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Growth performance and estimation of single celled protein in spirulina (*Spirulina platensis*) cultured in supernatant of digested rotten apple (*Malus domestica*)

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Abstract

Spirulina platensis was cultured in supernatant of three different concentrations of digested rotten apple (*Malus domestica*) and Kosaric medium (KM) to see the growth performance and crude protein content in the cells. The three different supernatant media were 1.25, 2.50 and 5.0% digested rotten apple (DRA) added with 9.0 g/L $NaHCO_3$, 0.50 ml/L micronutrients and 0.20 g/L urea. Culture of *S. platensis* was performed in 1.0 L flasks in four treatments, three from supernatant of DRA and one KM as control, each with three replications under fluorescent light in light: dark =12 hr :12 hr condition for a period of 14 days from May 20 to June 04, 2018. Growth performances of *S. platensis* varied from one medium to another. The initial cell weight of *S. platensis* was found to be increased 0.0023 mg/L and a maximum cell weight of 12.44 mg/L was found in KM followed by 12.359 mg/L in supernatant of 2.50% DRA on the 10th day of culture. The chlorophyll a content of *S. platensis* was found to be increased from the initial value of 0.0015 mg/L to the highest content of 10.54 mg/L when cultured in KM followed by 10.468 mg/L grown in supernatant of 2.50% DRA on 10th day of culture. Similar trend was followed in the case of total biomass. A decreasing trend of cell weight was observed from 10th day of culture. The growth of *S. platensis* was significantly ($p<0.05$) better in supernatant of 2.50% DRA than other concentrations of DRA (5.0 and 1.25%). Spirulina contained high crude protein (58.52%) when grown in KM which was almost similar to those grown in 2.50% DRA medium (crude protein 57.25%). From the results obtained in the present study, it is summarized that the growth and production of crude protein (around 57%) in a single cell of spirulina found better when grown in supernatant of 2.50% DRA than other concentrations of DRA. So it is concluded that the concentration of 2.50% DRA is most cost effective and suitable for spirulina culture and production than compared with standard KM. The rotten apple is easily available, mostly free and inexpensive in the country. Therefore, supernatant of digested rotten apple can be used for commercially and economically viable mass culture of *S. platensis.*

Key words: Spirulina, Protein, Supernatant, Rotten apple

Introduction

Microalgae not only play an important role in aquaculture as feed source but together with bacteria, they also have an important role in the O_2 and CO_2 balance in water (Habib *et al*., 2008 and Sukumaran *et al*., 2018). They contain all the essential amino acids (except systine and methionine) in sufficient amount to be utilized as human, fish and animal food (Habib *et al*., 200 and Sarr *et al*. 2019). These live foods are considered to be the best food for fishes. Fish larvae grow better on living foods than non- living diets (Rosas *et al*. 2019). Microalgae are a potential source of minerals in fish diets, which can replace a mixture of minerals if incorporated in small amounts (Abouelezz *et al*., 2017). In aquatic system. fish also gets protein, lipids and minerals by feeding on rotifers, which cause bioaccumulation of nutrients by directly feeding on algae (Habib and Rahman, 1993, Habib and Kohinoor, 2018 and Khalila *et al*., 2018). The number of species of microalgae is estimated to be 22,000-26,000 out of which about 50 have been studied in detail with regard to their biochemistry and echo-physiology (Clesceri *et al*., 1989 and Habib *et al*., 2008). Most of the microalgal species are autotrophic. But microalgae represent all photosynthetic, prokaryotic and eukaryotic microorganisms. They are at the beginning of the food chain in nature and produce oxygen during photosynthesis. Algae act as an ideal waste remover in nature (Rejdalje *et al.*, 1989). Some researchers used algae to remove toxic and recalcitrant compounds from the aquatic bodies to make the environment free from hazardous materials (Rejdalje *et al*., 1989, Habib *et al*., 2008 and Habib and Kohinoor, 2018).

