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Culture and production of spirulina (*Spirulina platensis*) in supernatant of digested rotten potato

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Abstract. An experiment was conducted to evaluate culture and growth performance of spirulina (*Spirulina platensis)* in supernatant of three different amount of digested rotten potato (DRP), and Kosaric medium (KM) as control. Three different concentrations such as 25, 50 and 75% of DRP were digested under aeration and the reddish white coloured supernatant was collected. Spirulina was inoculated in supernatant of DRP with the addition of 9.0 g/L $NaHCO_3$ and micronutrients, and KM for a period of 14 days. The cell weight of spirulina was attained a maximum of 11.48 $\pm$1.25 mg/L in KM followed by 11.46$\pm$1.03, 9.16$\pm$0.84 and 8.13$\pm$0.73 mg/L in supernatant of 50, 25 and 75% DRP, respectively on the 10th day of culture. Similar trend was also observed in the cases of optical density, chlorophyll a, total biomass, specific growth rates and total biomass of spirulina. Cell weight of spirulina grown in these media had highly significant ($p<0.01$) correlation with the chlorophyll a content and total biomass. The growth performance of spirulina grown in supernatant of 50% DRP was significantly higher than that of spirulina grown in supernatant of 25 and 75% DRP. The percentage of crude protein (55.15%) of spirulina grown in supernatant of DRP was little bit lower than that of spirulina cultured in KM (58.70%). The crude lipids (17.15%) of spirulina cultured in supernatant of 50% DRP was almost two and half times higher than that of spirulina grown in KM (6.33%). It indicates that for the production of spirulina with high lipid content, supernatant of DRP may be used. Therefore, mass culture of *S. platensis* may be done in supernatant of 50% DRP.
Key words: Spirulina, Rotten potato, Kosaric medium

Introduction

Spirulina platensis is a multicellular, blue-green alga. It is very small and microscopic and 300-500μm in length. This blue - green algae contains 50-70% protein, 10-12% carbohydrate, 6% fat, 7% minerals and a lot of vitamins (Lim 1990, Habib *et al*. 1997, Shuvo 2001). A considerable amount of phosphorous, magnesium, zinc and pepsin is found in *Spirulina*. It also consists of 6-11% polysaccharide, 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%). It is rich in B vitamins, minerals and trace elements, chlorophylls and enzymes (Li and Qi 1997, Habib *et al*. 2003). The cell wall of spirulina consists of polysaccharide which has a digestibility of 86% that could be easily absorbed in human body. The Wuhan Botanical Institute has collaborated with the Changed Central School of Physical training to study the effect of oral intake of spirulina pill on the physical status of athletes (Li 1995, Abed *et al*. 2016). After taking 10 g spirulina pills per day for four weeks, female athletes showed increase in their hemochrome level, whereas the male athletes did not show any apparent increase. The lung capacity of juvenile weight lifting and jujutsu athletes was improved. Spirulina could serve as an auxiliary cure for many diseases which have shown by clinical traits. Spirulina capsule has

improved the coalitions of in lowering blood lipid level and in decreasing white blood corpuscles after radiotherapy and chemotherapy as well as lowering immunological function (Ruan *et al*. 1990, Abed *et al*. 2016). It has been used for last ten years as a model organism in many studies on outdoor cultivation of algal biomass as a source of proteins and chemicals (Richmond 1988, Kim 1990, Li 1995). Spirulina species not only contribute in human health but also play considerable role in fish and animal feed (Rosas *et al*. 2019, Sarr *et al*. 2019). The yellowness and redness in broiler flesh increased when *Spirulina* was fed with diet (Habib *et al*. 2008, Sujatha and Narahari 2011, Rosas *et al*. 2019). In China, spirulina is used as substitute of imported forage to promote the growth, immunity and viability of prawns (Li 1995, Li and Qi 1997). Spirulina increase shell thickness of scallop. The survival rate of abalone was improved from 37.4 to 85.0%. Feeding on *Spirulina* helped to improve disease resistance of some high valued fish resulting in increased survival rate (Kebede and Ahlgren 1996, Sarr *et al*. 2019). When spirulina was added to forage for poultry and livestock, their growth rate was improved (Ross and Dominy 1985, Sujatha and Narahari 2011, Abouelezz *et al*. 2019).

