Asian Australas. J. Biosci. Biotechnol. 2018, 3 (3), 208-224

Asian-Australasian Journal of Bioscience and Biotechnology
ISSN 2414-1283 (Print) 2414-6293 (Online)
www.ebupress.com/journal/aajbb

Article

Evaluation of rotten orange as a source of inorganic nutrients for blue green algae, *Spirulina platensis* culture

Md. Mijanur Rahman[1], Md. Lemon Mia[1*], Baadruzzoha Sarker[2], Md. Fakhruddin[2], Md. Ahsan Bin Habib[1], Md. Rezaul Karim[1] and Nazmul Hoque[1]

[1]Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[2]Production Office, BRAC Fish Hatchery, Satgao, Sreemongal, Moulvibazar, Sylhet 3214, Bangladesh

*Corresponding author: Md. Lemon Mia, Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh. Phone: +8801761131871; E-mail: mmlemon16@gmail.com

Received: 01 December 2018/Accepted: 24 December 2018/ Published: 31 December 2018

Abstract: A study was conducted to examine the culture and growth performance of *Spirulina platensis* in three different concentrations (25, 50 and 75 percent) of digested rotten orange (DRO) and Kosaric medium (KM) as control for three months. Each experiment was done in triplicates under fluorescent light in light: dark (12 hour: 12 hour) condition for a period of 14 days. Cell weight, chlorophyll a content of *Spirulina platensis* and physico-chemical parameters of cultured media (temperature, pH, light intensity) were measured at every alternative day. During the culture period, temperature was more or less similar and ranged from 30.1 to 31.4˚C. The range of light intensities was recorded from 2660 to 2770 lux/m^2/s during the culture period. The maximum pH was recorded 9.9 in 25% digest rotten orange (DRO) on 8th day of the culture and minimum was recorded 8.2 in all the medias on the initial day of the culture. The growth rate of *Spirulina platensis* cultured in supernatant of DRO and KM were varied. The initial cell weight of *Spirulina platensis* was 0.0023 mg/L that attained maximum cell weight of 12.44 mg/L in Kosaric medium on the 10th day of culture. Besides that, the maximum cell weight was 7.679, 12.366, and 9.455 mg/L when cultured in supernatant of 25, 50, and 75% in DRO on the 10th day of the culture. While, the initial chlorophyll a content of *Spirulina platensis* was 0.0015 mg/L which attained the highest content of 10.54 mg/L when grown in KM. The highest chlorophyll a content of *Spirulina platensis* was 6.926 mg/L when grown in 25% DRO, it was 10.476 mg/L when grown in 50% DRO, and it was 9.140 mg/L when grown in 75% DRO on the 10th day of the culture. This study showed that, the growth performance of *Spirulina platensis* was higher in supernatant of 50% DRO than 25 and 75% DRO. This concentration of medium gave satisfactory results compared with standard KM. This variation probably occurred because of differences in nutrient concentrations and composition of the medium. Therefore, it can be concluded that, the concentration of 50% DRO is suitable and favorable for *Spirulina platensis* culture.

Keywords: rotten orange; organic nutrients; *Spirulina platensis*; aquaculture

1. Introduction

The number of species of micro algae is estimated at 22000 to 26000 out of which 50 have been studied in detail with regard to their biochemistry and eco physiology (Callegari, 1989). *Spirulina* is an important Cyanophyta known as *Spirulina* rich in high quality protein around (60-65%), essential lipids, vitamins, minerals and many biologically active substances (Becker, 1984). The ability of *Spirulina* to grow in hot and alkaline environments ensures its hygienic status, as no other organisms can survive to pollute the waters in which this alga thrives. *Spirulina* is one of the cleanest, most naturally sterile foods found in nature. It has been used as feed for fish, poultry, and farm animals (Tragut *et al*., 1995). *Spirulina* has reported to prevent oxidative damage and hence can indirectly reduce cancer formation in human body. In this respect, the increased consumption of foods

characterized by free radical scavenging activity, leads up to a doubling of protection against many common types of cancer formation (Cooke *et al*., 2002; Romay *et al*., 2003; Anbarasan *et al*., 2011). *Spirulina* (*Spirulina platensis*) is a "super-food" among the most plants and even good quality animal food. It has a rich, vibrant history, and occupies an intriguing biological and ecological niche in the plant kingdom. *Spirulina* is a spiral-shaped, blue-green microalgae that grows naturally in the wild in alkaline lakes, seawater, and saltwater. Its deep blue-green color is what gives the water its greenish hue. *Spirulina* is also cultivated and harvested in man-made reservoirs around the world. For centuries, civilizations the world over have cultivated and cherished *Spirulina* for its health-improving benefits (Habib, 1998). However, According to the researchers 1kg *Spirulina* is similar to 100 kg other vegetables in nutrition. These blue green algae contain 50-70% protein, 10-12% carbohydrate (in dry condition), 6% fat, 7% mineral, and a lot of vitamins (Shuvo, 2001). It was reported that *Spirulina* contains 6-11% of polyunsaturated fatty acids; the predominant are palmitic (16:0, 44.6-54.1%) gamalinolenic or GLA (18:3, 8.0-31.7%), linoleic (18:2, 10.8-30.7%) and oleic acids (18:1, 1-15.5%). Now a day, *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 and Vonshak, 1991). The growth of *Spirulina* and the composition of the biomass produced depend on many factors, the most important of which are nutrient availability, temperature, and light (Cornet *et al*., 1992). In addition, *Spirulina* requires relatively high pH values between 9.5 and 9.8 (Belkin and Boussiba, 1971), which effectively inhibits contamination by most algae in the culture. Production of *Spirulina* with reduced costs is necessary when considering large-scale cultivation for industrial purposes. The cost of nutrients is considered the second major factors influence the cost of *Spirulina* biomass production after labor (Vonshak, 1997). Zarrouk's medium has successfully served as the standard medium (SM) for *Spirulina* culture for many years (Zarrouk, 1996). Consequently, many media have been developed using seawater (Faucher *et al*., 1979), sewage water (Saxena *et al*., 1982) and industrial effluents (Tanticharoen *et al*., 1993). *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 health drink, tablets, etc. because of its alimentary value (Ruiz *et al*., 2003). *Spirulina* required light intensities during its growth phase. Currently, the commercial production of *Spirulina* is mainly oriented towards the health food market, utilizing a chemically defined medium (Belay *et al*., 1993). Mass culture of *Spirulina platensis* and used in various purpose in African country, some South East Asian countries, India etc. In Bangladesh the culture of *S. platensis* is first initiated by Dr. Flora Mazid (Chairman, Bangladesh Council of Scientific and Industrial Research, BCSIR). The ultimate goal of this experiment is to develop low cost media for large-scale production of *Spirulina*. Therefore, rotten orange can be considered as a media in producing *Spirulina* as rotten orange can be obtained cheaply from the market. In addition, rotten orange contains significant amount of inorganic nutrients, which can be used as the source of nutrient in *Spirulina* culture. This study was conducted to evaluate the growth performances of *Spirulina* in supernatant of digested rotten orange medium (DROM); and to analyze the proximate composition of *Spirulina* that grows on digested rotten orange medium (DROM).

