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Growth Performance and Nutritional Value of *Chlorella ellipsoidea* in Fertilizer Factory Effluent Media

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Abstract

The growth performance of green alga, *Chlorella ellipsoidea* was studied in a laboratory in different concentrations of fertilizer factory effluent media (FFEM). Five different concentrations viz. 40, 45, 50, 55 and 60% of FFEM and bold basal medium (BBM) (control) were used with three replications for a period of three months. Each trial was done for a period of 16 days. The initial cell density of *C. ellipsoidea* was 2.5×10^5 cells·ml^{-1} which attained a maximum density of $198.49\text{x}10^5$ cells·ml^{-1} in BBM followed by 182.07, 157.41, 142.34, 137.57 and 121.35 ($\text{x}10^5$cells· ml^{-1}) in 50, 55, 45, 60 and 40 % FFEM, respectively on the 10th day of culture. A similar trend was observed in the case of chlorophyll *a* content and the range was 5.85 to 9.39 mg·l^{-1}. The specific growth rate (SGR, μ·day^{-1}) on the basis of cell number and chlorophyll *a* were found at 0.40 to 0.44 and 0.41 to 0.45, respectively and was significantly ($p<0.05$) higher in BBM. The total biomass was found at 629.13 and 541.36 mg·l^{-1} in BBM and 50% FFEM, respectively. The overall growth performance of *C. ellipsoidea* was significantly ($p<0.05$) higher in 50% FFEM than in other concentrations of FFEM. The cultured microalga was found nutritionally rich.

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Introduction

Chlorella ellipsoidea is a very common microalga in Bangladesh as well as all over the world. It is a fast growing microalga rich in different kinds of nutrients (Soeder 1980; Geldenhuys et al. 1988; Habib 1998). *Chlorella* is one of the nutritious foods in nature, and is one of the simplest, primitive cells, yet science may never be able to unravel all its mysteries (Jensen 1989). It contains all the essential amino acids and about 14 - 19 minerals (Fabregas and Herrero 1986). Using inorganic media, laboratory based microalgal culture in Bangladesh is very costly due to the unavailability of different ingredients of media. So, it is essential to find inexpensive culture media using available ingredients. Anaga and Abu (1996) conducted an experiment on a laboratory scale cultivation of C*hlorella* using waste effluent from a fertilizer factory in Nigeria. They obtained approximately 6.1 $mg \cdot l^{-1}$ (dry wt.) *Chlorella* in the effluent media. Microalgae have been successfully grown in Industrial waste water such as rubber and palm oil mill effluents (Phang and Ong 1988; Yusoff et al. 1997; Habib et al. 1997). The constant increasing aquaculture production and the intensification of the process have raised the need for a much larger supply of particular microalgae than just the amount that can be harvested from natural habitats (Vonshak 1994). Fertilizer factory effluent is largely available in Bangladesh. In fact, five big fertilizer factories are discharging huge amounts of effluent in nature every year during production season, which partially polluted the aquatic environment and increased both biological oxygen demand (BOD) and chemical oxygen demand (COD) in the aquatic environment. The present experiment was undertaken to culture *C. ellipsoidea* in fertilizer factory effluent and to analyze the nutritional values of the cultured microalga.

Materials and Methods

Collection and preparation of fertilizer factory effluent (FFE)

Fertilizer factory effluent (FFE) was collected from the Jamuna fertilizer factory, Tarakanda, Jamalpur, Bangladesh. For urea production methane gas and air were used as the main raw ingredients. The factory personnel did not disclose the total production process due to business secret. However, a diagram of the production of urea wastes is given in

figure 1. The waste effluent was collected and decomposed within 15 days by aeration. To know the chemical status of waste effluent, different chemical parameters such as pH, phosphate phosphorus (PO_4-P), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonia nitrogen (NH_3-N), total suspended solids (TSS), total dissolved solids (TDS), total solids (TS), dissolved oxygen (DO), chemical oxygen demand (COD) of the raw collected sample of FFE were analyzed (Table 1) before decomposition. Then the decomposed liquid portion was filtered and diluted using distilled water in different concentrations viz. 40, 45, 50, 55 and 60%. The media was mixed well and sterilized at 120^{o}C for 15 minutes with moist heat by autoclave. Bold basal medium (BBM) was used as control medium whose chemical composition is shown in table 2.