Among microalgae, high protein (around 60%) (Phang *et al*., 2000, Becker 2007, Habib *et al*., 2008, Jias *et al*., 2016 and Sarr *et al*., 2019) and lipids (18-20%) were found in spirulina when smartly grown in sago waste (Phang *et al*., 2000), digested poultry waste (Parvin, 2006), digested rotten potato (Habib *et al*., 2019), in fermented Thai rice noodle factory waste water (Veteyasuporn, 2004) and matters industrially produced (Muys *et al*., 2019). Spirulina contains high amount of poly-unsaturated fatty acids (PUFAs) (1.5-2.0%) of its 5-6% total lipids (Lu and Takeuchi, 2004 and Sarr *et al*., 2019); γ-linolenic acid about 36% of total PUFAs (Ayachi *et al*., 2004, Muys *et al*., 2019); and is rich in antioxidant (Anbarasan *et al*., 2011, Raji *et al*., 2018, Rosas *et al*., 2019). It also contains the essential nutrients like carotene & phycocyanin (Habib *et al*., 2008, Walter *et al*., 2011, Rosas *et al*., 2019 and Wicaksono *et al*., 2019), vitamins (Venkataraman and Beckar, 1986; Bhattacharya and Shivaprakash, 2005 and Jias *et al*., 2016). It contains all essential minerals and works as a chelatizing agent (Venkataraman and Beckar 1986; Maeda and Sakaguchi 1990 and Habib *et al*., 2008), removes chromium (Doshi *et al*., 2009) and heavy metals from the environment (Jias *et al*., 2016). According to some researchers, one gram of spirulina protein is equivalent to one kilogram of assorted vegetables. The amino acid composition of spirulina protein was ranked high among the best plant in the world, more than that of soyabean (Raji *et al*., 2018). Gamma-linolenic acid contained in this algae were reported to stimulate prostaglandin synthesis and induction of the regulation of blood pressure, cholesterol synthesis, inflammation and cell proliferation (Habib *et al*., 2008 and Borowitzka and Borowitzka, 2010). Spirulina provides all essential nutrients without excess calories and fats. It is recommended to control obesity & premenstrual stress, and chronic leukemia (Subhashini *et al*,. 2004*).* Many herbal cosmetics like face creams, bio-

lipsticks, hair lotion etc. have been formulated from phycocyanin pigment found in spirulina. The beta carotene and other carotenoids are suggested to have role in the control of cancer in human and enhancement of pigmentation of eggs and meats of hens and birds (Alvarenga *et al*,. 2011 and Zahroojian *et al.,* 2013) and coloration of ornamental fish and fish fillet (Rosas *et al*., 2019).

Spirulina is used as a potential health food for humans and other animals (Becker 1984; Alvarenga *et al*., 2011; Zahroojian *et al*., 2013 and Abed *et al*. 2016). In Mexico, spirulina is used to enrich candies. In Australia and New Zealand, beverages of this substance are marketed. In Japan, India, Singapore and South Africa, spirulina enriched appetizers are sold specially to pregnant women (Becker, 1984 and Niang *et al*., 2017), anaemic and malnourished children (Abed *et al*., 2016) and elderly people (Habib *et al*., 2008). Spirulina is a good health food (Becker, 1986) but also a natural colouring agent in Japanese chewing gums (Habib *et al*. 2008). Countries like Chile, France, Cuba, Germany, Switzerland, Spain, Portugal, Sweden, Holland, Belgium, Denmark, United Kingdom, Australia, and New Zealand market food complements which include spirulina as the main component (Habib *et al*., 2008). Internationally, skin care products, shampoos, dyes, masks, creams and tonics containing this micro-organisms are marketed. In Sweden low calorie bread enriched with spirulina is sold, and in France a vegetable paste, made of spirulina is sold as bread spread. In China, spirulina is used as a substitute of imported forage to promote the growth, immunity and viability of prawn (Habib *et al*. 2008 and Lu *et al*. 2011). Spirulina (*Spirulina platensis*) has been used as a model organism in many studies. In Biological Research Division, BCSIR, Dhaka, spirulina was cultured at pilot plant scale for over 19 years in Bangladesh (Jahan *et al*., 1994). Some media were developed in the same laboratory for domestic scale culture of spirulina in Bangladesh (Khatun *et al.,* 2006). Spirulina is used to replace fish meal in diets of fish post-larvae/fry which resulted good growth performances (Habib and Kohinoor, 2018).

In Bangladesh, a lot of waste materials and effluents of agroindustrial products are available which have nutritional value (Habib *et al*., 1998). Among these sugar mills, fertilizer factory, biscuit factory, sago factory, poultry industry etc. are important (Habib *et al*., 200; Satter, 2017; Habib and Kohinoor, 2018 and Sukumaran *et al*., 2018). Spirulina is grown well in supernatant of digested rotten potato in the laboratory (Habib *et al*., 2019), in good nitrogen regimes & temperature (Costa *et al*., 2007), ammonia & urea (Soletto *et al*., 2005), urea as source of nitrogen in real environment (Sukumaran *et al*., 2018), CO_2 & light intensity (Soletto *et al*., 2008), and pH (Ogbonda *et al*., 2007). It was produced in supernatant of digested poultry waste with high lipids (Parvin, 2006 and Habib and Kohinoor, 2018), in sea water with high pigment production (Mary *et al*., 2010), and used in diets of stinging catfish fry (Habib and Kohinoor, 2018). The quality of about 12-15% apples (*Malus domestica*) become deteriorated during storage and marketing, and are sold for human consumption producing considerable amount of waste. Apple contains high carbohydrate, protein, lipid, vitamin, mineral and phosphorus. This phosphorus might help to produce high phospho-lipids and ultimately increased the amount of total lipids (Lu and Takeuchi 2004, Habib and Kohinoor, 2018). These wastes are easily available nationwide all the time and can be collected from the market.

