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Culture of *Chlorella ellipsoidea* (Gerneck) in Supernatant of Different Concentrations of Digested Waste Potato Powder

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Abstract. The growth performance of *Chlorella ellipsoidea* was evaluated in supernatants of different concentrations of digested waste potato powder (DWPP) medium, Bold basal medium (BBM) as control and tap-water (TW) without added nutrient in the laboratory for three months. Three different amounts of waste potato powder such as 0.80, 1.00 and 1.20 g/l were allowed to digest for 10 days to get clear supernatant. The initial cell density of *C. ellipsoidea* was 2.5×10^5 per ml which attained a maximum density of 227.45×10^5 cell/ml in BBM, 220×10^5 cell/ml in supernatant of 0.80 g DWPP/l followed by 187.33×10^5 cell/ml in supernatant of 1.00 g DWPP/l and 158.67×10^5 cell/ml in supernatant of 1.20 g DWPP/l and 126.823×10^5 cell/ml in tap-water without added nutrient. Similar trend was observed in the cases of chlorophyll a of this microalga and optical density of media containing *C. ellipsoidea*. The proximate composition of waste potato powder was found to contain (on dry matter basis) 8.67% moisture, 13.31% crude protein, 1.60% crude lipids and 4.55% ash. *C. ellipsoidea* grown in various concentrations of DWPP, TW and BBM was analyzed and it was found that the amount of moisture, crude protein, crude lipids and ash varied from 7.15 to 8.00%, 23.4 to 48.11%, 8.63 to 14.23%, and 2.66 to 4.05%, respectively. Cell number of *C. ellipsoidea* was significantly ($p<0.05$) higher when grown in supernatant of 0.80 g/l DWPP than in supernatant of other concentrations of DWPP, TW and BBM. Chlorophyll a of *C. ellipsoidea* and optical density of culture media of three concentrations of DWPP, TW and BBM followed almost similar trend. However, cell number was significantly ($p < 0.05$) correlated with chlorophyll a ($r = 0.965$).

Keywords: *Chlorella ellipsoidea,* waste potato powder, culture medium

Introduction

Microalgae play very important role in aquaculture as live food for larval stages of many species of crustaceans and fish, all stages of bivalves and for the zooplankton (Islam *et al.*, 2004; Lubzens, 1987; Spektorova *et al.*, 1986). They are used as live food for late larval and juvenile fish and crustaceans in hatcheries (Renaud *et al.*, 1991). Microalga such as *Chlorella* is rich in nutrients especially protein, lipid and minerals (Khan *et al.*, 2006; Geldenhuys *et al.*, 1988; Becker and Venkataruman, 1984; Soeder, 1980). *Chlorella vulgaris* Beijerinck is an autospore forming, fast growing and nutritionally rich microalga (Habib, 1998). It contains all the essential nutrients for growth and development of both aquatic and terrestrial animals. So, people of many countries such as Malaysia, Thailand, Philippines, Indonesia, China and Japan have been using seaweeds and other algae as a source of food. Having perceived the miraculous nutritional quality of *Chlorella*, Japanese firms manufactured *Chlorella* tablets in 1959. Since that time, numerous enterprises have emerged worldwide as specialized industries with a view to producing health foods, food additives, animal feed, biofertilizers and an assortment of natural products from algae (Sasson, 1997).

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From several nutritional data analysis, it is established that *Chlorella* is the store-house of valuable nutrients. It contains long chain polyunsaturated fatty acids that make it valuable food for marine invertebrates and fishes (Watanabe *et al.*, 1983). It is usually added together with rotifers to marine larvae rearing tanks (Liao, 1975; Nash and Kuo, 1975). For such purposes *Chlorella* is cultured with special care. Bold basal medium (BBM) is the most recognized and effective medium for *Chlorella* culture, however its availability is not consistent and it is expensive. Thus for mass culture of *Chlorella* its use will not be feasible.