Bangladesh is now producing plenty of potato and huge amount of potato are being sold in cheap price. About 15-20% potato get rotten in the market during selling every year especially in peak season (Uddin *et al*. 2007). These rotten potatoes or spoiled potatoes are thrown outside as waste material which decompose and create environmental hazards. However, it contains high broken organic and inorganic nutrients, and high biological oxygen demand (BOD) and chemical oxygen demand (COD), total dissolved solids, total suspended solids, nitrate, phosphate and also inorganic nutrients (Habib 1998, Habib and Kohinoor 2018). These organic and inorganic nutrients rich in carbon can help grow spirulina in supernatant after aerobic or anaerobic digestion of potatoes. The present work was undertaken to study the culture and growth performance and proximate composition of *S. platensis* in the supernatant of digested rotten potato.

Materials and Methods

The rotten potatoes were selected as medium for *Spirulina platensis* culture due to presence of high organic as well as inorganic nutrients specially carbohydrate. The rotten potatoes were collected from KR market, BAU, Mymensingh.

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

***Preparation of supernatant of digested rotten potato (DRP) and Kosaric medium (KM)*:** The collected rotten potatoes were cut into small pieces, air and oven dried (40°C), ground, packed in polythene bag and kept in the laboratory for future use. Then 90 g/4.0 L dry rotten potato was allowed to decompose in 5.0 L glass bottle for 22 days in the laboratory under aerobic condition with aeration. A light reddish white coloured supernatant from the bottle was screened through a net of 30 μm mesh size, mixed with 9.0 g/L sodium bicarbonate and 0.20 ml/L micronutrient, then diluted and made three concentrations as shown in (Table I). The

supernatant of three different concentrations were taken in 2.0 L flask with three replications. Simultaneously, the medium in flasks were mixed well and autoclaved.

Table I. Experimental design for *S. platensis* culture using supernatant of three different concentrations of digested dry rotten potato (DRP)

Types of medium	Treatments	Amount of dry rotten potato (g/L DRP) (%)	Duration of culture (days)
Supernatant of DRP	1	2.025 (25%)	14
	2	4.050 (50%)	
	3	6.075 (50%)	
Kosaric medium	4	Different inorganic chemicals and micronutrients (Table II)	14

Kosaric medium (KM) was prepared for *S. platensis* culture as a control (Table II). For the preparation of KM, the nutrients from No. 1-8 mentioned in Table II was weighed and took in a 1.0 L conical flask. Then 0.5 ml micronutrient solution was pipetted in the flask and distilled water was added to make the volume 1.0 L. Mixing, autoclaving and cooling were carried out pursuing the procedure used during the preparation of digested rotten potato medium.

Table II. Composition of Kosaric medium (Modified after Zarrouk 1996) for *S. platensis* culture

Sl. No.	Chemicals	Concentration in stock solution g/L	9. A_5 micronutrient solution[a]	0.5ml/L
1.	$NaHCO_3$	9.0	A, A_5 micronutrient solution	g/L
2.	K_2HPO_4	0.250	i) H_3BO_4	2.86
3.	$NaNO_3$	1.250	ii) $MnCl_2.4H_2O$	1.81
4.	K_2SO_4	0.50	iii) $ZnSO_4.7H_2O$	0.22
5.	NaCl	0.50	iv) $CuSO_4.5H_2O$	0.08
6.	$MgSO_4.7H_2O$	0.10	v) MoO_3	0.01
7.	$CaCl_2$	0.02	vi) $CoCl_2.6H_2O$	0.01
8.	$FeSO_4.2H_2O$	0.005		

***Culture of spirulina*:** Four treatments, three from supernatant of DRP at three different concentrations (25, 50 and 75%) and KM as control each with three replications were used to culture spirulina 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 was 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 Animal Nutrition laboratory.

The culture flasks were continuously aerated using electric aerator (Daivo pump). Four sub-samplings 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 glasswares used in the experiment were sterilized with dry heat at 70°C overnight.

Estimation of cell dry weight of Spirulina: Sample containing 20 ml spirulina suspension was filtered through a Sartorius filter paper of mesh size 0.45 μm and diameter 47 mm. The filter papers were dried in an oven for 24 hrs or overnight at 70°C and weighed prior to filtration. The filtered samples were washed three times to remove insoluble salts using 30% NaCl (Clesceri *et al*. 1989).