Therefore, the supernatant of this inexpensive waste material may be used to produce spirulina (*Spirulina platensis*). The present study was conducted to culture spirulina in supernatant of digested rotten apple (*Malus domestica*) medium, to record the growth performances of spirulina and to produce of single celled protein with the following specific objectives:

a) To evaluate the growth performances of spirulina grown in supernatant of digested rotten apple (SDRA); and
b) To analyze crude protein bioaccumulation and the proximate composition of spirulina grown in SDRA.

Materials and methods

The experiment was conducted in the laboratories of the Department of Aquaculture, Faculty of Fisheries, and Department of Animal Nutrition, Bangladesh Agricultural University (BAU), Mymensingh. The rotten apple was selected as medium for spirulina (*Spirulina platensis*) culture due to presence of high organic as well as inorganic nutrients specially carbohydrate. The rotten apple was collected from KR market, BAU campus, Mymensingh.

A. Maintenance of pure stock culture of spirulina

Pure stock culture of spirulina was maintained in the laboratory in Kosaric medium (KM) (Modified after Zarrouk, 1996). Growth of spirulina was monitored at every alternate day and was checked under a microscope to confirm its purity following the keys of Bold and Wynne (1978), Yamaguchi (1992), Vymazal (1995) and Phang and Chu (1999).

B. Preparation of supernatant of DRP and Kosaric medium (KM)

The collected rotten apple (*Malus domestica*) were cut into pieces, air and oven dried (50°C) overnight, ground, packed in polythene bag and kept in the laboratory for future use. Then 100 g/4.0 L dry rotten apple was allowed to decompose in 5.0 L glass bottle for 22 days under aerobic condition with aeration in the laboratory. Then a light reddish white coloured supernatant from bottle was screened through a net of 30 µm, mixed with 9.0 g/L sodium bicarbonate and 0.50 ml/L micronutrient from the stock solution and 0.20g/L urea (Khan *et al*), and made three concentrations at the rate of 25% (digested 2.50g dry apple/100 L), 50% (digested 2.5g dry apple/100 L) and 75% (digested 5.0 g dry apple/100 L) digested rotten apple (Table 1). The 600 ml/L supernatant of three different concentrations were taken in 1.0 L flask with three replications. Simultaneously, Kosaric medium (KM) using different laboratory grade inorganic chemicals was prepared for spirulina culture as a control (Table 2). The medium in flasks were mixed well and sterilized at 121°C for 15 minutes with moist heat by autoclave (Express Equipment, Dixon's Surgical Instrument Ltd.). After autoclaving, the media were kept for 72 hrs to make sure about any contamination before culture of microalgae.

Table 1: Experimental design for *Spirulina platensis* culture using supernatant of three different concentrations of digested rotten apple (DRA)

Types of medium	Treatments	Amount of dry rotten Apple, g/L DRA (% w/v)	Replications	Duration of culture (days)
Supernatant of DRA	1	1.25 (25%)	3	14
	2	2.50 (50%)	3	
	3	5.0 (75%)	3	
Kosaric medium	4	Different inorganic chemicals and micronutrients (Table 2)	3	14

For the preparation of Kosaric medium (KM), the mentioned amount (Table 2) of inorganic chemicals from no. 1 to 8 was weighed and took in a 1.0 L conical flask. Then 0.50 ml/L previously prepared micronutrient solution was pipetted in the flask and then distilled water was added up to the mark to make the volume 1.0 L (Zarrouk, 1996). Mixing, autoclaving at 12°C for 15 minutes and cooling were carried out pursuing the procedure used during the preparation of digested rotten apple (DRA) media.

C. Culture of spirulina (Spirulina platensis) in supernatant of DRA and KM

Four treatments, three from supernatant of digested rotten apple for three different concentrations viz. 1.25, 2.50 and 5% DRA, and one KM as control each (60 ml/L) with three replications were used to grow microalgae, *S. platensis* in 1.0 L volumetric flask. Spirulina was inoculated in each culture flask to produce a culture containing 10% spirulina suspension (Optical density, OD at 620 nm = 0.20) (Habib, 1998). Twenty ml of spirulina suspension needed for getting the required density. All the flasks were kept under two fluorescent tube lights (TFC, FL-40 SD/38 day light, Taiwan) (2000 lux/m^2/s) in light: dark (12h:12h) conditions in the laboratory.