Microalgae grow very well in different digested agro-industrial wastes like sugar mill effluents (Khan *et al.*, 2006; Parvin and Habib, 2005), fertilizer factory waste (Khan *et al.*, 2006; Toyub *et al.*, 2005; Toyub *et al.*, 2003), agricultural products like digested bean seed powder (Karmakar *et al.*, 2001) and digested cabbage powder (Islam *et al.*, 2004). Bangladesh is an agrarian country and here about 40% potatoes become unsuitable for human consumption due to fungal and bacterial spoilage every year which are thrown away. This easily available cheap potatoe waste may be used as medium instead of BBM. It can be collected from different sources (e.g. whole sale market, retail market, food processing industries, potato chip making companies etc.), processed and used to culture microalgae. The prime objective of the present study is to establish waste potato as an indigenous inexpensive ingredient for culture of *Chlorella ellipsoidea* (Gerneck).

Materials and Methods

The microalga, *Chlorella ellipsoidea* (Gerneck) was cultured in supernatant of three concentrations of digested waste potato powder in flasks in the Live Food Culture Laboratory, Department of Aquaculture, Bangladesh Agricultural University (BAU), Mymensingh for a period of three months. Waste potatoes were collected from a retailer of BAU campus market, Mymensingh. The potatoes were peeled and chopped by a slicer into thin and small pieces and then primarily dried in sunshine for about five days. For complete drying, the pieces of potato were kept in a tray in an oven at 40 °C, overnight. The dried samples were powdered by mortar and pestle. For getting very fine particles of potato powder, it was sieved through 500 μm mesh. The powder was put in plastic packets and kept in refrigerator for future use. Prior to media preparation, the proximate composition of powder was analyzed in the Nutrition Laboratory, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh following standard methods of Horwitz (1989). Then three different amounts of waste potato powder such as 0.80, 1.00 and 1.20 g/l were mixed with distilled water and digested for 10 days using aeration (Table 1). After 10 days, a clear supernatant was found which was transferred in 2.0 l flask in triplicates after filter. No bacteria was inoculated to digest the potato powder. However, it was understood that environment friendly bacteria freely grew which digested potato powder in glass jars. Prior to autoclaving, these media were neutralized using both 0.1 N NaOH and 0.1 N HCl.

Microalga, *C. ellipsoidea* was taken from the stock in the laboratory of the Department of Aquaculture, BAU, Mymensingh. Pure stock culture of the collected sample of *C. ellipsoidea* was maintained in the laboratory in standard Bold basal medium (BBM). Growth of *C. ellipsoidea* was monitored daily and checked under microscope to confirm its purity. Digested waste potato powder (DWPP), tap-water (TW) and Bold basal medium (BBM) (Thompson *et al.*, 1988) were prepared for culture of *C. ellipsoidea*. The supernasant of three different amounts of waste potato powder, 0.80, 1.00 and 1.20 g/l (Table 1) and tap-water (TW) without added nutrient (used as 4th treatment) and BBM were used for culture. The composition of BBM is given in Table 2. The experimental design of culture media for *C. ellipsoidea* is shown in Table 3.

Cell densities of *C. ellipsoidea* were determined using an improved Neubauer ruling haemacytometer according to Clesceri *et al.* (1989). Optical densities of the samples were determined at 620 nm by using UV Spectrophotometer, Milton Roy, Spectonic 1001 Plus (Clesceri *et al.*, 1989). The physical and chemical parameters such as light intensity, temperature, pH and dissolved oxygen of culture media were recorded on alternate days. The analysis of proximate composition of cultured *C. ellipsoidea* was carried out following the standard

Table 1. Different amounts of waste potato powder digested for 10 days for preparation of supernatant

Treatments	Organic material	Amount per litre (g)	Amount of urea (g)
1	WPP	0.80	0.20
2	WPP	1.00	0.20
3	WPP	1.20	0.20

Table 2. Composition of Bold basal medium (BBM) for *Chlorella ellipsoidea* culture (Phang and Chu, 1999).

