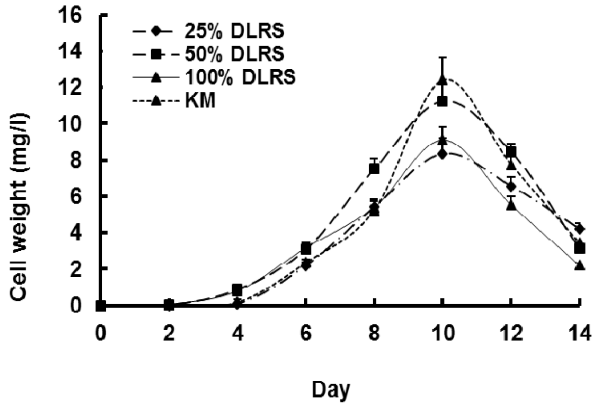

Fig. 8: Mean values of cell weight (mg/L) of *S. platensis*
Chlorophyll a of Spirulina

Chlorophyll a of *Spirulina* was found also higher on 10th day of culture than other days of culture of all the media and a down trend was also found after that day (Fig. 9).

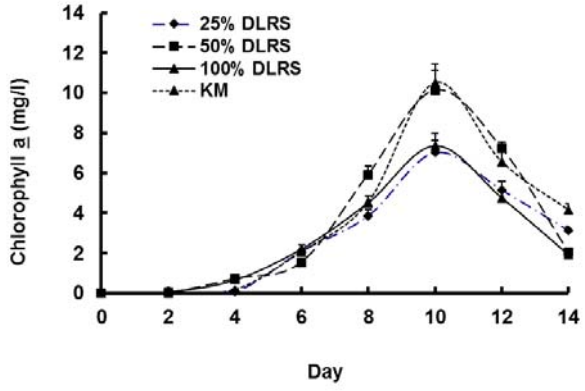

Fig 9: Mean values of chlorophyll a (mg/L) of *S. platensis*

Total biomass of Spirulina

Total biomass of *Spirulina* was increased from initial day (first day) up to 10th day (679.51 ± 9.25mg/L) in T_1 and then decreased up to 14th day (204.95 ± 5.13 mg/L) of experiment. However, the highest total biomass of *Spirulina* was recorded (471.15 ± 8.105mg/L) on 10th day of culture and then decreased up to 14th day (210.58 ± 4.45mg/L) in T_2. Again, total biomass in T_3 was increased from first day up to 10th day (493.72± 8.30mg/L) and then decreased up to 14th day (128.64 ± 5.18 mg/L). The highest total biomass was found to be (705.51± 9.45 mg/L) on 10th day and then decreased up to 14th day (278.72± 3.10 mg/L) in T_4 (Fig. 10).

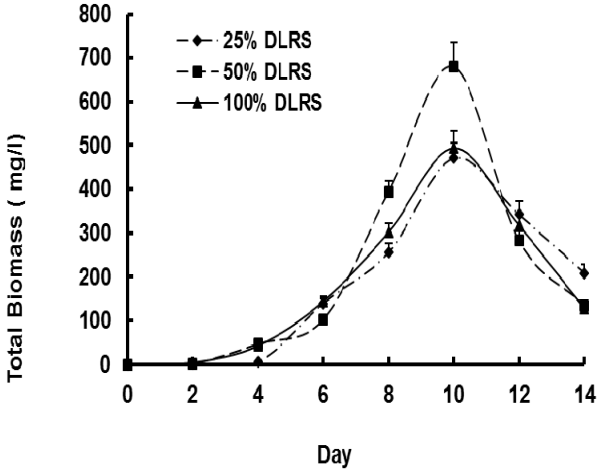

Fig. 10: Mean values of total biomass (mg/L) of *S. platensis*

Comparison of growth parameters of Spirulina (S. platensis) of 10th day of culture

Optical density of media contained Spirulina

Optical density of T_1 and T_4 contained *Spirulina* (*S. platensis*) was significantly ($P < 0.01$) higher than that of two other media T_2 and T_3. There was no significant ($P > 0.01$) difference among optical density of T_1 and T_4 among T_2 and T_3 during the study (Table 9).

