Borneo Journal of Resource Science and Technology (2022), 12(2): 63-73
DOI: https:doi.org/10.33736/bjrst.4695.2022

Utilisation of Fermented Wheat Bran Extract Medium as A Potential Low-cost Culture Medium for *Chlorella ellipsoidea*

TASLIMA AKTER*[1], MD. MAHADI HASAN[1], MOUSUMI DAS[1], MD. NURUNNABI MONDAL[2], SAHADAT HOSSAIN[1], MOHAMMAD BODRUL MUNIR[3], MD. AMZAD HOSSAIN[1]

[1]Department of Aquaculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur - 1706, Bangladesh; [2]Department of Fisheries Management, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur - 1706, Bangladesh; [3]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
*Corresponding author: taslima@bsmrau.edu.bd
Received: 17 May 2022 Accepted: 12 October 2022 Published: 31 December 2022

ABSTRACT

Microalgae, *Chlorella ellipsoidea* is an excellent energy source for food and biofuel production. Nevertheless, the production cost of *C. ellipsoidea* using Bold's Basal Medium (BBM) is expensive, which led to the exploration of alternative low-cost medium for large-scale production. Low-cost fermented wheat bran extract medium (FWBEM), which has good nutritional properties, might be an alternative feedstock for mass production of *C. ellipsoidea*. The present study was conducted to evaluate the growth and production of *C. ellipsoidea* using different concentrations of FWBEM. Wheat bran was fermented at the concentration of 8.33, 6.66, and 5.00 g/L water labelled as T_2, T_3, and T_4, respectively. The BBM was used as the control medium (T_1). The growth and production of *C. ellipsoidea* were monitored for three days in terms of cell dry weight, specific growth rate, optical cell density, chlorophyll *a* content, and cell numbers. Those growth data revealed that *C. ellipsoidea* cultured at 6.66 g/L (T_3) did not vary significantly with the standard inorganic BBM. However, T_2 and T_4 showed substantially lower cell growth and chlorophyll *a* content than control and T_3. Compared to the BBM, a significant reduction in production cost was obtained in the FWBEM. Based on the cell biomass growth, pigmentation, and production cost, FWBEM at a 6.66 g/L could be used as an alternative medium. Therefore, FWBEM has excellent potential to be used for the low-cost production of *C. ellipsoidea*.

Keywords: *Chlorella ellipsoidea*, culture medium, low-cost production, microalgae, wheat bran extract

INTRODUCTION

Microalgae are considered the essential primary producer of the food chain, particularly in the aquatic ecosystem (De-Silva *et al.*, 2018). They are used to purify heavy metals and nutrient load from various types of wastewater, especially aquaculture wastewater (Munoz & Guieysse, 2006; Posadas *et al.*, 2017; Khatoon *et al.*, 2018; Cardoso *et al.*, 2021; Pavithra *et al.*, 2020). They are also used to produce biofuel, several valuable chemicals, and pharmaceutical products (Illman *et al.*, 2000; Spolaore *et al.*, 2006; Chew *et al.*, 2017). In addition, microalgae are important food sources for the human, animal, and aquatic organisms for their higher digestibility and nutrition status (Görs *et al.*, 2010; Hemaiswarya *et al.*, 2011). Therefore, microalgae are used as a vital feed ingredient and also as live food in the aquaculture industry for larvae of fish, molluscans, and crustaceans (Roy & Pal, 2015; Jusoh *et al.*, 2020).

Among several microalgae species, *Chlorella ellipsoidea* is regarded as one of the most excellent food sources for human and aquacultural species, especially for fish larvae and bivalves (Bai *et al.*, 2001; Kim *et al.*, 2002). *Chlorella ellipsoidea* is a ubiquitous single-celled green freshwater microalga belonging to the division of Chlorophyta. It contains all the nutrients necessary to sustain life especially protein, lipid, and minerals (Rahman *et al.*, 2005; Toyub *et al.*, 2007). In addition, it is also a rich source of polyunsaturated fatty acids and essential amino acids (Mondal *et al.*, 2005). *Chlorella ellipsoidea* is also well known for its antitumor, anticarcinogenic, antiviral, anticataract, antiulcer, and antioxidative properties (Shibata *et al.*, 2003). However, for

the large-scale cultivation of microalgae, the availability and cost of nutrients in the culture medium is the prime issue of recent research (Rizal *et al.,* 2017; Akter *et al*., 2019).