***Estimation of chlorophyll a and total biomass of spirulina*:** The optical density (OD) of prepared samples were taken at 664, 647 and 630 nm by using a UV spectrophotometer against blank (Milton Roy, Spectronic 1001 plus) (Clesceri *et al*. 1989). Chlorophyll a content was calculated by the formula: Chlorophyll a (mg/L)= 11.85 (OD 664) – 1.54 (OD 647) – 0.08 (OD 630). Total biomass was calculated using the formula given by Vonshak and Richmond (1988): Total biomass = Chlorophyll a x 67.

***Analyses of physico-chemical properties of digested rotten potato and supernatant*:** The physico-chemical properties of DRP and supernatant such as pH, total suspended solids, total dissolved solids, total alkalinity, nitarte-N (NO_3-N) and phosphate-P (PO_4-P) were analyzed following Clesceri *et al*. (1984).

***Analyses of proximate composition of rotten potato and spirulina*:** The proximate composition of rotten potato (RP) 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).

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) followed by Duncan's Multiple Range Test (DMRT) (Zar 1984) were performed using statistical package.

Results and Discussion

Physico-chemical properties of liquid of rotten potato and of supernatant of digested rotten potato (DRP) were almost high in quantity (Table III) which indicate that the agricultural waste materials contain high organic loads due to presence of organic carbon, PO_4-P, NO_3-N and other micronutrients (Khan *et al*. 2018a). The loads of these inorganic nutrients in supernatant of DRP were reduced at least 10 times than the raw liquid rotten potatoes which has the similarity with the findings of Habib *et al*. (1998), Satter (2017) and Khan *et al*. (2018b).

The sufficient amount of carbon, nitrogen and phosphorus were present in the supernatant which influenced the growth and production of any micro- and macro-algae. The ash content was high in rotten potatoes which indicated high macro- and micro-minerals contents (Table IV). These minerals are very helpful for the growth of any microalgae specially spirulina (Habib *et al*. 1997 and 2005, Uddin *et al*. 2007, Toyub *et al*. 2010, Khan *et al*. 2018b).

Table III. Physico-chemical properties of liquid rotten potato and of supernatant of digested rotten potato (after 22 days digestion in aerobic condition)

Sl. No.	Characteristics	Liquid rotten potato	Digested rotten potato
1	Colour	Reddish	Light reddish
2	Odour	Bad smell	-
3	Structure	Semi-solid	-
4	Temperature	28.30-28.70°C	27.20-27.40°C
5	pH	7.30-7.45	7.30-7.70
6	Total solids (TSS + TDS)	1634-1734 mg/L	248-265 mg/L
7	Alkalinity	312-345 mg/L	180-195 mg/L
8	Total N	2.14-2.25 mg/L	1.90-2.20 mg/L
9	Available N (NO_3-N)	1.30-1.45 mg/L	2.50-2.70 mg/L
10	Available P (PO_3-P)	4.40-5.16 mg/L	4.10-4.20 mg/L

Table IV. Proximate composition (%) of rotten potato on wet and dry matter basis

Composition	Wet basis (%)	Dry matter basis (%)
Moisture	80.00	9.59
Crude protein	2.16	10.80
Crude lipids	1.60	8.00
Ash	3.20	16.15
Crude fiber	3.80	19.0
NFE*	9.23	36.45

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

Spirulina was cultured in three different concentrations (25, 50 and 75%) of supernatant of DRP and KM as control. The cell weight of spirulina in supernatant of DRP were found 0.0022 to 8.35 mg/L in 25% digested rotten potato media (DRPM), 0.0022 to 11.25 mg/L in 50% DRPM , 0.0022 to 9.10 mg/L in 75% DRPM and 0.0023 to 12.40 mg/L in KM (Table V). The growth performance of spirulina in supernatant of 50% DRPM was found better than in 25% and 75% DRPM. This variation might be due to the differences in nutrient concentrations and composition of varied media. In controlled KM, spirulina 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 25% DRPM showed lower growth performance of spirulina in relation to 50 and 75% DRPM. This might be due to higher dilution and lower concentration of the nutrients in the media. The concentration of 50 and 75% DRPM which were suitable and favorable for the growth of spirulina because of the nutrient content.