2. Materials and Methods

2.1. Study area

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

2.2. Culture of microalgae

2.2.1. Selection and collection of rotten orange

The rotten orange was selected as media for *Spirulina platensis* culture. It was collected from the Kamal Ranjit Market, BAU campus, Mymensingh.

2.2.2. Analysis of proximate composition of rotten orange

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 in the laboratory of the Fish Nutrition, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh (Table 1).

2.2.2.1. 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).

2.2.2.2. 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).

2.2.2.3. 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).

2.2.2.4. 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 rotten orange.

Sl. No.	Name of the component (rotten orange)	Moisture basis (%)	Dry matter basis %
1	Moisture	90.17	9.83
2	Ash	0.47	4.76
3	Protein	0.88	8.97
4	Lipid	0.95	9.67
5	Crude fiber	0.89	9.06
6	Carbohydrate	6.64	67.55

2.2.3. Analysis of physico-chemical properties of digested rotten orange

2.2.3.1. pH

pH of digested samples of rotten orange was determined using pH meter (HI module, 2005, Model HI 98129, HANNA).

2.2.3.2. Dissolved oxygen

It was determined using the digital oxygen meter (Model Nutron DO-5509).

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

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

2.2.3.4. Alkalinity

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

2.2.3.5. Nitrate-N (Available N)

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

2.2.3.6. Phosphate-P (Available P)

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

2.2.4. Collection of *Spirulina platensis*

Microalgae, *Spirulina platensis* was collected from the imported stock of my respected teacher and research supervisor Dr. Md. Ahsan Bin Habib, Professor, Department of fish, Bangladesh Agricultural University, Mymensingh-2202.

2.2.5. Maintenance of pure stock culture of *Spirulina platensis*

Pure stock culture of *Spirulina platensis* was maintained in the laboratory in Kosaric medium (KM) (Zarrouk, 1996). Growth of *Spirulina 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).

2.2.6. Preparation of digested rotten orange medium (DROM) and Kosaric medium (KM)

Rotten orange medium (ROM) and Kosaric medium (KM) were prepared for culture of *Spirulina platensis*. Different concentration and composition of rotten orange medium and kosaric medium are shown in the table 2 and 3 respectively.

Table 2. Concentration of rotten orange medium (ROM) for *Spirulina platensis*.

Sl. No.	Medium	Concentration/dilution of ROM (%)
1.	Digested Rotten orange medium (DROM)	25
2.	Digested Rotten orange medium (DROM)	50
3.	Digested Rotten orange medium (DROM)	75

For the preparation of supernatant of rotten orange collected samples were digested firstly by aerating it into a 5 liter volumetric flask under 4 liter distilled water. The concentration of rotten orange of 60g/L was maintained during digestion. After 47 days (27.02.18 – 15.04.18), digestion of rotten orange was completed and its supernatant was taken from the flask by filtering it with plankton net. Then the digested rotten orange was diluted according to the above direction with three replications using distilled water. Then the medium was mixed well and sterilized at 115^{o}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 microalgae.

Table 3. Composition of Kosaric medium (Zarrouk, 1996) for *Spirulina platensis* culture.

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	$MgSO_4\,7H_2O$	0.10
7	$CaCl_2$	0.02
8	$FeSO_4\,2H_2O$	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) $ZnSO_4 7H_2O$	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 1.0 L. Mixing, autoclaving and cooling were carried out pursing the procedure used during the preparation of RSM.

2.2.7. Experimental design of *Spirulina platensis* culture

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

Table 4. Three different doses of supernatant of digested rotten rotten orange (DRO) through dilution to culture *Spirulina*.

Types of medium	Treatments	Replications	Amount%	Duration of culture (days)
	T_1	3	25	
Supernatant of DRO	T_2	3	50	14
	T_3	3	75	
Kosaric Medium(KM)	T_4	3	-	14

2.2.8. Culture of *Spirulina platensis* in supernatant of digested rotten orange medium DROM and Kosaric medium KM

Four treatments, three from supernatant of DROM for three different concentrations (25, 50, and 75%) 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). Eight sub-samplings (15ml vial) were 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.

2.3. Estimation of *Spirulina platensis* cell weight (dry weight)

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

2.4. Determination of Chlorophyll a of *Spirulina*

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

2.5. Total biomass of *Spirulina* (*Spirulina platensis*)

Total biomass was calculated using the following formula given by Vonshak and Richmond (1988), Total biomass = Chlorophyll a x 67.

2.6. Specific growth rate (SGR) on the basis of dry weight chlorophyll a content and total biomass of *Spirulina*

2.6.1. 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.

2.6.2. 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.

2.6.3. 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.

2.7. Analysis of physicochemical parameters

2.7.1. Physical parameters

The physical parameters (temperature and light intensity) of the culture media were recorded as follows:

2.7.1.1. Temperature

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

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

2.7.2. Chemical parameters
The chemical parameters such as pH of the culture media were recorded following the procedures given by Clesceri *et al*. (1989) in the laboratory.

2.7.2.1. pH
pH of the culture media was measured from each sub sample by an electric pH meter (Conning pH meter 445).

2.8. 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).

3. Results
3.1. Physico-chemical characteristics of rotten orange
3.1.1. Colour, Odour and Structure
The color of the rotten orange was yellowish with little bid bad smell (odour). The structure was almost semi-solid (Table 5).

3.1.2. Temperature (°C)
Temperature of rotten orange was little bid higher than normal ambient temperature. It was ranged from 28.10 to 28.30ºC (Table 5).

3.1.3. Total solid (TSS + TDS)
Total soild is the addition of total suspended solids and total dissolved soilds of semi liquid (rotten orange) which was ranged from 1523 to 1622 mg/L (Table 5).

3.1.4. pH
pH of digested rotten orange was ranged from 4.30 to 5.15 which was acidic in nature (Table 5).

3.1.5. Alkalinity
Alkalinity of digested rotten orange was quite high and ranged from 75 to 85 mg/L (Table 5).

3.1.6. Nitrate-N (NO_3-N)
Nitrate-N (Available N) of digested rotten orange was ranged from 2.20 to 2.35 mg/L (Table 5).

3.1.7. Phosphate-P (PO_4-P)
Phosphate-P (Available P) of the digested rotten orange was high and varied from 3.80 to 3.950 mg/L (Table 5).

Table 5. Characteristics of rotten orange after collection.