Bold's Basal Medium (BBM) is an inorganic and frequently used culture medium for planktonic freshwater microalgae like *Chlorella* (Connon, 2007). However, the formulation of BBM contains laboratory-grade inorganic chemicals that are expensive and are not always readily available. In this perspective, it is an emerging demand to find a practical, low-cost, and readily available alternative medium for the large-scale production of *C. ellipsoidea*.

Locally available agricultural by-products can be used for the large-scale production of *C. ellipsoidea.* Wheat bran (*Triticum aestivum*) has great potential because it represents the major milling agro-industrial by-product in many countries (Apprich *et al.,* 2014). Moreover, it has valuable nutritional properties, i.e., high value of protein, lipids (omega-3 fatty acid), crude fiber, and a rich source of vitamins, minerals (iron, zinc, manganese, magnesium, phosphorus), and bioactive compounds (low-molecular-weight phenolic acid compounds: p-coumaric and ferulic acid) (Abdel-Aal & Hucl, 2002; Singh *et al*., 2007; Pruska-Kedzior *et al.,* 2008; Anson *et al*., 2012; De Brier *et al*., 2015). In addition, the fermentation process improves the nutritional and functional properties of wheat bran (Katina *et al.,* 2012). Therefore, the fermented wheat bran extract media (FWBEM) could be a promising nutritional source for the production of *C. ellipsoidea.* This study focused on the effect of different concentrations of FWBEM on the growth and production of *C. ellipsoidea,* along with the impact on production cost.

MATERIALS AND METHODS

Microalgal Strain and Culture Media Preparation

A pure strain of *C. ellipsoidea* was obtained from Bangladesh Agricultural University, Mymensingh, Bangladesh. The stock culture was maintained in Bold's Basal Medium (BBM). BBM medium was prepared according to the standard composition of chemicals and then sterilised at 121 ºC for 15 min with moist heat in an autoclave and cooled for 24 hr. The purity of microalgae was observed under a light microscope (Primo Star; Carl Zeiss). Different concentrations of FWBEM were prepared according to Rahman *et al*. (2005) by fermenting 8.33 g (T_2), 6.66 g (T_3), and 5.00 g (T_4) wheat bran into 1 L of water, while standard BBM medium was used as control (T_1) presented in Table 1.

Table 1. Composition of Bold's Basal Medium (BBM) and fermented wheat bran extract medium (FWBEM) for *C. ellipsoidea* culture

Treatments	Chemicals/Compounds	Stock solution g/L (H_2O)	Amount in culture medium (ml/L)
T_1 (Control)	$NaNO_3$	25.00	10.0
	$MgSO_4.7H_2O$	7.50	10.0
	NaCl	2.50	10.0
	K_2HPO_4	7.50	10.0
	KH_2PO_4	17.50	10.0
	$CaCl_2.2H_2O$	2.50	10.0
	Trace elements		1.0
	i) $ZnSO_4.7H_2O$	8.82	-
	ii) $MnCl_2.4H_2O$	1.44	-
	iii) MoO_3	0.71	-
	iv) $CuSO_4.5H_2O$	1.57	-
	v) $Co(NO_3)_2.6H_2O$	0.94	-
	H_3BO_3	11.40	1.0
	EDTA-KOH solution		1.0
	i) EDT Na_2	50.00	-
	ii) KOH	31.00	-
	i) $FeSO_4.7H_2O$	4.98	1.0
	ii) Conc. H_2SO_4	1.00 ml/L	
T_2	Wheat bran	8.33	
T_3	Wheat bran	6.66	
T_4	Wheat bran	5.00	

In the first step, wheat bran was kept in 30 L water with the concentration of the respective treatments. After one week, 15 g of urea was added to each medium. Partially fermented wheat bran extract was filtered through a thin fine cloth (markin cloth), and the clear supernatant was siphoned after four weeks. Following, lime (CaO) was mixed at 2 g/L to make the extract transparent. pH was adjusted to 7.5 with H_2SO_4 and fermented for another two weeks. After the fermentation, the clear supernatant of the respective FWBEM treatment was collected by siphoning.

The biochemical parameters of the culture medium such as total solids, total dissolved solids, nitrate-nitrogen (NO_3-N) (mg/L), ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), and phosphate-phosphorus (PO_4-P) were assessed according to Clesceri *et al.* (1989). The free CO_2 from the culture media was determined by titrimetric and the total alkalinity by methyl orange indicator method (APHA, 1981).

Culture of *Chlorella ellipsoidea*

Chlorella ellipsoidea was cultivated in 1 L Erlenmeyer flask under four treatments with three replications at Live Food Culture Laboratory, Department of Aquaculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. At first, all the glassware were washed with distilled water and then sterilised in a hot air oven at 160 ^oC for 60 min.