Table V. Comparison of cell weight, chlorophyll a and total biomass of *S. platensis* grown in supernatant of different DRP and KM on 10th day of culture before stationary phase

Parameters	T1 (25% DRP)	T2 (50% DRP)	T3 (75% DRP)	T4 (KM)
Optical density	1.35 ± 0.12^b	2.25 ± 0.15^a	1.55 ± 0.13^b	2.60 ± 0.22^a
Cell weight (mg/L)	8.35 ± 0.20^b	11.25 ± 0.55^a	9.10 ± 0.45^b	12.40 ± 0.21^a
Chlorophyll a (mg/L)	7.02 ± 0.12^b	10.13 ± 0.35^a	7.37 ± 0.20^b	10.50 ± 0.16^a
Total biomass (mg/L)*	470.34 ± 8.15^c	678.71 ± 9.32^b	493.79 ± 8.33^c	703.50 ± 9.50^a

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

The comparative study of growth performance of spirulina in different concentration of the media indicates that higher dilution followed lower concentration of nutrients and lower growth performance. During culture of spirulina, the exponential phase was found up to 10th day from the beginning and then the cell weight declined i.e. stationary phase started. The physical properties such as light intensity, aeration and temperature played significant roles to the whole culture system. During the culture system the climate condition was more or less suitable and less suitable and favorable for the growth of spirulina. Satter (2017) recorded the cell weight and chlorophyll a content of spirulina significantly ($p < 0.05$) higher in 4.0 g/L digested poultry waste than other media where light intensity, aeration and temperature played significant role to the culture system. The present findings have the similarity with the findings of Holman and Malau-Aduli (2012) and Khan *et al*. (2018b). Dey (2004) had grown spirulina in mustard oil cake medium in the concentration of 3.0, 4.0, 0.5 mg/L and KM and found maximum growth as 451.0, 614.33, 403.4 and 719.0 mg/L, respectively. These findings are more or less similar to the present findings.

Zarrouk (1996) is the pioneer in detailed study on the response of *S. platensis* to light. He concluded that the highest growth of spirulina was saturated at the level of 25-30 klux/m^2/s. The highest growth of spirulina in the present study was found at light intensity of 2710 lux/m^2/s and 2740 lux/m^2/s at 5g/L concentration of the media and KM on the 10th day of culture. This variation might be due to difference in space and difference of light source. Khan *et al*. (2018b) used similar light intensity and found highest growth of *Chlorella vugaris* on 10th day. The initial cell weight was 0.0023 mg/L which attained a maximum cell weight of 12.40 mg/L which grown in KM and 8.34mg in 25% DRPM, 11.25 mg/L in 50% DRPM, 9.10 mg/L in 75% DRPM on the 10th day of the culture. The chlorophyll a content of inoculated *S. platensis* was 0.0015 mg/L which attained a high content of 10.50 mg/L which cultured in KM and 10.13 mg/L in 50% DRPM at the 10th day of culture (Table V). These findings are more or less similar to the findings of Phang *et al*. (2000), Habib *et al*. (2003) and Satter (2017).

In the present study the supernatant of 50% DRP showed maximum optical density on the 10th day of culture compared with KM which has the similarity with the findings of Habib *et al*. (1997, 2003) and Satter (2017). High amount of crude protein and lipids were bioaccumulated in spirulina when grown in supernatant of DRP which indicate that supernatant of 50% DRP is suitable for culture and production of spirulina. This finding has the similarity with the results of Satter (2017) when he cultured spirulina in supernatant of 4% digested poultry waste (DPW). It showed almost similar growth performances when grown in supernatant of DRP and KM. The availability of phosphate-phosphorus has been considered very important in cultured media of plankton production (Kebede and Ahlgren 1996, Phang*et al*. 2000). The phosphate-phosphorus was found higher on initial culture and minimum on the 10th day. The optical density was minimum in initial day and maximum on the 10th day (Fig. 1).Cell weight of spirulina grown in these media had highly significant ($p < 0.01$) correlation with the chlorophyll a content ($r = 0.993$) and total biomass ($r = 0.925$) of spirulina (Figs. 2 and 3). Chlorophyll a content was also highly and significantly ($p < 0.01$) correlated with total biomass ($r = 0.989$) of spirulina (Fig. 4). Though these growth parameters were all highly correlated with each other which indicate that spirulina grew in the supernatant of digested rotten potato successfully.