The culture flasks were continuously aerated using electric aerator (Daivo pump). Two sub-samplings were carried out at every alternate 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 were sterilized with dry heat at 70°C overnight.

D. Estimation of cell weight (*dry weight*) *of spirulina* (*Clesceri et al., 1989*)

Sample containing 20 ml spirulina suspension was filtered through a Sartorius filter paper (mesh size 0.45 µm and diameter 47 mm) using high velocity vacuum pump. The filtered samples were washed three times to remove insoluble salts using 30% NaCl. The filter papers were dried in an oven for 24 hrs or overnight at 45°C, then put in desiccator, and weighed carefully after cooling.

E. Estimation of dry weight, chlorophyll a, total biomass and specific growth rates of spirulina

The microalgae was collected every alternate day withdrawing 20 ml sample twice from every flask and kept in two centrifuge tubes of 20 ml capacity. Filter papers were dried in oven at 50°C overnight and put in desiccator in next day. Two sets of 20 ml sample in tubes were filtered using pre-weighed Sartorius microfilter paper (mesh size 0.45µm and diameter 47mm)

Table 2: Composition of Kosaric medium (Modified after 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) $CuSO_4.5H_2O$	0.08
	v) MoO_3	0.01
	vi) $CoCl_2.6H_2O$	0.01

using high velocity diaphragm vacuum pump (S: 2.40 m^3/h). Filter papers were carefully collected and folded (keeping algae inside). One filter paper was kept in glass petri discs and put in oven over night at 50°C. Next day, the dry filter papers were taken out and put in desiccator. Weight of spirulina with dry filter papers was weighed. Weight of spirulina was measured by subtracting weight of filter paper from dry weight of filter paper with spirulina. Then another set of 20 ml sample in tube was filtered using dry filter paper following the above procedure, cut into small pieces and put in plastic centrifuge tube (20 ml capacity). Ten ml acetone was put in plastic tube having the pieces of filter paper were broken into very minute pieces using glass rod. After breaking, microalgae were left attachment with filter paper and then acetone was put up to the mark of 20 ml of centrifuge tube. The tubes were wrapped with aluminium foil and kept at 4°C in freezer (LG Electronics Model No. GR-T312GE) overnight. On the following day, the tubes were taken out from the freezer and kept for some time to reach the samples at ambient temperature. Then the tubes were centrifuged at 3000 rpm for 10 minutes to settle down the small pieces of filter paper as ppt at the bottom of

the tubes. The supernatant from every tube was taken out using micropipette very carefully and kept in another centrifuge tube one by one. The collected supernatant was taken in cuvette, run through UV Spectrophotometer (Mittion Roy, Spectronic 1001 plus) to analyse chlorophyll-*a* of microalgae at three different OD (630, 647 and 664 nm) and data were recorded. The chlorophyll a was calculated using the formula: 11.85 (OD 664 nm) – 1.54 (OD 647 nm) – 0.08 (OD 630 nm). Total biomass was calculated using the formula given by Vonshak and Richmond (1988): Total biomass = Chlorophyll-*a* x 67.

All the microalgal samples were collected just before reaching the stationary phase. The stationary phase was recorded giving two growth trials of spirulina and then final experiment was conducted to collect spirulina before stationary phase. These samples were used to collect spirulina for the analyses of growth parameters and proximate composition of spirulina (Horwitz, 1984). All the analyses were done following Clesceri *et al*., (1989) and Habib (1998).
Specific growth rates (SGRs) on the basis of dry weight, chlorophyll a content and total biomass of spirulina were calculated using the following formulas (Clesceri *et al.,* 1989):

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 day.

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 day.

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 day.

F. Analyses of physio-chemical properties of digested rotten apple and supernatant

The physico-chemical properties of digested rotten apple (DRA) were analyzed using different chemicals and equipments. These properties such as pH, total suspended solids, total dissolved solids, total alkalinity, nitrate-N (NO_3-N) and phosphate-P (PO_4-P) of DRA were analyzed in the laboratory of the Department of Aquaculture, BAU, Mymensingh following Clesceri *et al*. (1989).

G. Analyses of proximate compostion of rotten apple and spirulina

The proximate composition of rotten apple and spirulina such as moisture, crude protein, crude lipids, ash and nitrogen free extract (NFE) were analyzed in triplicates following the standard methods (Horwitz, 1984).