Chlorella ellipsoidea was inoculated into a flask with 10% suspension rate (Optical Density, 620 = 0.20) from the pure stock culture, according to Habib (1998). The culture was incubated under natural light at approximately 12/12 hr light: dark cycles, with a constant aeration supply by an air pump (Sobo, Aquarium pump SB 348A) for 18 days.

Temperature (^oC), dissolved oxygen (mg/L), pH, and light intensity (lux/m^2/s) of the culture media were measured on every sampling day using a thermometer, dissolved oxygen meter (HQ40d multi), electric pH meter (sensIONTM+ PH3) and lux-meter (LX-9621), respectively.

Estimation of Cell Growth (Dry Basis)

Cell dry weight and chlorophyll *a* content were estimated following Clesceri *et al.* (1989). Briefly, 50 ml culture suspension was filtered through a Whatman GF/C filter paper (0.45 µm mesh size and 47 mm diameter) and weighed. The suspension was washed with 20 ml acidified water (pH 4) to remove insoluble salts during filtration. All samples were oven-dried at 40 °C for 24 hr. The dry weight of *C. ellipsoidea* was calculated using formula, Eq. (1).

$$W = \frac{FFW - IFW}{Amount\ of\ sample\ taken\ filtration\ (ml)} \times 100$$

Eq. (1)

Where, W = Cell dry weight in g/L; FFW = Final filter weight in g and IFW = Initial filter weight in g.

Estimation of Specific Growth Rate (SGR)

The following equation, Eq. (2) calculated the specific growth rate (SGR, µ/day) of *C. ellipsoidea* (Clesceri *et al.,* 1989).

$$SGR\ (\mu/day) = \ln (X_2 - X_1)/ T_2 - T_1 \qquad \text{Eq. (2)}$$

Where, X_1 = Initial biomass concentration; X_2 = Biomass concentration at the end of the experiment, and $T_2 - T_1$ = Elapsed time.

Estimation of Cell Number and Optical Density of *C. ellipsoidea*

The cell density of C. *ellipsoidea* was estimated using an improved Neubauer ruling hemocytometer method, which was slightly modified from Rahman (2005). One drop of *C. ellipsoidea* culture was put on each of the two chambers of a hemocytometer and covered with cover glass. The plankton cells were counted (cells/ml) under a microscope by following Eq. (3).

$$N = A \times 10{,}000 \qquad \text{Eq. (3)}$$

Where, N = Number of *C. ellipsoidea* cells per ml of culture medium, and A = Average number of *C. ellipsoidea* cells in mm^3.

The samples of *C. ellipsoidea* grown in different treatments were placed in the UV spectrophotometer (DR 5000) at 620 nm; the optical density (OD) of cells was recorded according to Toyub *et al.* (2007).

Measurement of Chlorophyll *a* Content

For chlorophyll *a* measurement, 10 ml sample of *C. ellipsoidea* was filtered and then ground with a glass rod and mixed with 10 ml of 100% redistilled acetone. Then the sample mixers were homogenised and centrifuged at 4000 rpm for 10 min. The chlorophyll *a* content was calculated by recording OD at 664, 647, and 630 nm using a UV spectrophotometer (DR 5000) with Eq. (4).

Chlorophyll *a* (mg/L) = 11.85 (OD_{664}) - 1.54 (OD_{647}) - 0.08 (OD_{630}) Eq. (4)

Statistical Analysis

All the data were analysed statistically by one-way analysis of variance (ANOVA), while Tukey's post hoc test at 5% significance level test was applied in the case of significant differences using the Statistix 10 statistical package.

RESULTS

The biochemical composition of BBM and different concentrations of FWBEM are summarised in Table 2. All the biochemical compositions, particularly the nutrient requirement for *C. ellipsoidea* growth, were found adequate in FWBEM (T_2-T_4) viz. NO_3-N (51.3 to 60.4 mg/L); NO_2-N (15.65 to 18.65 mg/L); PO_4-P (79.36 to 110.45 mg/L). The mean values of physicochemical parameters in BBM and FWBEM in this study are summarised in Table 3. The treatments were found to significantly vary physicochemical parameters ($p > 0.05$).