Chemicals/Compounds	Concentration in stock solution (g/l)	Amount in culture medium (ml/l)
$NaNO_3$	25.00	10.0
$MgSO_47H_2O$	7.50	10.0
NaC1	2.50	10.0
K_2HPO_4	7.50	10.0
KH_2PO_4	17.50	10.0
$CaC1_2.2H_2O$	2.50	10.0
Trace elements		1.0
a) $ZnSO_4.7H_2O$	8.82	-
b) $MnC1_2.4H_2O$	1.44	-
c) MoO_3	0.71	-
d) $CuSO_4.5H_2O$	1.57	-
e) $Co(NO_3)_2.6H_2O$	0.94	-
H_3BO_3	11.40	1.0
EDTA-KOH solution		1.0
a) EDTA Na_2	50.00	
b) KOH	31.00	
a) $FeSO_4.7H_2O$	4.98	1.0
b) Conc. H_2SO_4	1.0 ml/l	10.0

Table 3. Experimental design of *Chlorella ellipsoidea* culture

Types of media	Conical flask (l)	Treatments	Replications	Duration of culture (days)
DWPP	2.0 l	3	3	20
TW	2.0 l	1	3	20
BBM	2.0 l	1	3	20

DWPP = digested waste potato powder; TW = tap-water; BBM = Bold basal medium procedure of Horwitz (1989). Data of *C. ellipsoidea* such as cell density, chlorophyll a, and optical density (OD) of media, crude protein and crude lipid of alga were compared in respect to treatments using one way analysis of variance (ANOVA) followed by Duncan multiple range test (DMRT) (Gomez and Gomez, 1976).

Results and Discussion

Chlorella ellipsoidea (Gerneck) was cultured in supernatants of three different concentrations (0.80, 1.00 and 1.20 g/l) of digested waste potato powder (DWPP), tap-water and Bold basal medium (BBM) as control. In every medium, the inoculated initial cell number of thie alga 2.5×10^5/ml attained the maximum density of 227.45×10^5 cell/ml, in BBM on the 6th day, and 200×10^5 cell/ml, in the supernatant of 0.80 g/l DWPP on the 18th day. (Fig. 1). Parvin and Habib (2005) recorded the maximum cell growth of *C. ellipsoidea* $199.88\text{x}10^5$/ml, when grown in sugar mill waste effluent. This variation might be due to the difference in nutrient composition of different media. The total biomass of *C. ellipsoidea* was higher when grown in the supernatant of digested 0.80 g/l DWPP than cultured in other concentrations of DWPP, TW and BBM (Table 4). Cell number was significantly ($P < 0.01$) correlated with chlorophyll a ($r = 0.965$) (Fig. 2) which indicates that the nutrients of media satisfied good growth of this alga. However, the optimum levels of physical facilities such as aeration, light intensity and temperature played a vital role in the total culture system.

Similar experiments were performed by Hussain (2001) and Khan *et al.* (2006) on cultur of green alga, *Chlorella ellipsoidea* with some differences in cell growth and optical density which might be due to the culture conditions and chemical properties of the culture media. Hossain (1999), and Alam *et al.* (2003) found lower cell densities of *Chlorella* spp. when cultured in different media.

Values of light intensities and dissolved oxygen (DO) for the growth of *C. ellipsoidea* were more or less similar to those recorded by Karmakar *et al.* (2001) and the range of pH was almost same as in case of Khan *et al.* (2006), Alam *et al.* (2003), Habib (1998), Mayo (1997), and Haq (1988). Temperature recorded during the culture period was 27.4 to 30 °C, similar to (Alam *et al.* (2003), Khan (1996), Martinez-Jeronimo and Espinosa-Chavez (1994) and Thinh (1994).

The highest protein content of *Chlorella ellipsoidea* was 48.11%, when grown in supernatant of 0.80 g/l DWPP, but the highest lipid content was 14.23%, in the supernatant of 1.00 g/l DWPP during the study (Table 5) which is more or less similar to the findings of Habib (1998) and Vass and Bhanou

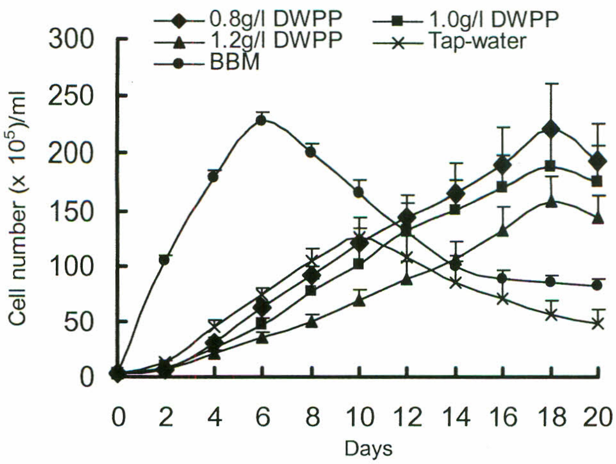

Fig. 1. Mean (± SD) cell number of *C. ellipsoidea* grown in supernatant of different amounts of digested waste potato powder, tap-water and BBM.