Culture of Chlorella ellipsoidea

Stock culture of *Chlorella. ellipsoidea* maintained at the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh was used for this study. The cells were separated with the help of microcapillary using compound microscope. *Chlorella ellipsoidea* was inoculated initially in prepared BBM. Six treatments, five from different concentrations (40, 45, 50, 55, and 60%) of FFEM and one from BBM (control) were used to grow microalga, *Chlorella ellipsoidea* in 1.0 liter volumetric flask with three replications for each treatment. The microalga were inoculated into each culture flask from the stock (Optical density at 620 nm = 0.20) (Habib 1998) to get 10% suspension of *Chlorella ellipsoidea*. All the flasks were kept under fluorescent lights (light: dark = 12 h: 12 h) in the Live Food Culture Laboratory. The culture flasks were continuously aerated using electric aerator. Nine sub-samplings were carried out every alternate day from each flask to observe cell density, chlorophyll *a*, optical density and physico-chemical properties of culture media viz. temperature (^{o}C), light intensity, dissolved oxygen (DO), pH, phosphate-phosphorus (PO_4-P), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), and ammonia-nitrogen (NH_3-N). All the glassware used in the experiment were sterilized in dry heat in an oven at 70°C, overnight. After successful completion of laboratory culture, mass culture was performed. During mass culture microalga was harvested before stationary phase and centrifuged at 3000 rpm for 10 minutes to separate the microalga. The separated microalga was used for the analysis of proximate composition.

Estimation of physico-chemical properties of culture media

Temperature, light intensity, dissolved oxygen and pH were determined using respective meters. Phosphate-phosphorus, nitrate-nitrogen,

nitrite-nitrogen and ammonia-nitrogen were determined using the Hach kit (DREL/2000) following Clesceri et al. (1989).