The cell biomass growth rate of *C. ellipsoidea* in BBM (T_1) and the FWBEM (T_2-T_4) is shown in Figure 1, which is expressed as cell dry weight (mg/L). The growth curve showed an initial lag phase for all the treatments, whereas the peak was found on the 15th day of culture when exponential growth occurred in the present study. At the end of the exponential growth phase, maximum cell growth (58.93 ± 3.26 mg/L) was found in the treatment T_1, where a standard BBM medium was used and then followed by T_3 (58.28 ± 1.34 mg/L), T_2 (55.93 ± 45 mg/L), and T_4 (51.83 ± 15 mg/L), respectively. Cell dry weight in T_3 and T_2 was statistically at par with the control but lower in T_4, where wheat bran was mixed at the lowest (5.00 g/L) concentration. After the 15th day of culture, the microalgae biomass production decreased in all the treatments.

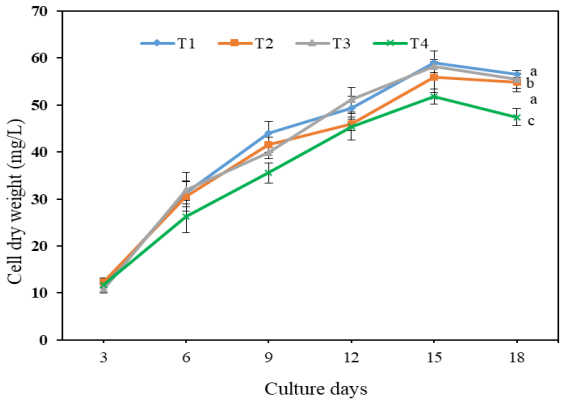

Figure 1. Cell dry weight (mg/L) of *C. ellipsoidea* under different treatments during the experimental period. Values are mean ± standard deviation (SD), *n* = 3. The letters at the end of the trend line represent significant ($p<0.05$) differences among the treatments

The specific growth rate (SGR, μ/day) of *C. ellipsoidea* in all the treatments ranged from 0.198 to 0.211 μ/day (Figure 2). Significant ($p<0.05$) difference was observed in terms of SGR (μ/day) among the treatments. The highest and lowest SGR (μ/day) were seen in T_1 and T_4, respectively. However, in treatment T_3, where 6.66 g/L wheat bran was used, the SGR did not vary significantly from the control (T_1).

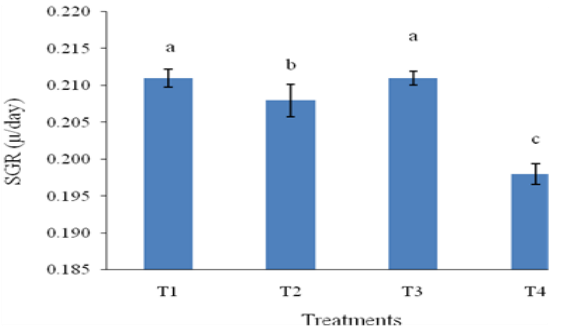

Figure 2. Specific growth rate (μ/day) in different treatments based on biomass content of *C. ellipsoidea.* Values are mean ± standard deviation (SD), *n* = 3. The letters at the end of the trend line represent significant ($p<0.05$) differences among the treatments.

The highest optical density (OD) of the *C. ellipsoidea* culture was recorded in T_1 (1.34 g/L), which was not significantly varied with T_3 (1.18 g/L) on the 15th day of culture (exponential phase). On the other hand, T_2 and T_4 showed significantly lower OD than the other treatments (Figure 3).

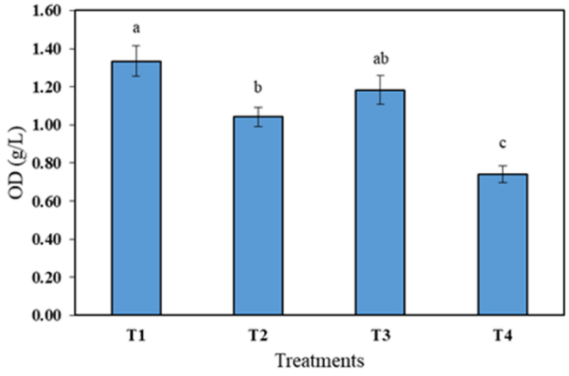

Figure 3. Mean optical density (g/L) of *C. ellipsoidea* under different treatments at 15th days of culture. Values are mean ± standard deviation (SD), *n* = 3. The letters at the end of the trend line represent significant (p<0.05) differences among the treatments

Cell density (× 10^5 cells/ml) of *C. ellipsoidea* cultured in BBM and FWBEM is represented in Figure 4. The result revealed that the maximum cell density (86.30×10^5 cells/ml) of *C. ellipsoidea* was in T_1, where BBM was used and followed by T_3, T_2 and T_4, respectively. Conversely, the number of *C. ellipsoidea* cells was significantly lower in T_2, T_3, and T_4 compared to T_1.