Cell weight of Spirulina

Highest cell weight (mg/L) of *Spirulina* grown in T_4 was recorded (Table 9). Cell weight of *Spirulina* grown in T_4 and T_1 was varied significantly ($P < 0.01$) from that cultured in T_2 and T_3. However, there was no significant ($P > 0.01$) difference of cell weight of *Spirulina* grown in T_2 and T_3 (Table 9).

Chlorophyll a of Spirulina

Chlorophyll a (mg/L) of *Spirulina* grown in T_4 and T_1 was significantly ($P < 0.01$) higher than that of *Spirulina* cultured in T_2 and T_3. There was no significant difference among the Chlorophyll a of *Spirulina* grown in T_4 and T_1 and among the same of *Spirulina* cultured in supernatant of T_2 and T_3 (Table 9).

Total biomass of Spirulina (S. platensis)

Total biomass (mg/L) of *Spirulina* cultured in T_4 and T_1 was significantly ($P < 0.01$) higher than that of *Spirulina* grown in T_2 and T_3. There was no significant difference found among the total biomass of *Spirulina* cultured in T_4 and T_1 (Table 9).

Table 9: Comparison of optical density, cell weight, chlorophyll a and total biomass of *S. platensis*

Parameters	T1 (25% DLRS)	T2 (50% DLRS)	T3 (100% DLRS)	T4 (KM)
Optical density	2.257 ± 0.16[b]	1.347 ± 0.12[c]	1.554 ± 0.12[c]	2.61 ± 0.22[a]
Cell weight (mg/L)	11.255 ± 0.53[b]	8.352 ± 0.21[c]	9.109 ± 0.43[c]	12.42 ± 0.21[a]
Chlorophyll a (mg/L)	10.142 ± 0.32[b]	7.032 ± 0.11[c]	7.369 ± 0.20[c]	12.42 ± 0.21[a]
Total biomass (mg/L)*	679.51 ± 9.25[b]	471.15 ± 8.105[c]	493.72 ± 8.30[c]	705.51 ± 9.45[a]

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

Correlation among the growth parameters of Spirulina (S. platensis)

Cell weight of *Spirulina* (*S. platensis*) had highly significant ($P < 0.01$) direct correlation with chlorophyll a ($r = 0.8452$) of *Spirulina* grown in the supernatant of different digested liquid rice starch media and Kosaric medium during the study (Fig. 11). Similarly, total biomass of *S. platensis* was highly ($P < 0.01$) and directly correlated with chlorophyll a ($r = 0.823$) of *Spirulina* cultured in the supernatant of various digested liquid rice starch mediums and Kosaric medium (Fig.12). Again, total biomass of *Spirulina* was found to be highly ($P < 0.01$) and directly correlated with the cell weight ($r = 0.852$) of *Spirulina* grown in the supernatant of different digested liquid rice starch mediums and Kosaric medium (Fig.13).

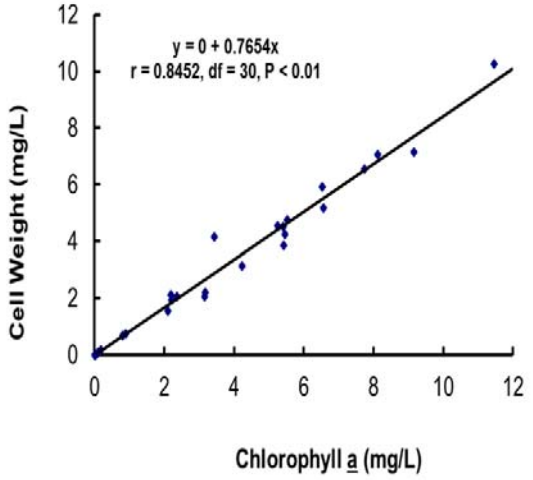

Fig 11: Correlation coefficient (r) of cell weight (mg/L) of *S. platensis with* chlorophyll a (mg/L)