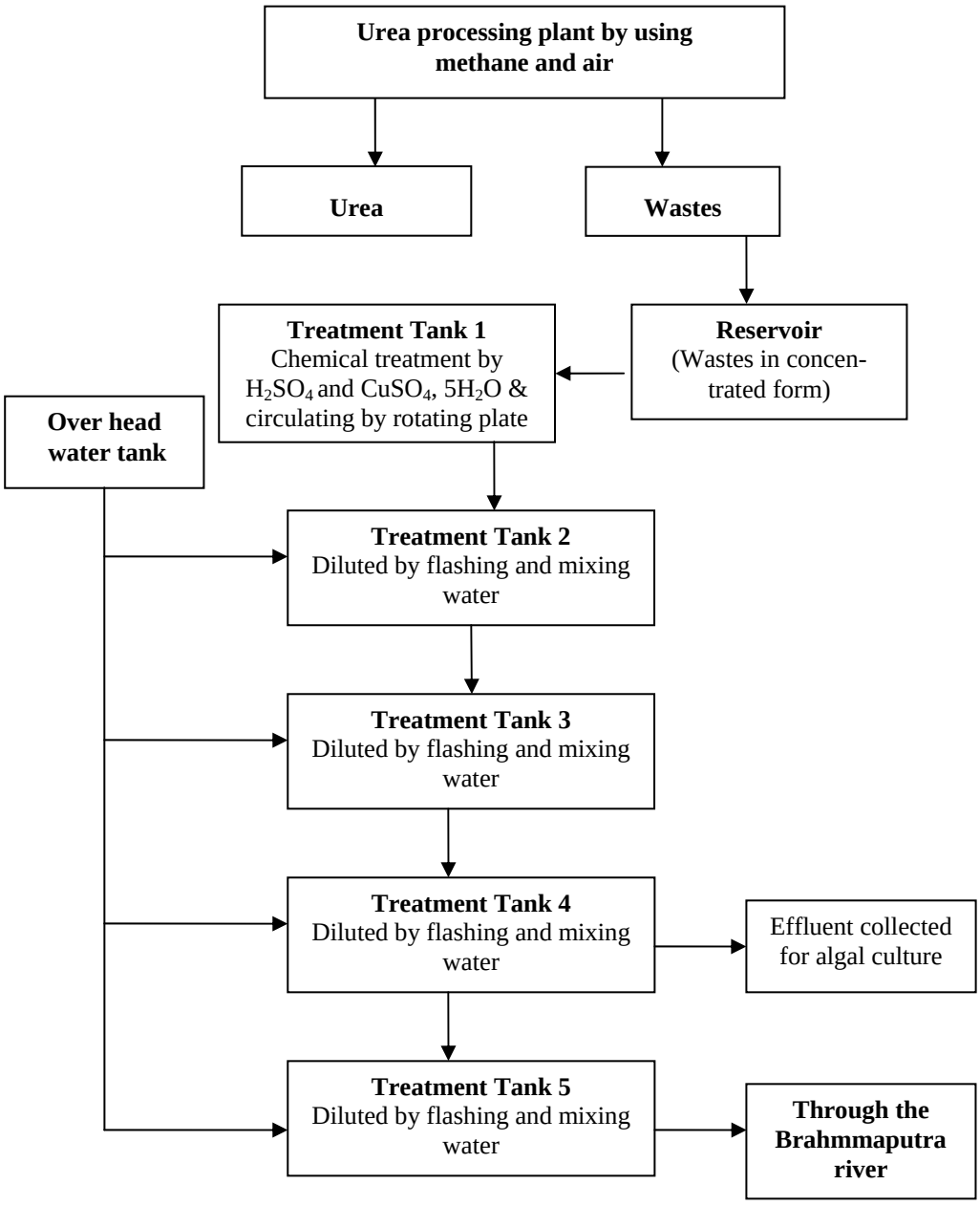

Fig. 1 Flow chart in the production of urea factory waste

Table 1. Mean (± SD) chemical composition (mg/l, except pH) of collected raw fertilizer factory effluent (FFE)

Composition	Fertilizer factory effluent (FFE)
pH	7.51 ± 0.04
Phosphate phosphorus (PO_4-P)	11.62 ± 0.28
Nitrate nitrogen (NO_3-N)	29.73 ± 0.40
Nitrite nitrogen (NO_2-N)	19.65 ± 0.09
Ammonia nitrogen (NH_3-N)	12.46 ± 0.22
Total suspended solids (TSS)	37.00 ± 1.00
Total dissolved solids (TDS)	785.00 ± 3.61
Total solids (TS)	838.33 ± 4.04
Dissolved oxygen (DO)	0.33 ± 0.06
Chemical oxygen demand (COD)	3686.00 ± 50.48

Table 2. Composition of bold basal medium (BBM) for *Chlorella ellipsoidea* culture

Chemicals	Concentration in stock solution ($g \cdot l^{-1}$)	Amount in culture medium ($ml \cdot l^{-1}$)
$NaNO_3$	25.00	10.0
$MgSO_4.7H_2O$	7.50	10.0
NaCI	2.50	10.0
K_2HPO_4	7.50	10.0
KH_2PO_4	17.50	10.0
$CaCI_22H_2O$	2.50	10.0
Trace element		1.0
a) $ZnSO_4.7H_2O$	8.82	-
b) $MnCl_2.4H_2O$	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	-
KOH	31.00	-
a) $FeSO_4.7H_2O$	4.98	
b) Conc. H_2SO_4	$1ml \cdot l^{-1}$	1.0

Estimation of Chlorella ellipsoidea cell density

Chlorella ellipsoidea cells were counted using an improved Neubauer ruling haemacytometer and cell density of algal culture were estimated according to the following formula (Clesceri et al. 1989):

$$\text{No. of cells} \cdot ml^{-1} = \frac{\text{No. of cell counted in 16 chamber}}{10^{-4}} \text{ x dilution factor}$$

Estimation of chlorophyll a

Optical densities of the prepared samples were determined at 664, 647 and 630 nm wave length by using UV spectrophotometer. A blank with 100% acetone was run simultaneously. Chlorophyll *a* content was calculated following this formula (Clesceri et al. 1989):

Chlorophyll *a* ($mg \cdot l^{-1}$) = 11.85 (OD 664)-1.54 (OD 647)-0.08 (OD 630)

Estimation of specific growth rate (SGR, $\mu \cdot day^{-1}$) of microalga

The specific growth rate (SGR $\mu \cdot day^{-1}$) of cultured microalga was calculated following this equation (Clesceri et al. 1989):

$$SGR\ (\mu \cdot day^{-1}) = In\ (X_1 - X_2)/\ t_2 - t_1$$

Where,

X_1 = Biomass concentration at the end of selected time interval

X_2 = Biomass concentration at the beginning of selected time interval, and

$t_2 - t_1$ = Elapsed time between selected time in the day.