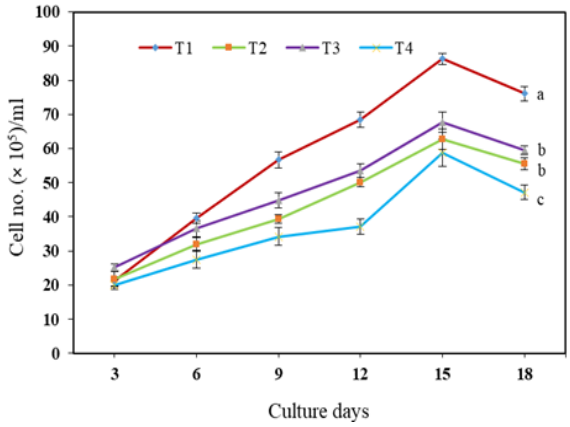

Figure 4. Mean cell densities (× 10^5 cells/ml) of *C. ellipsoidea* in different treatments. Values are mean ± standard deviation (SD), *n* = 3. The letters at the end of the trend line represent significant (p<0.05) differences among the treatments

Regarding algal chlorophyll *a* content, the present study showed that the mean values of chlorophyll *a* content of *C. ellipsoidea* in BBM and FWBEM ranged between 1.47 mg/L and 5.65 mg/L (Table 4). Chlorophyll *a* content increased gradually with the increase in the day of the exponential culture period. Significantly (p<0.05) highest chlorophyll *a* content was found in T_1 (5.65 mg/l), while the lowest pigmentation was recorded in T_4 (4.33 mg/L) at the end of the 15th day of the exponential growth phase. After the exponential growth phase the chlorophyll *a* content in all the treatments continue to decrease up to the end of the culture period.

Table 5 provides a straightforward cost analysis of FWBEM and BBM media utilised in various treatments. When compared to inorganic BBM, the production costs of FWBEM were significantly reduced (p<0.05). It costs 1.92 (USD) to produce 1 L of BBM medium in T_1, as opposed to 0.0024 to 0.0014 (USD) (T2 to T4).

DISCUSSION

The variation of physicochemical parameters, i.e., temperature, DO, pH, and light intensity, could influence the growth of microalgae. The optimum range of temperature, DO, pH, and light intensity for *C. ellipsoidea* production are 25 – 33ºC (Mayo, 1997), 3.5 – 5.5 mg/L (James *et al*., 1988), 7.5 – 8.5 (Khan *et al*., 1996) and 2000 – 2280 Lux/m^2/sec, respectively. The range of temperature, DO, pH, and light intensity in different treatments in this study was at the optimum level for *C. ellipsoidea*. These results are comparable with the finding of various researchers (Rahman *et al*., 2005; Mohshina *et al*., 2017). Therefore, the result indicates that different concentrations of FWBEM did not affect the culture environment of *C. ellipsoidea* in this study.

The growth and quality of the microalgae are greatly influenced by the availability of organic or inorganic carbon sources, nitrogen, phosphorus, iron, and other minerals (Mg, Zn, K) in the culture medium (Grobbelaar, 2004; Khan *et al*., 2018).

Table 2. Biochemical composition (Mean ± SD) of BBM and FWBEM used for the culture of *C. ellipsoidea*

Biochemical parameters	Culture Medium			
	Bold's Basal Medium (BBM) (T_1)	FWBEM (T_2)	FWBEM (T_3)	FWBEM (T_4)
pH	7.50 ± 0.03	8.51 ± 0.04	8.13 ± 0.02	7.80 ± 0.03
Free CO2 (mg/L)	N/A	132 ± 0.02	121 ± 0.07	102 ± 0.01
Total alkalinity (mg/L)	200.5 ± 24	421.1 ± 0.04	410.5 ± 0.04	407.2 ± 0.04
Nitrate nitrogen (NO_3-N) (mg/L)	41.28	60.4 ± 0.02	57.0 ± 0.03	51.3 ± 0.01
Nitrite nitrogen (NO_2-N) (mg/L)	N/A	17.65 ± 0.05	18.65 ± 0.01	15.65 ± 0.03
Ammonia nitrogen (NH_3-N) (mg/L)	N/A	10.3 ± 0.20	7.8 ± 0.29	8.1 ± 0.12
Phosphate phosphorus (PO_4-P) (mg/L)	163.02	110.45 ± 0.05	95.38 ± 0.02	79.36 ± 0.01
Total suspended solids (TSS) (mg/L)	57.5 ± 5.2	43.6 ± 0.21	39.2 ± 0.18	40.1 ± 0.25
Total dissolved solids (TDS) (mg/L)	6370 ±2.88	525.0 ± 1.04	472.0 ± 2.186	379.0 ± 1.81
Total solids (TS) (mg/L)	7152 ± 5.03	8152 ± 5.03	7152 ± 5.03	7152 ± 5.03