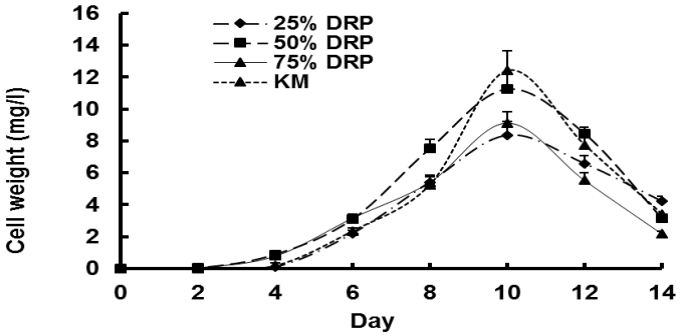

Fig. 1. Mean values of cell weight of *S. platensis* grown in supernatant of three different digested rotten potato, and Kosaric medium. Vertical bars represent standard errors.

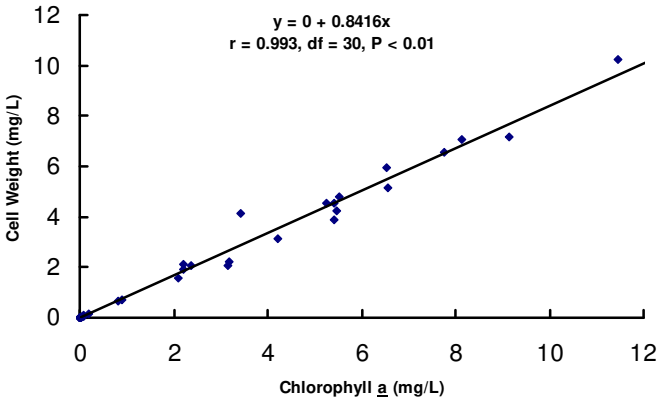

Fig. 2. Correlation coefficient (r) of cell weight (mg/L) with chlorophyll a (mg/L) of spirulina grown in supernatant of three digested rotten potato, and Kosaric medium.

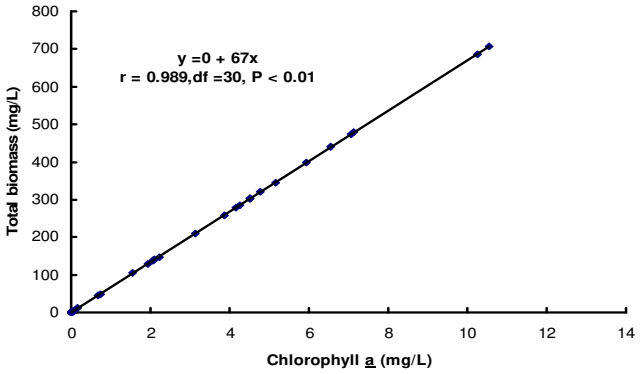

Fig. 3. Correlation coefficient (r) of total biomass (mg/L) with chlorophyll a (mg/L) of spirulina grown in supernatant of three digested rotten potato, and Kosaric medium.

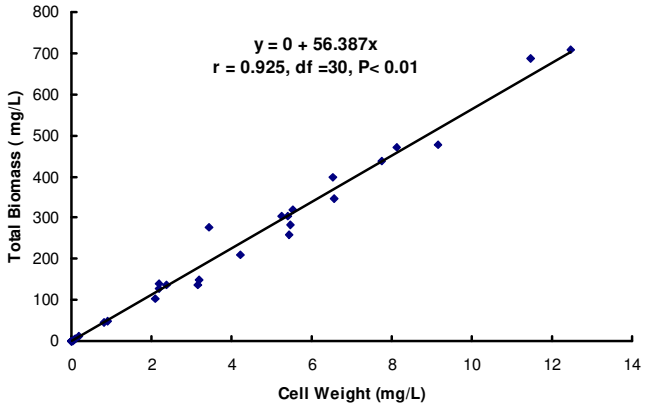

Fig. 4. Correlation coefficient (r) of total biomass (mg/L) with cell weight (mg/L) of spirulina grown in supernatant of three digested rotten potato, and Kosaric medium.

It indicates that for the production of spirulina with high lipid content, supernatant of DRPM may be used. Therefore, mass culture of *S. platensis* may be done in supernatant of 50% digested rotten potato medium.

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