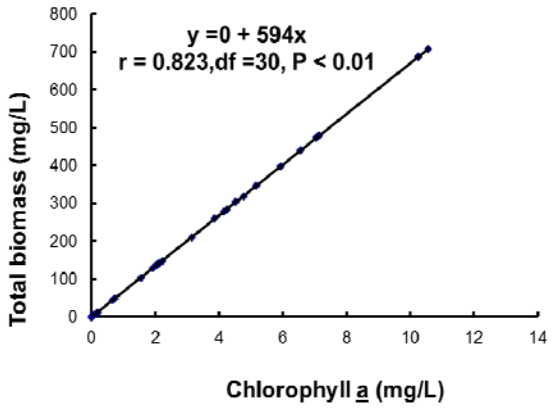

Fig. 12: Correlation coefficient (r) of total biomass (mg/L) of *S. platensis* with chlorophyll a (mg/L)

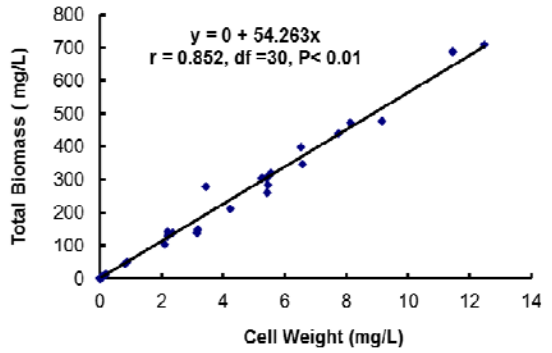

Fig 13: Correlation coefficient (r) of total biomass (mg/L) of *S. platensis* with cell weight (mg/L)

Specific growth rates (SGR) of Spirulina (S. platensis)

SGR in respect to cell weight of Spirulina

Specific growth rate (SGR) in respect to cell weight of *Spirulina* grown in T_4 and s T_1 was significantly ($P < 0.01$) higher than that of *Spirulina* cultured in T_2 and T_3. There was no significant ($P > 0.01$) difference among the SGR of cell weight of *Spirulina* grown in T_4 and T_1 and among the same of *Spirulina* cultured in T_2 and T_3 (Table 10).

SGR in respect to Chlorophyll a of Spirulina

The SGR in respect to Chlorophyll a of *Spirulina* cultured in T_4 and T_1 was significantly ($P < 0.01$) varied from that of *Spirulina* grown in T_2 and T_3. It had no significant difference when *Spirulina* grown in T_4 and T_1 and similar thing happened when *Spirulina* cultured in T_2 and T_3 (Table 10).

SGR in respect to total biomass of Spirulina

The SGR in respect to total biomass of *Spirulina* cultured in T_4 and T_1 was significantly ($P < 0.01$) varied from that of *Spirulina* grown in T_2 and T_3 (Table 10). There was no significant ($P < 0.01$) difference recorded among the SGRs on the basis of total biomass of *S. platensis* grown in T_1 and T_4. Similarly, it had no significant variation among the SGR on the basis of total biomass of *Spirulina* when cultured in T_2 and T_3.

Table 10: Specific growth rates (SGR) on the basis of cell weight, chlorophyll a and total biomass of *S. platensis*

Parameters	T_1 (25% DLRS)	T_2 (50% DLRS)	T_3 (100% DLRS)	T_4 (KM)
SGR of cell weight	0.30 ± 0.021[a]	0.25 ± 0.022[b]	0.26 ± 0.014[b]	0.31 ± 0.021[a]
SGR of Chlorophyll-a	0.28 ± 0.012[b]	0.24 ± 0.014[a]	0.25 ± 0.011[b]	0.29 ± 0.014[a]
SGR of total biomass	0.82 ± 0.031[a]	0.74 ± 0.022[b]	0.75 ± 0.018[b]	0.81 ± 0.023[a]

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

Proximate Composition (%) of Spirulina (S. platensis)

Moisture

Moisture of *Spirulina* grown in the supernatant of three different digested liquid rice starch media and Kosaric medium was varied from 8.22 to 8.23 % (Table 11).