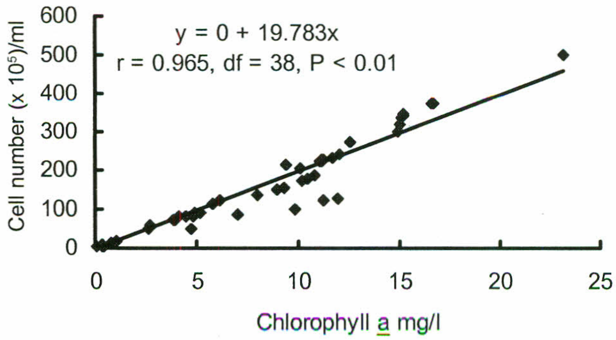

Fig. 2. Correlation of cell number of *C. ellipsoidea* with chlorophyll a in supernatant of DWPP 0.8 g/l.

(1973). However, Parvin and Habib (2005) reported protein 42.70% and lipid 17.50%, when cultured in supernatant of digested sugar mill waste. According to Vass and Bhanou (1973), *Chlorella* contains appreciably higher protein, around 50% on dry matter basis. But Habib (1998) reported that the range of crude protein of *C. vulgaris* grown in different dilutions of latex concentrate rubber effluent (LCRE) and NPK, was 34.90 to 39.92%, crude lipid 11.72 to 16.32%, ash 7.49 to 15.72% and moisture 5.55 to 5.66%, which is not consistent with the present findings. Khan *et al.* (2006), recorded highest protein content 43.69% of *Chlorella vulgaris,* when grown in 50% sugar mill effluent but 48.11% protein of *C. vulgaris* was recorded when cultured in supernatant of 0.80 g/l digested waste potato powder. The observed variations might be due to the variation in the cultured species. Mean cell densities and optical densities of *C. ellipsoidea* in supernatant of 0.80 g/l DWPPwere significantly ($p<0.01$) better than in other concentrations of DWPP. Again, *Chlorella*

Table 4. Maximum mean values (± SD) of cell number ($\times 10^5$)/ ml, Chlorophyll a content (mg/l), total biomass and optical density (at 620 nm) of *Chlorella ellipsoidea* in different media

Parameters	Supernatant of digested waste potato powder (g/l)			Tap water	BBM
	0.80	1.00	1.20		
Cell number (x10^5)	$500.00^a \pm 2.0$	$347.33^b \pm 2.0$	$238.67^c \pm 2.0$	$226.82^b \pm 1.47$	$127.45^a \pm 3.19$
Chlorophyll a (mg/l)	$22.50^a \pm 1.45$	$16.75^b \pm 1.20$	$14.20^c \pm 0.90$	$14.10^c \pm 0.90$	$14.20^c \pm 0.85$
Optical density at 620 nm	$1.79^a \pm 0.01$	$1.41^b \pm 0.01$	$1.13^c \pm 0.03$	$1.18^c \pm 0.04$	$1.524^b \pm 0.01$
Total biomass* (67 x Chlo-a)	1507.75 ± 97.15	1122.25 ± 80.40	951.40 ± 60.30	944.70 ± 60.30	951.40 ± 56.95

* = Vonshak and Richmond (1988); a,b,c in superscript do not differ significantly at 5% level of probability

Table 5. Proximate composition (% on dry weight basis) of *Chlorella ellipsoidea* grown in different media

Proximate composition	Supernatant of digested waste potato powder (g/l)			Tap water	BBM
	0.8	1.0	1.20		
Crude protein	48.11	39.36	33.21	23.40	38.68
Crude lipid	11.01	14.23	10.82	10.95	8.63
Moisture	8.00	7.87	7.15	7.35	7.23
Ash	2.82	2.96	4.05	2.66	3.45

ellipsoidea cultured in DWPP contained considerable amounts of protein, lipid and ash.

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