H. Statistical anatysis

Analysis of variance (ANOVA) of mean cell weight and chlorophyll- *a*, and crude protein, crude lipid and ash of *S. platensis* cultured in different media (treatments) were done and to find whether any significant difference among treatment means was done by Tukey test using statistical package following Zar (1984).

Results and discussion

A lot of fruits, vegetables, agricultural products like apple, banana, orange, potato, tomato, cauliflower, onion, garlic etc. are spoiled (rotten) in the market (Khan *et al*., 2018a and Habib *et al*., 2019). Among the fruits, apple is an important item which is spoiled in store and during marketing. These rotten apples were collected, dried, ground, and aerobically digested in glass jar in the laboratory following Habib and Kohinoor (2018). The proximate composition (Table 3) and physico-chemical properties of rotten apple (Table 4) were done. The supernatant of digested rotten apple contained carbon, nitrogen and other inorganic nutrients almost 10 times lower than raw apple (Table 5). The rotten apple contained enough carbon in collected sample and digested samples of rotten apple which worked as an important source of carbon helped for algal growth but contained low nitrogen. Usually these types of agricultural products contain high carbon and low nitrogen (Habib *et al*., 2019). To compensate nitrogen deficiency, 0.20 g /L urea was added (Khan *et al*., 2018b; Mia *et al*., 2018 and Habib *et al.* 2019). The most important algal species, *Spirulina platensis* commonly known as spirulina was cultured in supernatant of three concentrations of digested rotten apple (DRA) such as 1.25, 2.50 and 5.0 g/L on dry weight basis, and in Kosaric medium (KM) as control at ambient temperature in the laboratory. The experiment was developed to evaluate culture and growth performance of *S. platensis* in the laboratory. The initial cell weight of *S. platensis* was 0.011 mg/L in all the treatments which finally attained a maximum to 12.359 mg/L when cultured in supernatant of digested 2.50% DRA, 9.102 mg/L in supernatant of digested 5.0% rotten apple (DRA), 7.679 mg/L in supernatant of 1.25% DRA and 12.44 mg/L in Kosaric medium (KM) (Table 6, Figs. 1 & 2). The growth of cell was found to vary from one to other media. This variation in the cell weight happened most probably due to variation in composition in media and differences in nutrient concentrations (Ogbonda *et al*., 2007; Sukumaran *et al*., 2018; Habib *et al*., 2019 and Muys *et al*. 2019). The growth rate of *S. platensis* was found higher in KM than other different concentrations of supernatant of DRA. The higher cell weight and chlorophyll-*a* content of *S. platensis* was observed in supernatant of 5.0% DRA than other two concentrations of DRA. It might be happened due to suitable nutrient quantity and nutrient composition for growth of cell than other concentrations of DRA during the culture. The concentrations of 1.25 and 2.50% DRA were not suitable and favorable for growth of *S. platensis*. Habib *et al*. (2008) and

Habib and Kohinoor (2018) recorded that agro-industrial wastes such as poultry waste, biscuit factory waste effluents, other agro-industrial wastes contain organic as well as inorganic nutrients very favourable for growth of spirulina which has similarity with the present findings. Phang *et al*., (2000) recorded very good nutritional status of spirulina when cultured in sago waste effluent. Habib *et al*., (2019) cultured *Spirulina platensis* in supernatant of digested rotten potato and got very good production which has the similarity with the present results.

Table 3: Proximate composition (%) of rotten apple on moisture and dry weight basis

Composition	**Moisture basis (%)**	**Dry basis (%)**
Moisture	87.31	10.61
Crude protein	0.418	3.30
Crude lipids	0.780	6.15
Ash	0.603	4.75
Crude fiber	0.770	6.07
NFE*	10.11	69.11

*NFE (nitrogen free extract) = 100 - (moisture + crude protein + crude lipids + ash).

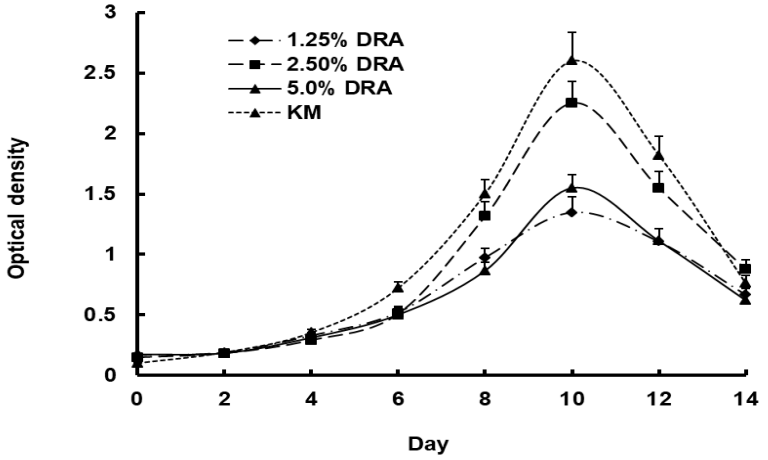

Fig. 1: Mean values of optical density of media contained *Spirulina platensis* in supernatant of three different digested rotten apple and Kosaric medium. Vertical bars represent standard errors