Crude protein

There was no significant variation among the crude protein of *Spirulina* grown in the supernatant of three different digested liquid rice starches. But, crude protein of *Spirulina* cultured in T_4 (58.55 ± 0.45) was significantly ($P < 0.01$) higher than that of *Spirulina* grown in the supernatant of three other digested liquid rice starch media. The percentage of crude protein of *Spirulina* was 57.48 ± 0.44, 54.25 ± 0.40 and 54.66 ± 0.55 when grown in T_1, T_2 and T_3 (Table 11).

Crude lipids

Crude lipids (%) of *Spirulina* cultured in T_1 (14.50 ± 0.23%) varied significantly ($P < 0.01$) from that of *Spirulina* grown in T_2 (10.20 ± 0.30%) and T_3 (11.22 ± 0.20%) followed by T_4 (6.30± 0.13%) (Table11). There was no significant difference of crude lipids of *Spirulina* when cultured in T_2 and T_3.

Ash

Ash (%) of *Spirulina* grown in T_3 (9.55 ± 0.25%) had significant ($P < 0.01$) difference from that of *Spirulina* cultured in T_1 (10.30 ± 0.20%) and T_2 (9.25 ± 0.20%), and T_4 (13.50 ± 0.14%) (Table 11). There was no significant ($P > 0.01$) difference among the ash of *Spirulina* grown in T_1 and T_4.

Nitrogen free extract (NFE) of Spirulina

Nitrogen free extract (%) of *Spirulina* cultured in T_1 (8.77 ± 0.30%) and T_3 (15.59 ± 0.25%) varied significantly ($P < 0.01$) from that of *Spirulina* grown in T_4 (12.70 ± 0.30%) and then T_1 (7.35 ± 0.10%) (Table 9). There was no significant variation among the NFE of *Spirulina* grown in T_2 and T_3.

Crude fiber of Spirulina

Very small amount of crude fiber (%) was found in *Spirulina* grown in the supernatant of three different digested liquid rice starch media (DLRSM) and Kosaric medium (Table 11).

Table 11. Proximate composition (% in dry matter basis) of *S. platensis*

Treatments	T1 (25% DLRS)	T2 (50% DLRS)	T3 (100% DLRS)	T4 (KM)
Moisture	8.22 ± 0.08	8.22 ± 0.08	8.23 ± 0.07	8.22 ± 0.08
Crude Protein	57.48 ± 0.44[a]	54.25 ± 0.40[b]	54.66 ± 0.55[b]	58.55 ± 0.45[a]
Crude Lipids	14.50 ± 0.23[b]	10.20 ± 0.30[b]	11.22 ± 0.20[b]	6.30± 0.13[c]
Ash	10.30 ± 0.20[b]	9.25 ± 0.20[b]	9.55 ± 0.25[b]	13.50 ± 0.14[a]
NFE*	8.77 ± 0.30[c]	17.34 ± 0.10[a]	15.59 ± 0.25[a]	12.70 ± 0.30[b]
Crude Fibre	0.72 ± 0.04	0.73 ± 0.03	0.74 ± 0.04	0.72 ± 0.03

*NFE (Nitrogen Free Extract) = 100 - (Moisture + Crude protein + Crude lipids + Ash). Fig.s in common letters in the same row do not differ significantly at 1% level of probability.

DISCUSSION

S. platensis was cultured in three different concentrations of digested liquid rice starch media (DLRSM) viz. 25%, 50% and 100% DLRSM and Kosaric medium (KM). The ranges of cell weight of *S. platensis* in three concentrations of DLRSM and KM were found from 0.0022 to 11.25 mg/L in 25% DLRSM, 0.0022 to 8.352 mg/L in 50% DLRSM, 0.0022 to 9.109 mg/L in 100% DLRSM and 0.0022 to 12.42 mg/L in KM. The growth performance of *S. platensis* in 25% DLRSM was found better growth than other 50% DLRSM than 100% DLRSM. This variation might be due to the differences in nutrient concentrations and added 0.4 g/L urea composition of varied media. In controlled KM *S. platensis* showed the highest growth performance. It may be happened due to suitability and availability of the nutrients for the growth of the species. On the other hand 50% and 100% DLRSM showed lower growth performance of *S. platensis* in relation to 25% DLRSM.