Sl. No.	Characteristics of past of rotten orange	Comments
1	Colour	Yellowish
2	Odour	Little bid bad
3	Structure	Semi-solid
4	Temperature	28.10-28.30ºC
5	pH	4.30-5.15
6	Total solids (TSS + TDS)	1523-1622 mg/L
7	Alkalinity	75-85 mg/L
8	Total N	2.76-2.95 mg/L
9	Available N (NO_3-N)	2.20-2.35 mg/L
10	Available P (PO_3-P)	3.80-3.950 mg/L

3.2. Physico-chemical properties of supernatant of digested rotten orange

3.2.1. Temperature

Temperature of supernatant of digested rotten orange (DRO) used to culture *Spirulina* was varied from 28.40 to 29.70°C (Table 6).

3.2.2. pH

pH of supernatant of DRO used for *Spirulina* culture was found to range from 5.20 to 5.40 (Table 6).

3.2.3. Total solid (TSS + TDS)

Total solid (TSS + TDS) of supernatant of DRO used to culture *Spirulina* was reduced due to decomposition which was ranged from 115 to 133 mg/L (Table 6).

3.2.4. Alkalinity

Alkalinity of supernatant of DRO used to culture *Spirulina* was high which was ranged from 110 to 120 mg/L (Table 6).

3.2.5. Nitrate N (NO_3-N)

Nitrate N (Available N) of supernatant of DRO used for *Spirulina* culture was also high in amount and varied from 2.55 to 2.80 mg/ (Table 6).

3.2.6. Phosphate P (PO_4-P)

Phosphate P (Available P) of supernatant of DRO used to culture *Spirulina*was very high and found to vary from 3.30 to 3.50 mg/L (Table 6).

3.2.7. Total N

Total N of supernatant of DROM used for *Spirulina* culture was found also high in amount and ranged from 2.30 to 2.45 mg/L (Table 6).

Table 6. Physico-chemical properties of supernatant of digested rotten orange after digestion in aerobic condition.

Sl. No.	Characteristics	Range
1	Temperature(°C)	28.40-29.70
2	pH	5.20-5.40
3	Total solid (TSS + TDS) (mg/L)	115-133
4	Alkalinity (mg/L)	110-120
5	Total N (mg/L)	2.30-2.45
6	Available N (NO_3-N) (mg/L)	2.55-2.80
7	Available P (PO_3-P) (mg/L)	3.30-3.50

3.3. Proximate composition of rotten orange on dry basis

3.3.1. Moisture

It was measured from dry orange was 9.83% (Table 7).

3.3.2. Crude protein

Crude protein of rotten orange was 8.97% (Table 7) which quite high in percent.

3.3.3. Crude lipid

Crude lipid of rotten orange was 9.67% (Table 7) which quite high in percent.

3.3.4. Ash

Ash of rotten orange was 4.76 % (Table 7).

3.3.5. Crude fiber

Crude fiber in rotten orange was not high in amount and it was 9.06% (Table 7).

3.3.6. Nitrogen free extracts (NFE)

The NFE of rotten orange was high in amount and it was 67.90% (Table 7).

Table 7. Proximate composition (%) of rotten orange on moisture and dry weight basis.

Composition	Moisture basis (%)	Dry bsis (%)
Moisture	90.17	9.83
Crude protein	0.882	8.97
Crude lipids	0.950	9.67
Ash	0.468	4.76
Crude fiber	0.890	9.06
NFE*	6.63	67.90

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

3.4. Physico-chemical properties of different media contained *Spirulina* (*Spirulina platensis*) culture

3.4.1. Light intensity

It was varied slightly in different days in all the four culture media. However, light intensity (lux/m^2/s) was varied from 2754 ± 24 on first day to 2770 ± 27 lux/m^2/s on the last day with slight variation in other days in T_1. It was varied from 2740 ± 26 on first day to 2766 ± 25 lux /m^2/s on the last day in T_2. Similarly, it was observed 2725 ± 30 on the first day and 2727 ± 30 on the last day (14th day) in T_3. Light intensity was found to be 2725 ± 26 lux /m^2/s on first day in T_4 and 2660 ± 15 lux /m^2/s on the last day (14th day) of experiment (Figure 1).

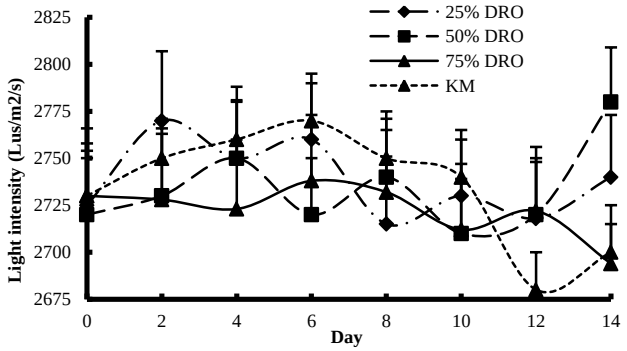

Figure 1. Mean values of light intensity (Lux/m^2/s) during culture of *Spirulina platensis.*

3.4.2. Temperature

The temperature round the culture in T_1 was found 30.1 ± 0.24 (lowest) on the first day to 30.8 ± 0.26 at the end (14th day) of experiment with slight up on 2nd, 6th and 10th day of experiment. It was also follow the similar trend of fluctuation from first to last day in T_2 and T_3. But, it was recorded 30.3 ± 0.32 °C on the first day of experiment to 31.2 ± 0.42 °C at the end of experiment in T_4 (Figure 2).

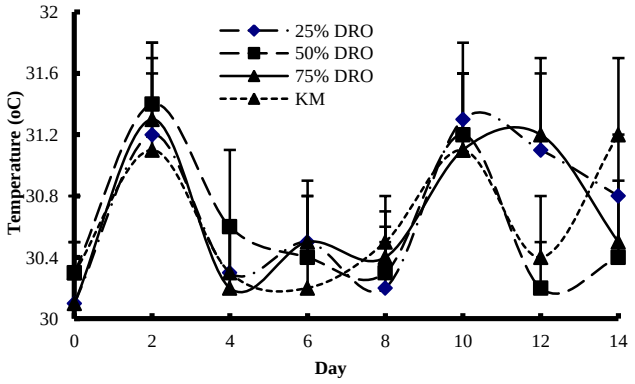

Figure 2. Mean values of temperature (°C) during culture of Spirulina platensis.