Analysis of proximate composition

Proximate composition of algal samples were analyzed in the nutrition laboratory, Faculty of Fisheries, Bangladesh Agricultural University, following standard methods (Horwitz 1984).

Statistical analysis

Mean and standard deviation were calculated from the experimental data. The data were analyzed through one-way analysis of variance (ANOVA) using SPSS followed by Duncan's Multiple Range Test (DMRT) to determine significant difference among the treatment means (Zar 1984).

Results

The maximum ($p<0.05$) cell number of *Chlorella ellipsoidea* 198.49×10^5 cells$\cdot ml^{-1}$ was recorded in control BBM, followed by 50, 55, 45, 60 and 40 % FFEM (Fig. 2) on the 10^{th} day of the culture. Similar trend was observed in the case of chlorophyll *a* content and optical density. The

range of chlorophyll *a* content was 5.85 to 9.39 $mg \cdot l^{-1}$ (Fig. 3) and optical density (at 620 nm wave length) was 0.98 to 1.95 at the peak period of cell growth. Highest pH of the media was recorded on the stationary phase, which ranged from 7.94 to 8.42 for all the treatments (Fig. 4). An increasing trend of dissolved oxygen (DO) was recorded up to the stationary phase, after that it decreased and on the 10^{th} day of culture DO was recorded at 5.17 to 5.47 $mg \cdot l^{-1}$ for all treatments. The range of light intensity and temperature was 2170 to 2205 $lux \cdot m^{-2} \cdot s^{-1}$ and 28.0 to 29.9°C, respectively during the whole culture period of the study. The specific growth rate (SGR, $\mu \cdot day^{-1}$) on the basis of cell number and chlorophyll *a* content were found in the range of 0.40 to 0.44 and 0.41 to 0.45, respectively for all the treatments. Total biomass of *C. ellipsoidea* in respect to chlorophyll *a* content was found 392.17 to 629.13 $mg \cdot l^{-1}$ and it was maximum when grown in BBM followed by 50, 55, 45, 60 and 40% FFEM (Table 3).

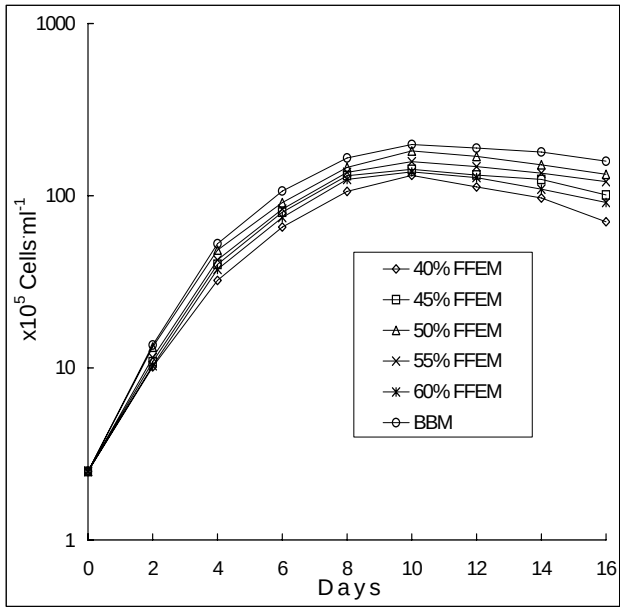

Fig. 2. Semilogarithmic growth curves based on cell number ($x10^5 cells \cdot ml^{-1}$) of *Chlorella ellipsoidea* grown in different concentrations of FFEM and BBM