Table 4: Physico-chemical characteristics of ground rotten apple

Sl. No.	Characteristics of rotten apple paste	Properties
1	Colour	Reddish white
2	Odour	Little bid bad
3	Structure	Semi-solid
4	Temperature	28.30-28.60ºC
5	pH	6.30-6.45
6	Total solids (TSS + TDS)	1954-2135 mg/L
7	Alkalinity	132-142 mg/L
8	Total N	1.55-1.76 mg/L
9	Available N (NO_3-N)	1.10-1.15 mg/L
10	Available P (PO_3-P)	2.90-3.30 mg/L

Table 5: Physico-chemical properties of supernatant of digested rotten apple after digestion in aerobic condition

Sl. No.	Characteristics	
1	Temperature	28.20-29.50°C
2	pH	6.80-6.90
3	Total solid (TSS + TDS)	125-153 mg/L
4	Alkalinity	140-160 mg/L
5	Total-N	1.20-1.40 mg/L
6	Available-N (NO_3-N)	1.05-1.10 mg/L
7	Available-P (PO_3-P)	2.40-2.70 mg/L

Table 6: Comparison of cell weight, chlorophyll a and total biomass of *Spirulina platensis* grown in supernatant of three different concentrations of digested rotten apple (DRA), and Kosaric medium on the 10 day of culture before stationary phase

Parameters	T_1 (1.25% DRA)	T_2 (2.50% DRA)	T_3 (5.0% DRA)	T_4 (KM)
Optical density	1.331 ± 0.12^b	2.270 ± 0.15^c	1.568 ± 0.12^c	2.63 ± 0.20^a
Cell weight (mg/L)	7.679 ± 0.23^b	12.359 ± 0.52^c	9.102 ± 0.42^c	12.44 ± 0.21^a
Chlorophyll-*a*(mg/L)	6.919 ± 0.14^b	10.468 ± 0.32^c	7.360 ± 0.20^c	10.54 ± 0.14^a
Total biomass (mg/L)*	463.57 ± 8.13^b	701.36 ± 9.28^c	493.12 ± 8.30^c	706.18 ± 9.50^a

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

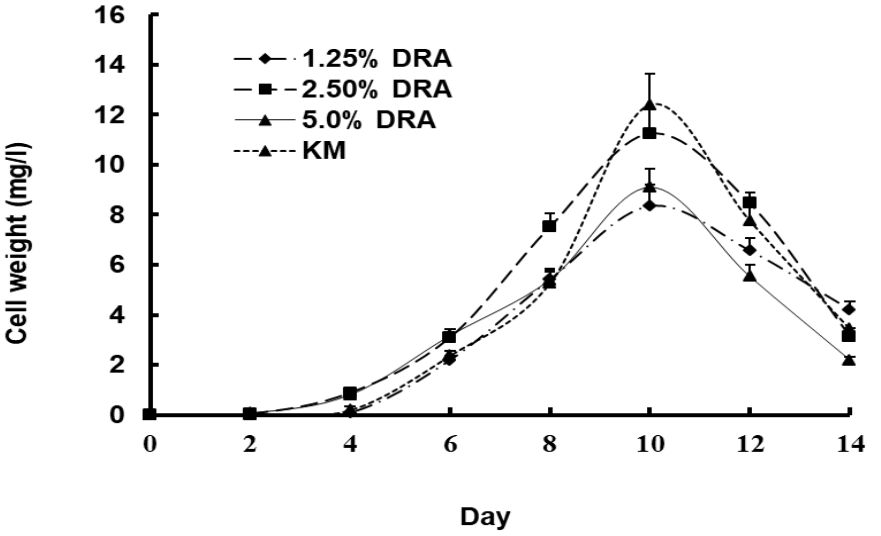

Fig. 2: Mean values of cell weight of *Spirulina platensis* grown in supernatant of three different digested rotten apple, and Kosaric medium. Vertical bars represent standard errors