Table 3. Average range of physicochemical parameters of different culture media during the culture period of *C. ellipsoidea*

Treatments	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Light Intensity (Lux/m^2/sec)
T_1	26.44±0.10	4.19±0.15	7.50 ±0.04	2220 ± 0.41
T_2	26.48±0.09	4.53±0.15	7.62±0.13	2222 ± 0.82
T_3	26.56±0.14	4.56±0.06	7.6 ±0.14	2221 ± 0.47
T_4	26.57±0.11	4.13±0.12	7.61±0.02	2225 ± 0.24

Values are expressed as mean ± SD, n = 3

Table 4. Chlorophyll *a* content (mg/L) of *C. ellipsoidea* in different treatments during the experimental period

Treatments	Culture period (Days)					
	3rd	6th	9th	12th	15th	18th
T_1	1.75±0.06^a	2.93±0.04^a	3.95±0.10^a	4.67±0.04^a	5.65±0.03^a	5.51±0.02^a
T_2	1.76±0.05^a	2.75±0.00^a	3.91±0.02^a	4.25±0.03^b	5.36±0.05^a	4.29±0.05^b
T_3	1.65±0.03^a	2.87±0.07^a	3.81±0.19^a	4.33±0.04^b	5.10±0.02^a	4.34±0.02^b
T_4	1.47±0.06^b	2.64±0.03^b	3.15±0.00^b	4.08±0.03^c	4.33±0.08^b	4.30±0.08^b

Values are expressed as mean ± SD, n = 3. Values with different superscript letters in each column indicate significant differences.

Table 5. Average cost analysis of *C. ellipsoidea* culture media for different treatments

Treatments	Cost of *C. ellipsoidea* production (USD/L)			Total Cost for mass production USD/1000L
	BBM	FWBEM	TotalCost (USD/L)	
T_1	1.92	0.00	1.92.00^a	1920
T_2	0.0024	0.25	0.0024^b	2.4
T_3	0.0019	0.20	0.0019^b	1.9
T_4	0.0014	0.15	0.0014^b	1.4

The maximum cell dry weight of *C. ellipsoidea* was recorded in BBM medium (58.93 ± 3.26 mg/L) (T_1) and T_2 and T_4 vary significantly from T_1. Whereas, cell dry weight of *C. ellipsoidea* in T_3 (58.28 ± 1.34 mg/L) treatment did not vary significantly from the control (T_1). This may be due to the rich sources of nutrients and minerals (iron, zinc, manganese, magnesium, and phosphorus) of wheat bran that could provide suitable nutrients for *C. ellipsoidea* in the T_3 treatment compared to the other treatments. However, the higher and lower concentration of wheat bran in T_2 and T_4 treatment could cause lower nutrient availability and thus may cause a lower growth rate of *C. ellipsoidea*. The growth rate was about similar to the study of Feng *et al.* (2011) for the *C. zofingiensis* (58.4 mg/L) culture in outdoor flat plate photobioreactors. Scragg *et al.* (2002) obtained a growth rate of 40 mg/L and 41 mg/L for *C. vulgaris,* and *C. emersonii* cultured in 230 L pumped tubular indoors photobioreactor, which is lower than the present study. Kumaran *et al.* (2016) found the cell biomass of *C. vulgaris* in peat moss compost was 0.67 g/L, which was higher than the current study.

The significantly highest and lowest SGR (μ/day) resulted in T_1 and T_4, respectively. However, treatment T_3, where wheat bran was used at 6.66 g/L, did not vary significantly from the control T_1.

Machado *et al.* (2020) have cultured *Chlorella vulgaris* in four commercial organic substrates (OS) media, Nutrimais, Nutriverde, and EcoMix4 medium, they found the SGR values of 0.111, 0.075, 0.036, and 0.122 μ/day, respectively, which were lower compared to the present study. The difference may be due to the minerals, vitamin B , and bioactive compounds content in wheat bran besides organic carbon (Prueckler *et al.,* 2014). On the other hand, Kumar *et al*. (2010) found the highest SGR value of *C. vulgaris* (0.345 μ/day) in the culture medium of digested piggery effluent.