During the culture period a decreasing trend of both PO_4-P and NO_3-N was observed for all the treatments up to stationary phase and next it continued to increase. At the stationary phase the content of PO_4-P was recorded 1.59, 2.65, 2.98, 3.45, 4.45 and 4.99 $mg \cdot l^{-1}$ in 40, 45, 50, 55, 60% FFEM and BBM, respectively (Fig. 5). The range of NO_3-N was 7.27 to

15.20 $mg \cdot l^{-1}$ during the whole culture period. Significantly ($p<0.05$) higher amount of NO_3-N was recorded in 50 and 60% FFEM followed by 55, 45, 40% FFEM and BBM at the stationary phase (Fig. 6). On the other hand NO_2-N showed an increasing trend with the age of the culture. At the stationary phase NO_2-N was found 10.15, 10.61, 10.63, 10.66 and 11.63 $mg \cdot l^{-1}$ in 40, 45, 50, 55, and 60% FFEM, respectively and 0.17 $mg \cdot l^{-1}$ in BBM. A few amount of NO_2-N was recorded in BBM which was significantly ($p<0.05$) lower than that of all concentrations of FFEM. The content of NH_3-N gave the similar trend like NO_2-N and the range was 0.39 to 1.44 $mg \cdot l^{-1}$ at the stationary phase.

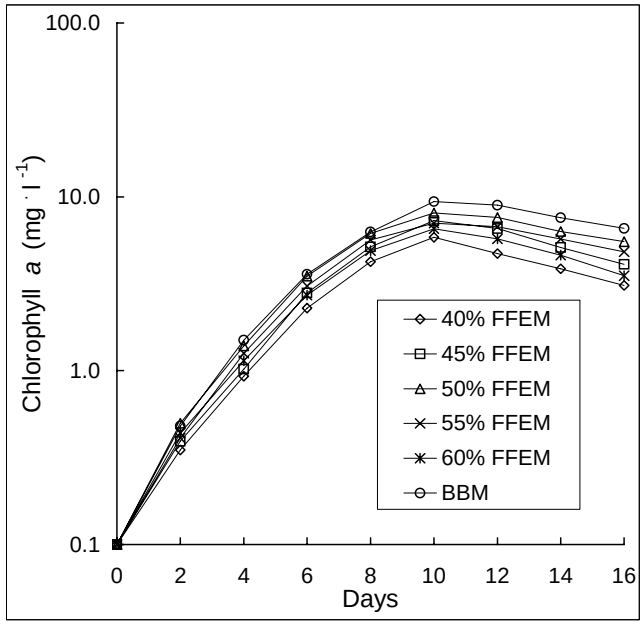

Fig. 3. Semilogarithmic growth curves based on chlorophyll *a* content ($mg \cdot l^{-1}$) of *Chlorella ellipsoidea* grown in different concentration of FFEM and BBM

The proximate composition of C. *ellipsoidea* cultured in different concentrations of FFEM as well as BBM was analysed. The crude protein, crude lipid, ash, crude fiber, moisture and NFE (%) were recorded and ranged from 33.26 to 44.63, 7.63 to 8.66, 10.49 to 13.14, 4.65 to 7.95, 9.73 to 10.25 and 17.35 to 26.64, respectively, for all the treatments including BBM (Table 4).

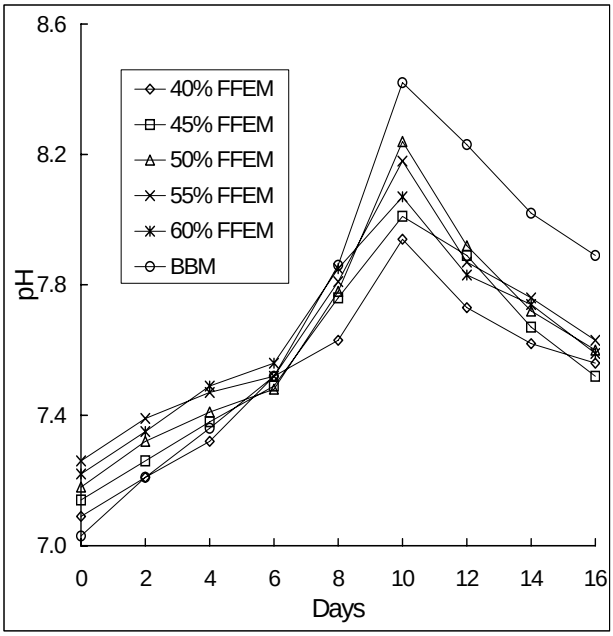

Fig. 4 pH of different concentrations of FFEM and BBM containing *Chlorella ellipsoidea*