3.4.3. pH

During the 14 days experiment, it was increased from 8.2±0.18 on first day to 9.9±0.23 on 8th day in T_1 and then it was decreased to 8.5±0.20 on last day (14^{th} day) of experiment. It was found 8.2±0.19 on the first day which was increased to 9.8±0.26 on 10^{th} day in T_2 and then decreased on 12^{th} day and again decreased on the

last day (14th day) of experiment. Similar trend of fluctuation of pH were observed in T_3. Nevertheless, it was increased from first day (8.2 ± 0.33) of experiment up to 10th day (9.9 ± 0.32) of experiment, and then decreased on the 12th day and again decreased on the last day (14th day) in T_4 (Figure 3).

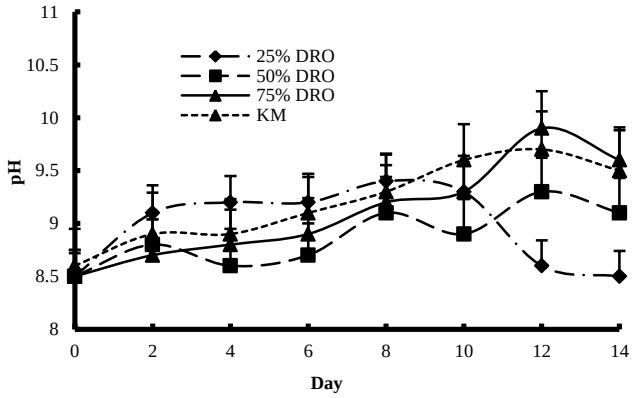

Figure 3. Mean values of pH of culture during *Spirulina platensis.*

3.4.4. Alkalinity

It was found 2260±102 mg/L on first day of experiment and then increased up to 2263±105 on the 2nd day and then gradually decreased up to 1660±210 mg/L on the 8th day, and then increased up to 1938±170 on the 14th day (last day) in T_1. Total alkalinity was recorded 1810±160 mg/L on first day of experiment and increased up to 10th day (2045 ± 185 mg/L), and then decreased on 12th day and again increased on 14th day in T_2. It was found 2110 ±170 mg/L on the first day of experiment, and then the concentrations of total alkalinity was followed a zig-jag trend up to 10th day of experiment and then decreased up to 14th day in T_3. The concentrations of total alkalinity was followed a zig-jag trend from first day up to 6th day of experiment and then decreased up to 14th day in T_4 (Figure 4).

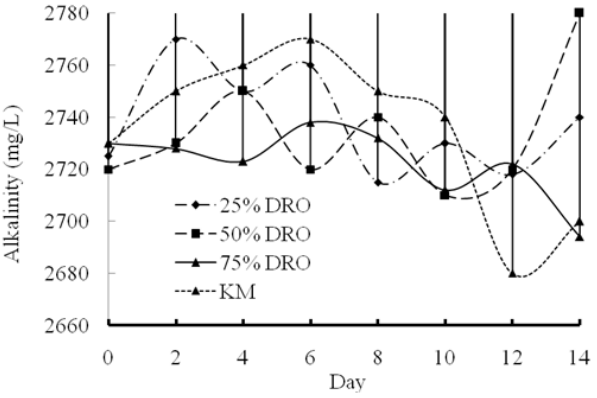

Figure 4. Mean values of alkalinity (mg/L) during culture of *Spirulina platensis.*

3.4.5. Nitrate N (NO_3-N)

It was positively increased from 1.76±0.14 mg/L (first day) to 3.37±0.26 mg/L (6th day) of experiment and then decreased to 2.60±0.20 (8th day) of experiment and then again increased up to 2.80±0.20 (12th day) of experiment and again decreased to 1.85±0.16 (14th day) of experiment in T_1. The trend of nitrate-N was found to decrease from first day (2.26±0.15 mg/L) to 8th day (1.27±0.14 mg/L) of culture and then increased up to 14th day in T_2. It was 3.34±0.20 mg/L on the first day of the experiment. Lowest amount of nitrate-N (2.99±0.26 mg/L) was recorded on the 2nd day of the experiment and it was increased up to 6.05±0.47 on the 14th day of the experiment which was highest amount of nitrate-N in T_3. It was found lowest (2.04 ± 0.18 mg/L) on 4th day of culture and it was found highest (3.63 ± 0.28 mg/L) on 14th day in T_4 (Figure 5).

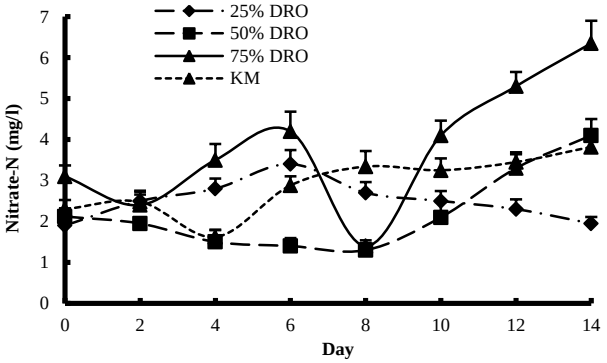

Figure 5. Mean values of Nitrate-N (mg/L) during culture of *Spirulina platensis.*

3.4.6. Phosphate-P (PO_4-P)

Phosphate-P (Available P) was high in amount in the media in first day (31.21 ± 3.03 mg/L) of experiment and gradually decreased in amount up to 10th day (10.55 ± 1.22 mg/L) in T_1, but increased from 12th day up to 14th day of culture. Similarly it was found to decrease from first day (46.40 ± 4.40 mg/L) of experiment to 10th day (15.55 ± 1.42 mg/L) but increased from 12th to 14th in T_2. However, Phosphate-P (Available P) was found to decrease from first day (60.35 ± 5.40 mg/L) of experiment to 12th day (18.20 ± 2.20 mg/L) and then again increased to 25.70 ± 2.40 mg/L on the 14th in T_3. Similar trend was sharply followed in T_4 (Figure 6).

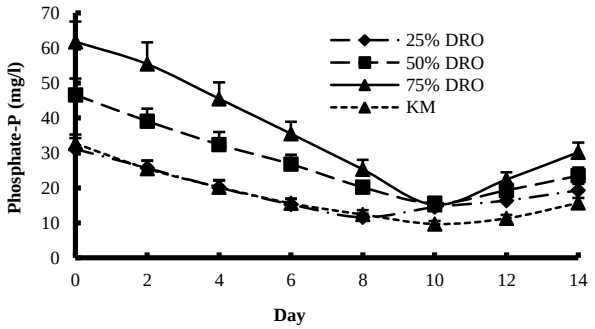

Figure 6. Mean values of Phosphate-P (mg/L) during culture of *Spirulina platensis.*

3.5. Growth parameters of *Spirulina* (*Spirulina platensis*)

3.5.1. 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 rotten orange (DRO) and Kosaric medium and then decreased up to 14th day of experiment. However, highest OD of T_1 was 1.325 ± 0.11 mg/L, where highest OD of T_2 was found 2.285 ± 0.16 mg/L. The highest OD of T_3 was 1.577 ± 0.13. The highest optical density of T_4 was 2.63 ± 0.20 mg/L (Figure 7).