The OD of *C. ellipsoidea* in the present experiment was comparable to Ashrafuzzaman (2006), who observed a maximum OD (1.33 g/L) of *Chlorella sp.* in urban waste effluents, while Fatemeh and Mohsen (2016) observed the highest OD 1.68 g/L in the outdoor culture of *C. vulgaris*. The variation in cell growth rate and OD from the previous study might be found due to variation in algal species, nutrient components in culture media, and culture techniques.

The OD of *C. ellipsoidea* culture in T_3 was not significantly varied with T_1, but the cell density of *C. ellipsoidea* in the control treatment with the standard BBM media had substantially higher compared to T_2, T_3, and T_4. It has been reported that, higher cell growth is related to higher OD. As T_1 and T_3 showed similar cell growth, cell dry weight OD also followed the same trend. Cells grown in T_3 may have a bigger size than those produced in the control treatment, leading to a higher cell biomass rate in T_3 treatment. The cell densities found in the present experiment are similar to Ashrafuzzaman (2006) and Rahman *et al.* (2005) when cultured in an urban waste effluent medium and whole pulse medium.

Ashrafuzzaman (2006) recorded chlorophyll *a* content of *C. ellipsoidea* ranging from 0.12 to 8.75 mg/L in different urban waste media concentrations which is comparable to the present study. The chlorophyll *a* content of *C. ellipsoidea* was significantly lower in the wheat bran treatments than in the cell grown in BBM medium. However, only the chlorophyll *a* content was measured in this study; total chlorophyll content may need to be measured in future research. The presence of iron (Fe) in the culture medium increases the chlorophyll content of cells due to the direct involvement of Iron (Fe) in the enzymatic reactions of photosystem I (PSI) and II (PSII) (Sun *et al.,* 2014). However, FWBEM may contain less iron than the standard BBM. In this case, supplementation of Fe in the FWBEM may be investigated in further study.

Production costs for control BBM were significantly higher ($p<0.05$) than for all other treatments (Table 5). However, the formulation of FWBEM required only 0.0024 to 0.0014 USD/L (T_2 to T_4), which was 1.92 USD/L for BBM medium. Therefore, using FWBEM at a concentration of 6.66 g/L (T_3) during the large-scale (1000 L) production of *C. ellipsoidea* will result in a reduction in the cost of culture medium from 1920 USD to 1.9 USD without affecting the growth and pigment contents. Moreover, wheat bran is a readily available agro by-product, which ensures its easy supply for the large-scale production of *C. ellipsoidea* compared to commercial BBM. So, FWBEM

could considerably ensure the availability of culture medium and lower the production cost for *C. ellipsoidea*.

CONCLUSION

The study showed that FWBEM at 6.66 g /L rate could be an effective alternative medium for culturing *C. ellipsoidea*, as it resulted better growth performance viz. cell dry weight, specific growth rate SGR, optical density (OD), cell densities ($\times 10^5$ cells/ml) and chlorophyll *a* content. Moreover, its significantly lower cost is favourable for culturing *C. ellipsoidea*. Consequently, FWBEM is beneficial in the production of *C. ellipsoidea* hence reducing the dependency on expensive standard BBM. This study reported that fermentation using 6.66 g wheat bran/L resulted better growth performance of *C. ellipsoidea*. However, the higher and lower amount of wheat bran significantly affected the production performances of *C. ellipsoidea*. Therefore, this study suggests that wheat bran (6.66 g/L) could be used as an alternative low-cost culture medium for *C. ellipsoidea*.

ACKNOWLEDGEMENTS

This study was funded by the Higher Education Quality Enhancement Project (HEQEP), supported by the World Bank under the subproject entitled "Establishment of a Live Food Culture Laboratory in the Department of Aquaculture, BSMRAU, Bangladesh" through the University Grants Commission of Bangladesh, implemented by Ministry of Education, Government of the People's Republic of Bangladesh. In addition, technical support from Fish Nutrition Laboratory, Department of Aquaculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh, is highly appreciated.

REFERENCES

Abdel-Aal, E.S.M. & Hucl, P. (2002). Amino acid composition and *in vitro* protein digestibility of selected ancient wheats and their end products. *Journal of Food Composition and Analysis*, 15(6): 737-747. DOI:10.1006 /jfca.2002.1094

APHA (American Public Health Association) (1981). *Standard Methods for the Examination of Water and Wastewater*. Washington, DC: American Public Health Association. pp. 1134.