During the culture period, exponential phase was found up to 10th 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 and pH played a significant role to the whole culture system. During the culture, the climatic condition was more or less suitable and favourable for the growth of *S. platensis*. Similar type of work was carried out by Mary *et al.* (2010) where the annual yield of biomass of *Spirulina maxima* strain 4MX grown in fertilized sea water in out door system was 7.36 mg i.e. 0.39 g/L/d which was higher than the present study. In the present study, the cell weight of *S. platensis* in supernatant of DRA, and KM were lower than the findings of Sukumaran *et al*., (2018) who grown spirulina in media enriched with urea in real environment. The variation in production 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 recorded that yield of spirulina sp. was 8 to 12 $g/m^2/d$. This yield found from the experiment was also much higher than the present findings. Sukumaran *et al*., (2018) reported that the biomass output rate in Chinese production plant was 7.0 $g/m^2/d$ which was almost similar with the results of the present study. Similarly, Tanticharoen *et al.*, (1991) reported that the addition of $NaHCO_3$ and nirogen fertilizer in waste water from the stabilization pond of topica starch factory raised the productivity up to 7-10 g/m/d which was much higher than the findings of the present study. The variation in the above results might occur due to nutrient composition of different media and physico-chemical factors involved in

the culture. Khan *et al*., (2018b) found that molasses is a good source of carbon where microalgae grew very well which has similarity with the present results. Crude lipids were bio-accumulated in spirulina about double than the same in spirulina cultured in Kosaric medium (Table 7) which might be due to presence of high inorganic carbon in media of DRA. Satter (2017) and Habib and Kohinoor (2018) stated that lipids were bioaccumulated in high amount (more than double) in spirulina (*Spirulina platensis*) when grown in supernatant of aerobically digested poultry waste than spirulina cultured in KM. This might be happened due to the formation of phospho-lipids in high amonut in this spirulina due to presence of high phosphorus in poultry waste (Satter, 2017 and Sukumaran *et al*., 2018).

Table 7: Proximate composition (% in dry matter basis) of *Spirulina platensis* cultured in supernatant of three different concentrations of digested rotten apple (DRA) and control as Kosaric medium

Treatments	T_1 (1.25% DRA)	T_2 (2.50% DRA)	T_3 (5.0% DRA)	T_4 (KM)
Moisture	8.20 ± 0.07	8.21 ± 0.07	8.21 ± 0.07	8.20 ± 0.07
Crude Protein	53.74 ± 0.38^b	57.25 ± 0.42^a	54.25 ± 0.52^b	58.52 ± 0.44^a
Crude Lipids	10.15 ± 0.28^b	14.62 ± 0.23^a	11.02 ± 0.19^b	6.31 ± 0.23^c
Ash	9.20 ± 0.16^b	10.14 ± 0.19^b	10.42 ± 0.24^b	13.52 ± 0.13^a
NFE*	17.99 ± 0.35^a	19.19 ± 0.18^a	15.35 ± 0.22^b	12.72 ± 0.28^c
Crude Fibre	0.71 ± 0.04	0.72 ± 0.03	0.74 ± 0.04	0.72 ± 0.03

*NFE (nitrogen free extract) = 100 - (moisture + crude protein + crude lipids + ash). Figures with common letters in the same row do not differ significantly at 1% level of probability.

Spirulina is a good source of single celled protein which may be produced in different media but bioaccumulation of protein and sometimes lipids may vary in respect to the inorganic nutrient concentrations of media. The contents of protein and lipids in spirulina were found higher (57.25% protein and 14.62% lipids) cultured in supernatant of DRA than standard Kosaric medium (Table 7) where Phang *et al*., (2000) recorded around 60% protein and 20% lipids in spirulina grown in supernatant of sago starch factory waste effluent. The present results has similarity with the findings of Tantichareon *et al*., (1991) when cultured spirulina in tapioca starch wastewater, Vetayasuporn (2004) grown spirulina in Thai rice noodle factory wastewater and Habib *et al*., (2019) cultured spirulina in supernatant of digested rotten potato. Cheirsilp and Louhasakul (1913) found very good growth of spirulina in different industrial wastes (palm oilmill waste, cerum latex rubber waste, mollasses and crude glycerol) and produced high lipids which directly transesterified into biodiesel production and also bioelectricity (Thong *et al*., 2019).