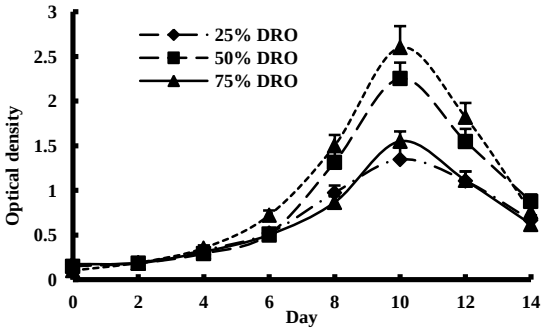

Figure 7. Mean values of optical density of media contained *Spirulina platensis.*

3.5.2. 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 (Figure 8). Cell weight of *Spirulina* increased from initial day (first day) up to 10th day (7.679 ± 0.23 mg/L) in

T_1 and then decreased up to 14th day (4.104 ± 0.18 mg/L) of experiment. However, the highest cell weight was found to be 12.44 ± 0.21 mg/L in T_2. Cell weight of *Spirulina* increased from initial day (first day) up to 10th day (9.455 ± 0.40mg/L) T_3 and then decreased up to 14th day 2.30 ± 0.13 mg/L of experiment. Highest cell weight of T_4 was 12.44 ± 0.21 mg/L on 10th day and then decreased up to 14th day of experiment.

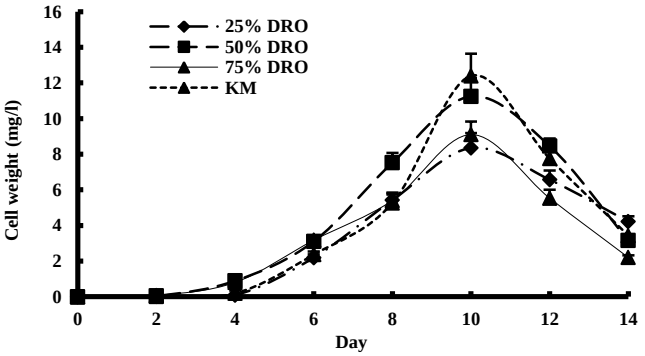

Figure 8. Mean values of cell weight (mg/L) of *Spirulina platensis.*

3.5.3. Chlorophyll a of *Spirulina*

Chlorophyll a of *Spirulina* increased from first day up to 10th day (6.926 ± 0.15 mg/L) in T_1 and then decreased up to 14th day (3.137 ± 0.15 mg/L). However, chlorophyll a in T_2 was 10.476 ± 0.31 mg/L on 10th day and then decreased up to 14th day (last day) of culture. Chlorophyll a in T_3 was 9.140 ± 0.22 mg/L on 10th day and then decreased up to 14th day (last day) of experiment, where the highest chlorophyll a in T_4 was 10.54 ± 0.14 mg/L on 10th day and decreased up to 14th day (last day) of experiment (Figure 9).

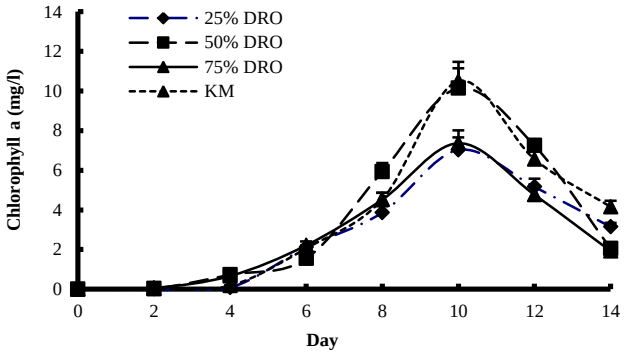

Figure 9. Mean values of chlorophyll a (mg/L) of *Spirulina platensis.*

3.5.4. Total biomass of *Spirulina*

Total biomass of *Spirulina* was increased from initial day (first day) (0.101 ± 0.003 mg/L) up to 10th day (464.04 ± 8.05 mg/L) in T_1 and then decreased up to 14th day (210.18 ± 4.45 mg/L) of experiment. However, the highest total biomass in T_2 was recorded 701.89 ± 4.33 mg/L on 10th day of culture and then decreased up to 14th day (209.84 ± 2.12 mg/L). Again, total biomass in T_3 was increased from first day up to 10th day (612.38 ± 4.30 mg/L) and then decreased up to 14th day (127.97 ± 2.17 mg/L). The highest total biomass in T_4 was found to be 706.18 ± 4.50 mg/L on 10th day and then decreased up to 14th day (278.05 ± 2.12 mg/L) (Figure 10).

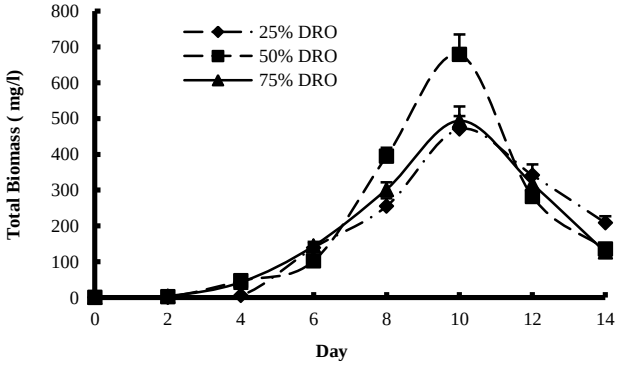

Figure 10. Mean values of total biomass (mg/L) of *Spirulina platensis.*

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

3.6.1. Optical density of media contained *Spirulina*

Optical density of T_2 and T_4 was significantly ($P < 0.01$) higher than that of two other media (25%) and (75%) (Table 8). There was no significant ($P > 0.05$) difference among optical density of T_1 and T_3 during the study.

3.6.2. Cell weight of *Spirulina*

Highest cell weight (mg/L) of *Spirulina* grown in T_4 was recorded (Table 8). Cell weight of *Spirulina* grown in T_4 and T_2 was varied significantly ($P < 0.01$) from that cultured in T_1 and T_3 (Table 8). However, there was no significant ($P > 0.01$) difference of cell weight of *Spirulina* grown in $T_1$and T_3.

3.6.3. Chlorophyll a of *Spirulina*

Chlorophyll a (mg/L) of *Spirulina* grown in T_4 and T_2 was significantly ($P < 0.01$) higher than that of *Spirulina* cultured in T_1 and T_3 (Table 8). There was no significant difference among the Chlorophyll a of *Spirulina* grown in $T_1$and T_3.