Table 3. Mean (± SD) specific growth rate ($\mu \cdot day^{-1}$) of cell and chlorophyll *a* (chlo-*a*), and total biomass ($mg \cdot l^{-1}$) of *Chlorella ellipsoidea* grown in different concentrations of FFEM and BBM

Parameters	40% FFEM	45% FFEM	50% FFEM	55% FFEM	60% FFEM	BBM
SGR of cell	0.40 ± 0.00 ^{e}	0.40 ± 0.00 ^{d}	0.43 ± 0.01 ^{b}	0.41 ± 0.00 ^{c}	0.40 ± 0.01 ^{de}	0.44 ± 0.00 ^{a}
SGR of chlo-*a*	0.41 ± 0.01 ^{d}	0.42 ± 0.01 ^{cd}	0.44 ± 0.01 ^{b}	0.43 ± 0.00 ^{c}	0.42 ± 0.01 ^{cd}	0.45 ± 0.00 ^{a}
Total biomass (Chlo-*a* x 67)	392.17 ± 29.18 ^{d}	463.86 ± 19.92 ^{c}	541.36 ± 3.55 ^{b}	473.69 ± 14.42 ^{c}	438.85± 21.44 ^{e}	629.13 ± 20.77 ^{a}

Different superscripts in each row indicate significant differences ($p<0.05$)

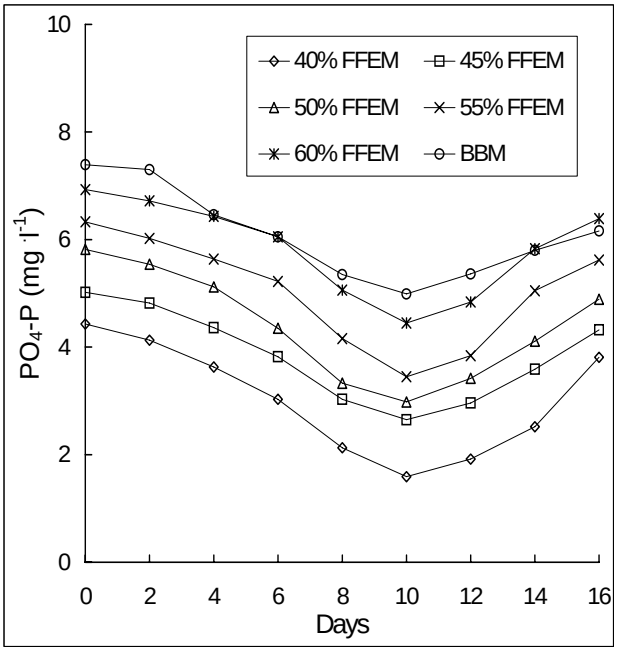

Fig. 5 Phosphate-phosphorus (PO4-P, $mg \cdot l^{-1}$) of different concentrations of FFEM and BBM containing *Chlorella ellipsoidea*

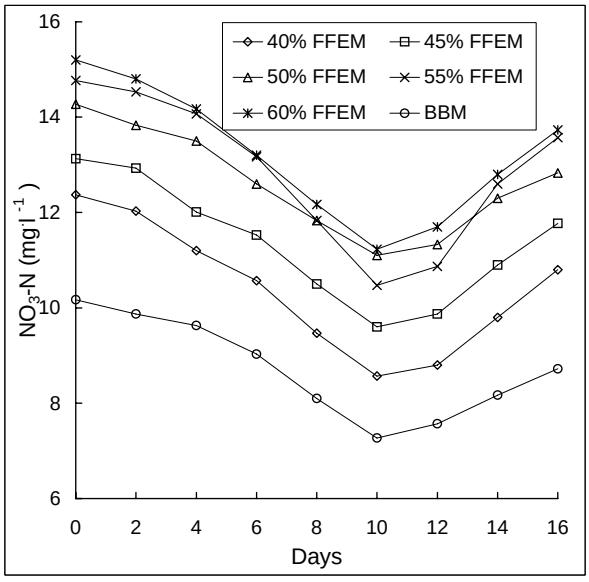

Fig. 6 Nitrate-nitrogen (NO_3-N, $mg \cdot l^{-1}$) of different concentrations of FFEM and BBM containing *Chlorella ellipsoidea*