Cell weight of spirulina (*Spirulina platensis*) had highly significant ($P< 0.01$) direct correlation with chlorophyll-*a* ($r = 0.942$) of spirulina grown in the supernatant of different digested rotten apple and Kosaric medium during the study (Fig. 3). Similarly, total biomass of *S. platensis*

was highly (P< 0.01) and directly correlated with chlorophyll-*a* (r = 0.961) of spirulina cultured in the supernatant of various digested rotten apple and Kosaric medium (Fig. 4). Again, total biomass of spirulina was found to be highly (P < 0.01) and directly correlated with the cell weight (r = 0.889) of spirulina grown in the supernatant of different digested rotten apple and Kosaric medium (Fig. 5). These correlations meant that cell weight, chlorophyll a and total biomass of spirulina were increasing and decreasing accordingly. Habib *et al*., (2019) recorded highly significant (P<0.01) correlation among cell weight and chlorophyll-*a* of spirulina, total biomass with chlorophyll-*a* of spirulina and total biomass with cell weight of spirulina which are more or less similar with the present findings. Costa *et al*. (2007), Jais *et al*., (2016) and Muys *et al*., (2019) also found similar results.

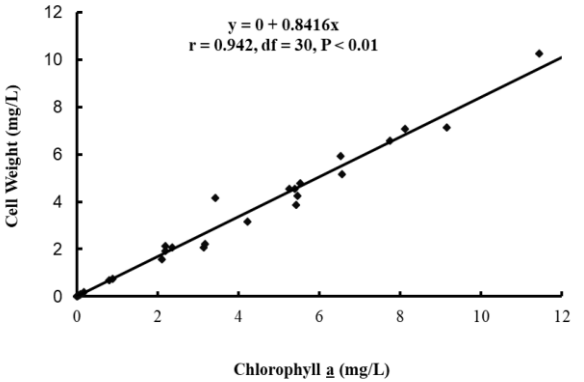

Fig. 3: Correlation coefficient (r) of cell weight (mg/L) with chlorophyll a (mg/L) of spirulina grown in supernatant of three digested rotten apple, and Kosaric medium

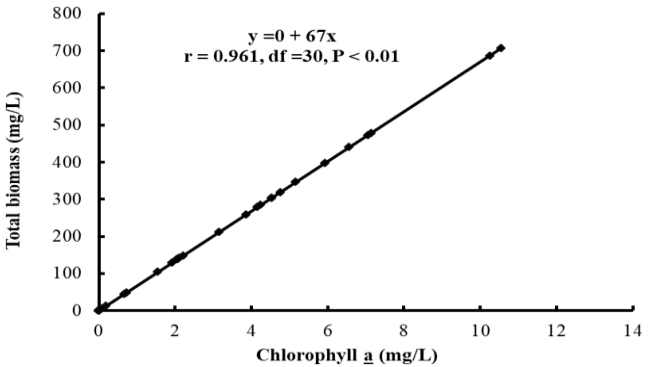

Fig. 4: Correlation coefficient (r) of total biomass (mg/L) with chlorophyll a (mg/L) of spirulina grown in supernatant of three digested rotten apple, and Kosaric medium

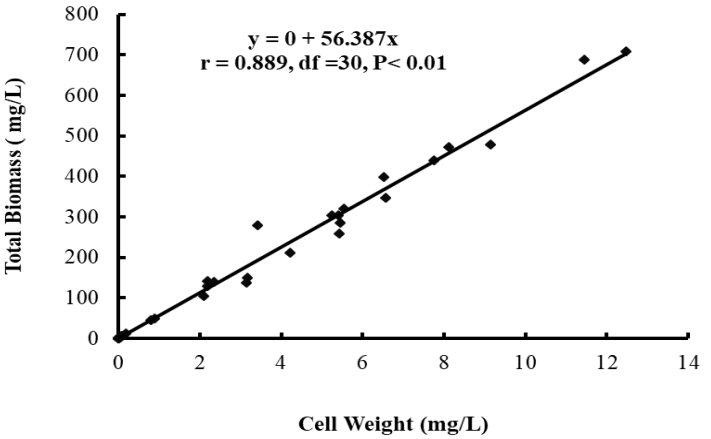

Fig. 5: Correlation coefficient (r) of total biomass (mg/L) with cell weight (mg/L) of spirulina grown in supernatant of three digested rotten apple, and Kosaric medium.

From the findings of this study, it is important to mention that spirulina may be successfully grown in agro-industrial waste materials like fruits, vegetables etc. and used in feed of different domestic animals like fishes, poultry, cattle etc. due to presence of high percentage of protein.

Conclusion

From the results obtained in the present study, it can be concluded that the growth of spirulina (*Spirulina platensis*) was better in the concentrations of 2.50% DRA than other concentrations of DRA. Thus, the concentration of 2.50% DRA was most suitable for *S. platensis* culture compared with standard Kosaric medium. These media are easily available and most inexpensive in contrast of Bangladesh. So digested rotten apple can be used for commercially and economically viable mass culture of spirulina.

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