This might be due to lower dilution and higher concentration of the nutrient media. The concentration of 100% DLRSM, which was not suitable and favorable for the growth of *S. platensis* because of the higher amount of nutrients content. Simillary type of work was carried out by Mario *et al.* (1986) where the annual yield of biomass of *Spirulina maxima* strain 4MX grown in fertilized sea water in out door system was 7.359 mg i.e. 0.39 $g/L^2/d$ which was higher than the present study . At present study the cell weight of *S. platensis* is various concentration of digested liquid rice starch media (DLRSM) and KM were lower than the findings of Mario *et al.* (1986).

The variation in result probably happened because of different nutrient component of media used in culture, different culture technique and different species cultured. An experiment conducted by Becker (1984) on algal culture in a series of different horizontal ponds and recorded that yield of *Spirulina* sp. was 8 to 12 $g/m^2/d$. The yield found from the experiment was also much higher than the present findings. From the findings of Li and Qi (1997) it was reported that the biomass output rate in Chinese production

plant was 7.0 g/m^2/d which was much higher than the results of present study. From the findings of Phan-Lan-Phuong and Thuoc (1988) reported that *S. platensis* grew well with a good protein content (57.58%) in a medium prepared from silk waste water and $NaHCO_3$ (8 g/L). It showed that 25% DLRSM same protein content (57.48%) in the present findings. Similarly, Tanticharoen *et al.* (1990) reported that the addition of $NaHCO_3$ and nirogen fertilizer in waste water from the stabilization pond of topics starch factory raised the productivity up to 7-10 g/m^2/d which was much higher than the findings of the present study.

Similarly, Jabber (2005) MS thesis showed that, culture and growth performance of *Spirulia platensis* in three different concentrations of seasame meal media (SeMM) for three month. *Spirulia platensis* was cultured in various concentrations viz., 40% 60%, 80% SeMM and control as Kosaric medium. *S. platensis* was 0.12 mg/L which attained the highest content of 11.01 mg/L when when cultured in KM and 10.01 mg/L in lowest concentration 40% SeMM/L than other concentrations of SeMM that showed the same result of 25% DLRSM in present study. Similarly, Satter (2017) studied on culture and production of housefly larvae and *Spirulina* using poultry waste, and their use as food for catfish post-larvae. He produced *Spirulina* and used as important feed ingredient to replace fish meal up to 100% but got very good growth of catfish post-larvae fed diet contained 25% fish meal, 50% *Spirulina* meal and 25% maggot meal. He also got good results when post-larvae fed diets contained 25% fish meal and 75% *Spirulina* meal, and another diet contained 100% *Spirulina* meal. During culture of *S. platensis* in 25%, 50% and 75% of digested of poultry waste (DPW) which was better growth in 25% DPW at the same result found in the present study. Similar type of work was observed, 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. The growth rate of *S. platensis* was found to vary in different media. The initial cell weight of *S. platensis* was 0.0004 g/L which a maximum weight of 0.720 g/L concentrations of PSPM, respectively on 8th day of culture period. He also observed that similar trend in case of chlorophyll a content of *S. platensis*.

The result indicated that the growth rate of *S. 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^{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.

CONCLUSIONS

The growth of *S. platensis,* was better the concentration in 25% of DLRSM than other concentrations in 50% and 100% of DLSRM. So, we can say the concentration of 25% DLRSM is most suitable and favorable law cost medium for *S. platensis* culture compared with standard Kosaric medium (KM). These media are easily available and inexpensive in contrast of Bangladesh. So, collection and preparation of liquid rice starch can be used for commercially and economically viable mass culture of *S. platensis* in Bangladesh.

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