3.6.4. Total biomass of *Spirulina (Spirulina platensis)*

Total biomass (mg/L) of *Spirulina* cultured in T_4 and T_2 was significantly ($P < 0.01$) higher than that of *Spirulina* grown in T_1 and T_3 (Table 8). There was no significant difference found among the total biomass of *Spirulina* cultured in T_1 and T_3.

Table 8. Comparison of cell weight, chlorophyll a and total biomass of *Spirulina platensis* grown in supernatant of three different concentrations of digested rotten orange (DRO), and Kosaric medium (KM) on 10th day of culture before stationary phase.

Parameters	T_1 (25% DRO)	T_2 (50% DRO)	T_3 (75% DRO)	T4 (KM)
Optical density	1.325 ± 0.11^{c}	2.285 ± 0.16^{b}	1.577 ± 0.12^{c}	2.63 ± 0.20^{a}
Cell weight (mg/L)	7.679 ± 0.23^{c}	12.366 ± 0.50^{a}	9.455 ± 0.40^{b}	12.44 ± 0.21^{a}
Chlorophyll a (mg/L)	6.926 ± 0.15^{b}	10.476 ± 0.31^{a}	9.140 ± 0.22^{a}	10.54 ± 0.14^{a}
Total biomass (mg/L)*	464.04 ± 8.05^{c}	701.89 ± 4.33^{a}	612.38 ± 4.30^{b}	706.18 ± 9.50^{a}

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

3.7. Correlation among the growth parameters of *Spirulina*

Cell weight of *Spirulina* (*Spirulina platensis*) had highly significant ($P < 0.01$) direct correlation with chlorophyll a ($r = 0.806$) of *Spirulina* grown in the supernatant of different digested rotten media and Kosaric medium during the study (Figure 11). Similarly, total biomass of *S. platensis* was highly ($P < 0.01$) and directly correlated with chlorophyll a ($r = 0.845$) of *Spirulina* cultured in the supernatant of various digested rotten orange and Kosaric medium (Figure 12). Again, total biomass of *Spirulina* was found to be highly ($P < 0.01$) and directly correlated with the cell weight ($r = 0.833$) of *Spirulina* grown in the supernatant of different digested rotten orange and Kosaric medium (Figure 13).

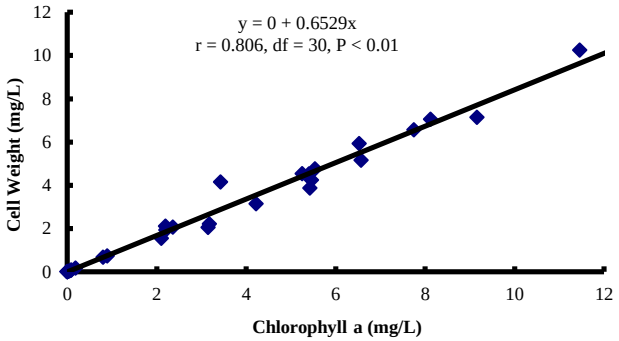

Figure 11.Correlation coefficient (r) of cell weight (mg/L) of *Spirulina platensis* with chlorophyll a (mg/L) of *Spirulina* grown in supernatant of three digested rotten orange, and Kosaric medium.

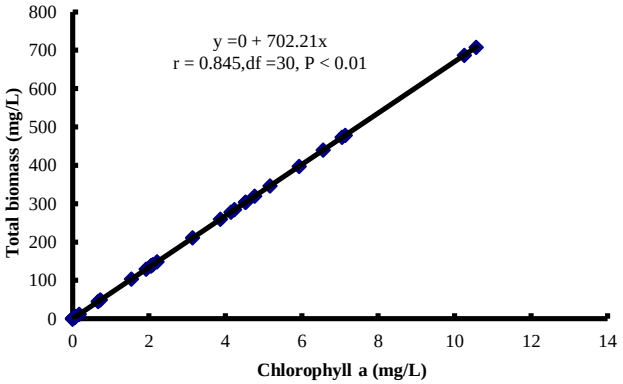

Figure 12. Correlation coefficient (r) of total biomass (mg/L) of *Spirulina platensis* with chlorophyll a (mg/L) of *Spirulina* grown in supernatant of three digested rotten orange, and Kosaric medium.

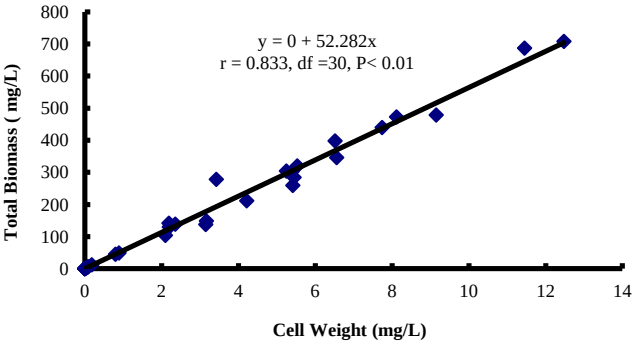

Figure 13. Correlation coefficient (r) of total biomass (mg/L) of *Spirulina platensis* with cell weight (mg/L) of *Spirulina* grown in supernatant of three digested rotten orange, and Kosaric medium.

3.8. Specific growth Rates (SGR) of *Spirulina* (*Spirulina platensis*)

3.8.1. SGR in respect to cell weight of *Spirulina*

Specific growth rate (SGR) in respect to cell weight of *Spirulina* grown in T_4 and T_2 was significantly ($P < 0.01$) higher than that of *Spirulina* cultured in T_1 and T_3 (Table 9). There was no significant ($P > 0.01$) difference among the SGR of cell weight of *Spirulina* grown in T_4 and T_2, and among the same of *Spirulina* cultured in T_1 and T_3.

3.8.2. SGR in respect to Chlorophyll a of *Spirulina (Spirulina platensis)*

The SGR in respect to Chlorophyll a of *Spirulina* cultured in T_4 and T_2 was significantly ($P < 0.01$) varied from that of *Spirulina* grown in T_1 and T_3 (Table 9). It had no significant difference when *Spirulina* grown in T_4 and T_2, and similar thing happened when *Spirulina* cultured in $T_1$and T_3.

3.8.3. SGR in respect to total biomass of *Spirulina*

The SGR in respect to total biomass of *Spirulina* cultured in T_4 and T_2 was significantly ($P < 0.01$) varied from that of *Spirulina* grown in T_1 and T_3 (Table 9). There was no significant ($P < 0.01$) difference recorded among the SGRs on the basis of total biomass of *S. platensis* grown in T_2 and T_4. Similarly, it had no significant variation among the SGR on the basis of total biomass of *Spirulina* when cultured in T_1 and T_3.