Table 4. Mean values (±SD) of proximate composition (%) of *C. ellipsoidea* cultured in different concentrations of FFEM and BBM

Composition	40% FFEM	45% FFEM	50% FFEM	55% FFEM	60% FFEM	BBM
Crude Protein	34.83 ± 0.06 ^{c}	37.27 ± 0.08 ^{b}	44.63 ± 0.11 ^{a}	34.86 ± 0.04 ^{c}	33.26 ± 0.11 ^{d}	43.47 ± 0.11 ^{a}
Crude lipid	10. 80 ± 0.06 ^{b}	10.65 ± 0.10 ^{b}	11.66 ± 0.06 ^{a}	11.56 ± 0.16 ^{a}	10.63 ± 0.19 ^{b}	10.85 ± 0.29 ^{b}
Ash	13.14 ± 0.12 ^{a}	10.71 ± 0.29 ^{b}	10.49 ± 0.53 ^{b}	13.06 ± 0.18 ^{a}	12.32 ± 0.14 ^{a}	10.51 ± 0.21 ^{b}
Crude fiber	4.77 ± 0.05^{cd}	4.65 ± 0.06^{d}	5.26 ± 0.03^{b}	5.13 ± 0.09^{b}	4.90 ± 0.02^{c}	7.95± 0.03^{a}
Moisture	9.99 ± 0.12^{b}	10.08 ± 0.19^{ab}	9.73 ± 0.11^{c}	9.85 ± 0.09^{bc}	10.25 ± 0.06^{a}	9.94 ± 0.15^{bc}
NFE	26.47 ± 0.26^{b}	26.64 ± 0.13^{b}	18.23 ± 0.28^{d}	25.54 ± 0.23^{c}	28.64 ± 0.37^{a}	17.35 ± 0.24^{d}

Different superscripts in each row indicates significant differences ($p<0.05$)

Discussion

In the study different concentrations of FFEM were used with control as BBM. The cell density was increasing up to the stationary phase and after that they were decreasing. The maximum cell number 198.49×10^5 cells$\cdot$ ml^{-1} was recorded on the 10th day of culture in BBM. The cell number 182.07×10^5 cells$\cdot$ml^{-1} grown in 50% FFEM was significantly ($p<0.05$) higher than that grown in other concentrations of FFEM. It could be due to the presence of sufficient amount of nutrients in 50% FFEM. Habib (1998), in his study on *Chlorella vulgaris* in rubber effluent media recorded the highest cell number of *C. vulgaris* 285.20×10^5 cells$\cdot$ml^{-1} on the 10th day of culture, which is higher than the present findings. Islam et al. (2004) found higher cell density (263.62×10^5 cells$\cdot$ml^{-1}) of *Chlorella ellipsoidea* in 0.25 mg$\cdot$l^{-1} cabbage powder media. Singha (2001) performed a similar type of work and he found that the green alga *C. vulgaris* attained a maximum cell density of 159.00×10^5 cells$\cdot$ml^{-1} in 50% sugarcane mill effluent medium (SMEM), 163.25×10^5 cells$\cdot$ml^{-1} in 1.0 g$\cdot$l^{-1} press mud medium (PMM) and 213.43×10^5 cells$\cdot$ml^{-1} in BBM. These variations could be due to the difference of media as well as the media concentrations.