Table 9. Specific growth rates (SGRs) on the basis of cell weight, chlorophyll a and total biomass of *Spirulina platensis* grown in supernatant of three different concentrations of digested rotten orange (DRO), and Kosaric medium

Parameters	T_1 (25% DRO)	T_2 (50% DRO)	T_3 (75% DRO)	T_4 (KM)
SGR of cell weight	0.23 ± 0.021^b	0.31 ± 0.020^a	0.28 ± 0.014^a	0.31 ± 0.021^a
SGR of Chlorophyll a	0.24 ± 0.012^b	0.28 ± 0.014^a	0.27 ± 0.011^a	0.29 ± 0.014^a
SGR of total biomass	0.70 ± 0.022^b	0.82 ± 0.031^a	0.78 ± 0.018^a	0.81 ± 0.023^a

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

3.9. Proximate Composition (%) of *Spirulina* (*Spirulina platensis*)

3.9.1. Moisture

Moisture of *Spirulina* grown in the supernatant of three different digested rotten orange and Kosaric medium was varied from 8.22 ± 0.06 to 8.27 ± 0.07 % (Table 10). The moisture content (%) was suitable for the preservation of the samples for future analysis.

3.9.2. Crude protein

There was no significant variation among the crude protein of *Spirulina* grown in the supernatant of three different digested rotten oranges (Table 10). But, crude protein of T_4 (58.55 ± 0.40%) was ($P < 0.01$) higher than that of *Spirulina* grown in the supernatant of three other digested rotten orange media. The percentage of crude protein of *Spirulina* was 53.74 ± 0.38%, 57.25 ± 0.42% and 56.35 ± 0.52% when grown in T_1, T_2 and T_3.

3.9.3. Crude lipids

Crude lipids (%) of *Spirulina* cultured in T_2 (14.75 ± 0.23%) varied significantly ($P < 0.01$) from that of *Spirulina* grown in T_1 (10.18 ± 0.28%) and T_3 (12.65 ± 0.19%) followed by T_4 (6.32 ± 0.22%) (Table 10).

3.9.4. Ash

Ash (%) of *Spirulina* grown in T_4 (13.55 ± 0.12 %) had significant ($P < 0.01$) difference from that of *Spirulina* cultured in T_1 (9.22 ± 0.15 %) and T_2 (10.18 ± 0.17 %), and T_3 (10.49 ± 0.22 %) (Table 10). There was no significant ($P > 0.01$) difference among the ash of *Spirulina* grown in T_2 and T_3.

3.9.5. Nitrogen free extract (NFE) of *Spirulina*

Nitrogen free extract (%) of *Spirulina* cultured in T_1 (17.89 ± 0.39%) varied significantly ($P < 0.01$) from that of *Spirulina* grown in T_4 (12.64 ± 0.26%) and then T_2 (8.83 ± 0.18%) and T_3 (11.52 ± 0.26%) (Table 10). There was no significant variation among the NFE of *Spirulina* grown in T_3 and T_4.

3.9.6 Crude fiber of *Spirulina*

Very small amount of crude fiber (%) was found in *Spirulina* grown in the supernatant of three different digested rotten orange media (DROM), and Kosaric medium (Table 10). However, it was varied from 0.71 ± 0.04% when *Spirulina* grown in T_1 and T_3 to 0.72 ± 0.03% when cultured in T_2.

Table 10. Proximate composition (% in dry matter basis) of *Spirulina platensis* cultured in supernatant of three different concentrations of digested rotten orange (DRO), and control as Kosaric medium.

Treatments	T_1 (25% DRO)	T_2 (50% DRO)	T_3 (75% DRO)	T_4 (KM)
Moisture	8.25 ± 0.07	8.26 ± 0.07	8.27 ± 0.07	8.22 ± 0.06
Crude Protein	53.74 ± 0.38^b	57.25 ± 0.42^a	56.35 ± 0.52^b	58.55 ± 0.40^a
Crude Lipids	10.18 ± 0.28^b	14.75 ± 0.23^a	12.65 ± 0.19^b	6.32 ± 0.22^c
Ash	9.22 ± 0.15^b	10.18 ± 0.17^b	10.49 ± 0.22^b	13.55 ± 0.12^a
NFE*	17.89 ± 0.39^a	8.83 ± 0.18^c	11.52 ± 0.26^a	12.64 ± 0.26^b
Crude Fiber	0.71 ± 0.04	0.72 ± 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 1% level of probability.

4. Discussion

One of the most important microalgae *Spirulina platensis* was cultured in supernatant of three concentrations of digested rotten orange media (DROM), such as 25, 50, and 75% and Kosaric medium (KM) as control. This experiment was conducted in order to evaluate culture and growth performance of *S. platensis* in the laboratory. The cell weight of *S. platensis* in three concentrations of DROM and KM were ranged from 0.0022 ± 0 mg/L to 7.679 ± 0.23, 0.0025 ± 0 mg/L to 12.366 ± 0.50 mg/L, 0.0022 ± 0 mg/L to 9.455 ± 0.40 mg/L, and 0.0023 ± 0 mg/L to 12.44 ± 0.21 mg/L when grown in 25% DROM, 50% DROM, 75% DROM and KM. Nevertheless, the cell growth was found to be varied in different media. Most likely, this variation in cell weight occurred because of composition of varied media and the differences in nutrient concentration. *S. platensis* growth rate was comparatively higher in KM than digested rotten orange media (DROM) in various concentrations. Besides that, higher growth rate was observed in concentration of 50% DROM than other concentrations of DROM (concentration 25% and 75%). This was probably happened due to suitable and favorable amounts of nutrients in 50% DROM than other concentrations of DROM during the culture of *S. platensis*. The concentration of 25 and 75 % DROM saw lower growth of *Spirulina platensis* due to lack of nutrients in 25 % DROM and more nutrients contain in 75% DROM than 50% DROM. The exponential phase was found up to 10^{th} day from the beginning and then the cell weight declined i.e. stationary phase started. The physico-chemical properties such as light intensity, aeration, temperature played a crucial role to the entire culture system. During the culture system, the climatic condition was more or less suitable and favorable for the growth of *S. platensis.* Similar 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 $L^{-1}d^{-1}$ which was higher than the present study. At present study the cell weight of *S. platensis* in DROM 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 7.0g/m^2/d which was much higher than the results of present study. Similarly, Tanticharoen *et al.* (1990) reported that the addition of $NaHCO_3$ and nirogen fertilizer in waste water from the stabilization pond of topics orange factory raise the productivity up to 7-10 g $m^{-2}d^{-1}$ which was much higher than the findings of the present study. The variation in results occurred due to nutrient composition of different media and physico-chemical factors involved in the culture. On the other hand, 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 *Spirulina 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. From above all the discussion it may be concluded that the chemical parameters, the physical properties and technical facilities used in the present study where more or less similar to those used by other researchers. The findings of the present work differed with other findings mentioned above due to the nutritional variation of the culture media.

5. Conclusions

After observing the results of the present study, it can be concluded that the growth performance of *Spirulina platensis* was higher in supernatant of 50% digested rotten orange (DRO) than 25% and 75% DRO. This concentration of media gave satisfactory results compared with standard Kosaric media. This variation probably occurred because of differences in nutrient concentrations and composition of the media. Therefore, the concentration of 50% DRO is suitable and favorable for *Spirulina platensis* culture.

Conflict of interest

None to declare.

References

Anbarasan V, KV Kishor, KP Satheesh and T Venkatachalam, 2011. In Vitro evaluation of antioxidant activity of blue green algae *Spirulina platensis*. Int. J. Pharma. Sci. Res., 2: 2616-2618.

AOAC, 2016. Official Methods of Analysis. In: Helrich, K., 20^{th} Edition, Association of Official Analytical Chemists, Arlington, VA.

APHA (American Public Health Association), 1976. Standard methods for the examination of water and waste water. American Public Health Association. Broadway. New York, 10019.

Becker EW, 1984. Production and utilization of the blue-green algae *Spirulina* in India. Biomass, 4: 105-125.

Belay AY, K Ota, Miyakawa and K Shimamatsu, 1993. Current knowledge on potential health benefits of *Spirulina*. J. App. Phycol., 5: 235-245.

Belkin S and S Boussiba, 1971. Resistance of *Spirulina platensis* (Cyanophyta) to high pH values. J. App. Phycol., 32: 953-958.

Bhuiyan MRR, H Zamal, MM Billah, MS Bhuyan, AA Asif and MH Rahman, 2018. Proximate composition of fish feed ingredients available in Shibpur Upazila, Narsingdi district, Bangladesh. *J. Entomol. Zool. Stud.*, 6: 1345-1353.

Bold HC and MJ Wynne, 1978. Introduction to the Algae: Structure and Reproduction. 2nd edn., Prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA. pp.706.

Callegari JP, 1989. Feu vert pour les microalgues. Biofutur (Paris), 76: 25-28.

Clesceri LS, AE Greenberg and RR Trussell, 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. pp.10-203.

Cohen Z and A Vonshak, 1991. Fatty acid composition of *Spirulina* and *Spirulina*-like cyanobacteria in relation to their chemotaxonomy. Phytochemistry, 30: 205-218.

Cooke MS, MD Evans, N Mistry and J Lunec, 2002. Role of dietary antioxidants in the prevention of in vivo oxidative DNA damage. Nutri. Res. Rev., 15: 19-41.

Cornet JF, CG Dussap and G Dubertret, 1992. A structured model for simulation of cultures of the cyanobacterium *Spirulina platensis* in photobioreactors. I. Coupling between light transfer and growth kinetics. Biotech. Bioengin., 40: 817-825.

EMSL-Ci, 2003. Methods for Chemical Analysis of Water and Wastes. 3rd Edition, Environmental Protection Agency, Cincinnati, Ohio 45268, EPA-600/4-79- 020, Method 354.1, Storet # Total 00615.

Faucher O, B Coupal and A Leduy, 1979. Utilization of seawater and urea as a culture medium for *Spirulina maxima*. Canadian J. Microbiol., 25: 752-759.

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

HI Module, 2005. Popular multipurpose pocket meters. Model HI 98129.Hanna Instruments Ltd., Eden Way, Pages Industrial Park, Leighton Buzzard, Bedfordshire, UK. pp.1-2.

HP Module, 1999. How to measure dissolved, suspended & total solids: Hydrology Project Training Module; Training module WQ – 10. World Bank & Government of The Netherlands funded. New Delhi, India. pp. 1-24.

Li DM and YZ Qi, 1997. *Spirulina* industry in China: Present status and future prospects. J. App. Phycol., 9: 25-28.

Mario R, T Papuzzo and S Tomaselli, 1986. Outdoor mass culture of *Spirulina maxima* in sea water. App. Microbiol. Biotechnol., 24: 47-50.

Phang SM and WL Chu, 1999. University of Malaya algae culture collection (UMACC). Catalogue of Strain. Institute of Postgraduate Studies and Research, University of Malaya, Kuala Lumpur, Malaysia. pp.77.

Rahman MH, MA Rahman, MMM Hossain, SM Yeasmin and AA Asif, 2015. Effect of feeding management of broodstock on breeding performance of bata (*Labeo bata*). *Asian J. Med. Biol. Res.*, 1: 553-568.

Romay C, R Gonzalez, N Ledon, D Remirez and V Rimbau, 2003. C-phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Curr. Protein Peptide Sci.*, 4: 207-216.

Ruiz FLE, EM Bujaidar, M Salazar and G Chamarro, 2003. Anticlastogenic effect of *Spirulina maxima* extract on the micronuclei induced by maleichydrazide in Tradescantia. Life Science, 72: 1345-51.

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.

Saxena PN, MR Ahmad, K Shyam and DV Amla, 1982. Cultivation of *Spirulina* in sewage for poultry feed. Experientia, 39: 1077-1083.

Shuvo AK, 2001. *Spirulina* is future food. 243, Professors Coprofessors, Current Affairs, June. pp.74.

Tanticharoen M, S Bhumiratana, N Jeyaskoke, B Bunnag, M Ruengiitehawaly, P Chithumsub, C Wantawin and S Lerttriluck, 1990. The cultivation of *Spirulina* using tapioca orange wastewater. In: 5th International Conference of the Society of Applied Algology. Recent advance in algal biotechnology. Israel, January 1990. pp. 136-140.

Tanticharoen M, B Bunnag and A Vonshak, 1993. Cultivation of *Spirulina* using secondary treated orange wastewater. Australasian Biotechnol., 3: 223-226.

Thermo Fisher Scientific Company, 2008. Alkalinity Test Kit, Code AC2046, pp.1-12.

Tragut V, J Xiao, EJ Bylina and D Borthakur, 1995. Characterization of DNA restriction- modification systems in *Spirulina platensis* strain pacifica. J. App. Phycol., 7: 561- 564.

Vonshak A and A Richmond, 1988. Mass production of the blue-green alga *Spirulina*: an overview. Biomass, 15: 233-247.

Vonshak A, 1997. *Spirulina platensis* (*Arthrospira*): Physiology, cell biology and biotechnology. Taylor and Francis, London ,UK. pp. 213-226.

Vymazal J, 1995. Algae and element cycling in wetlands. Inc. Boca Raton, Florida, USA. pp. 689.

Yeasmin MY, MH Rahman, MAR Rahman, AA Asif, MAM Farid and MM Billah 2018. Influence of feeding administration of brood-stock on breeding performance of common Carp (*Cyprinus carpio* Linnaeus, 1758). *J Aquacul. Engin. Fish. Res.*, 4:127 137.

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.