Chlorophyll *a* content and optical density were increasing up to the stationary phase and after that they were decreasing. The direct relation of the cell number, chlorophyll *a* and optical density proves that the culture was justified. The findings of Karmaker et al. (2001) regarding chlorophyll *a* content and optical density were lower than that of the present study. On the other hand, Habib (1998) and Islam et al. (2004) recorded higher chlorophyll *a* content and optical density than the present study. These variations could be due to the variations in media and difference of cell density grown in the media. In the present experiment, pH gave an increasing trend up to the stationary phase and highest pH was observed 8.42 on the 10th day of the culture. Karmaker et al. (2001) recorded the highest pH 7.83 in his study of *C. ellipsoidea* in ripe bean seed powder. Habib (1998) reported pH above 9 when *C. vulgaris* was grown in different concentrations of rubber and palm oil effluents. These variations could be due to the different composition and concentrations of media. Dissolved oxygen ranged from 4.37 to 5.09 during maximum cell growth of *C. ellipsoidea*. Singha (2001) and Karmakar et al. (2001) observed maximum DO 5.75 and 4.49 mg$\cdot$l^{-1} respectively with cultured *Chlorella sp*. in different organic media. Light intensity ranged from 2170.72 to 2205.61 lux$\cdot$m$^{-2}\cdot$s^{-1} during the whole

culture period of the study. Karmakar et al. (2001) used light intensity 2410 to 2490 $lux \cdot m^{-2} \cdot s^{-1}$ with cultured *Chlorella ellipsoidea*.

The SGR of *C. ellipsoidea* on the basis of cell no. was significantly ($p<0.05$) higher in BBM than in other treatments. The SGR and total biomass in 50% FFEM was significantly ($p<0.05$) higher than in other concentrations of FFEM (Table 2). Habib (1998) found SGR of *Chlorella vulgaris* 0.32 to 0.46 and 0.33 to 0.45 on the basis of cell number when cultured in different concentrations of latex concentrate rubber effluent (LCRE) and standard Malaysian rubber effluent (SMRE) respectively. On the basis of chlorophyll *a* the SGR were 0.30 to 0.42 and 0.30 to 0.40 respectively and the total biomass was 438.85 to 812.04 $mg \cdot l^{-1}$. These findings are more or less similar to the present findings.

Both the contents of NO_3-N and PO_4-P were higher on the first day of culture in different concentrations of FFEM and BBM. The values were decreasing up to the stationary phase for all the treatments, which could be due to the utilization of NO_3-N and PO_4-P by the cells. At the stationary phase the highest amount of NO_3-N (11.26 $mg \cdot l^{-1}$) was recorded at 60% FFEM which was significantly ($p<0.05$) higher than all the other treatments. At the stationary phase the content of PO_4-P recorded in BBM (4.99 $mg \cdot l^{-1}$) was significantly ($p<0.05$) higher than those recorded in 40, 45, 50, and 55% FFEM. Here it is clear that FFEM contained a higher amount of NO_3-N and lesser amount of PO_4-P than the control media, BBM. Similar findings were recorded by Hossain (2001) in the culture of *Chlorella* sp. The increasing trend of NO_3-N and PO_4-P after the stationary phase could be due to the presence of decomposed dead cells. The NO_2-N and NH_3-N showed an increasing trend with the age of culture in different concentration of FFEM and BBM. At the stationary phase a significantly ($p<0.05$) higher amount of NO_2-N and NH_3-N were recorded in all concentrations of FFEM than BBM.

Proximate composition analysis showed that a significantly ($p<0.05$) higher percentage of protein was recorded when *C. ellipsoidea* was cultured in 50% FFEM than in other treatments except BBM. The percentage of crude lipid of *C. ellipsoidea* grown in 50% FFEM was significantly higher than that grown in other treatments including BBM. The ash content of *C. ellipsoidea* grown in 50% FFEM and BBM varied insignificantly ($p<0.05$). *Chlorella* was found rich with protein, lipid and other minerals when cultured in some inorganic fertilizer (Geldenhuys et al. 1988). The present findings somehow agree with the findings of Soeder (1980), Geldenhuys et al. (1988) and Habib (1998). Considering the

proximate composition of the microalgae it may be concluded that *C. ellipsoidea* is nutritionally rich when cultured in different concentrations of FFEM but 50% FFEM showed the best performance.

Conclusion

This study proved the feasibility of FFEM as a *Chlorella* culture media and that FFEM is as effective as a commercially culture media. It was also revealed in this study that as this is an inexpensive algal culture media it can be broadly used in Bangladesh where culture media are expensive and largely unavailable.

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