Apprich, S., Tirpanalan, O., Hell, J., Reisinger, M., Böhmdorfer, S., Siebenhandl-Ehn, S., Novalin, S. & Kneifel, W. (2014). Wheat bran-based biorefinery 2: valorization of products. *LWT-Food Science and Technology*, 56(2): 222-231. DOI:10.1016/j.lwt.2013.12.003

Akter, T., Haque, M.I., Das, M. & Hossain, M.A. (2019). Growth performance analysis of *Spirulina platensis* production by substituting K_2SO_4-K of kosaric medium with MOP-K. *Bangladesh Journal of Botany*, 48(3): 529-535. DOI:10.3329/bjb.v48i3.47914

Anson, N.M., Hemery, Y.M., Bast, A. & Haenen, G.R.M.M. (2012). Optimizing the bioactive potential of wheat bran by processing. *Food and Function*, 3(4): 362-75. DOI:10.1039/c2fo10241

Ashrafuzzaman, M.D. (2006). *Culture and growth performance of Chlorella vulgaris in different urban waste effluents*. (Master thesis), Bangladesh Agricultural University, Bangladesh.

Bai, S.C., Koo, J.W., Kim, K.W. & Kim, S.K. (2001). Effects of *Chlorella* powder as a feed additive on growth performance in juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf). *Aquaculture Research*, 32: 92-98. DOI:10.1046/j.1355-557x. 2001.00008.x

Cardoso, L.G., Duarte, J.H., Costa, J.A.V., Assis, D.J., Lemos, P.V.F., Druzian, J.I. & Chinalia, F.A. (2021). *Spirulina* sp. as a bioremediation agent for aquaculture wastewater: production of high added value compounds and estimation of theoretical biodiesel. *BioEnergy Research*, 14(1): 254-264. DOI:10.1007/s12155-020-10153-4

Clesceri, L.S., Greenbergand, A.E. & Trussel, R.R. (1989). *Standard methods for the examination of water and wastewater* (Seventeenth Edition). American Public Health Association, American Water Works Association, and Water Works Pollution Control Federation, 1915, Washington DC, USA.

Chew, K.W., Yap, J.Y., Show, P.L., Suan, N.H., Juan, J.C., Ling, T.C. & Chang, J.S. (2017). Microalgae biorefinery: high value products perspectives. *Bioresource Technology*, 229: 53-62. DOI:10.1016/j.biortech.2017.01. 006

Connon, R. (2007). *Culturing of Chlorella vulgaris standard operating procedure, SOP on how to prepare Bold's Basal Medium (BBM)*. Daphnia Research group. University of Reading, United Kingdom.

De Brier, N., Hemdane, S., Dornez, E., Gomand, S.V., Delcour, J.A. & Courtin, C.M. (2015). Structure, chemical composition and enzymatic activities of pearlings and bran obtained from pearled wheat (*Triticum aestivum* L.) by roller milling. *Journal of Cereal Science*, 62: 66-72. DOI:10.1016/j.jcs.2014.12.009

De Silva, A.Z., sheriff, M., Banerjee, S. & Yusoff, F.M. (2018). Growth and quality enhancement of *Chlorella vulgaris* Beyerinck (Beijerinck) 1890 using simple cost-effective medium. *Asian Fisheries Science*, 31(1): 61-72.

Fatemeh, L. & Mohsen, D. (2016). Effects of environmental factors on the growth, optical density and biomass of the green algae *Chlorella vulgaris* in outdoor conditions. *Journal of Applied Sciences and Environmental Management*, 20(1): 133-139. DOI:10.4314/jasem.v20i1.16

Feng, P., Deng, Z., Hu, Z. & Fan, L. (2011). Lipid accumulation and growth of *Chlorella zofingiensis* in flat plate photobioreactors outdoors. *Bioresource Technology*, 102(22): 10577-10584. DOI:10.1016/j.biortech.2011.08.109

Grobbelaar, J.U. (2004). Algal nutrition: mineral nutrition. In Richmond, A. (Ed.) Handbook of microalgal culture: biotechnology and applied phycology. Oxford: Blackwell publication, London, United Kingdom. Pp. 97-115.

Görs, M., Schumann, R., Hepperle, D. & Karsten, U. (2010). Quality analysis of commercial *Chlorella* products used as dietary supplement in human nutrition. *Journal of Applied Phycology*, 22(3): 265-276. DOI:10.1007/s10811-009-9455-4

Habib, M.A. (1998). *Culture of selected microalgae in rubber and palm oil mill effluents and their use in the production of enriched Rotifers*. (Ph. D thesis). Universiti Putra Malaysia, Selangor, Malaysia.

Hemaiswarya, S., Raja, R., Ravi Kumar, R., Ganesan, V. & Anbazhagan, C. (2011). Microalgae: a sustainable feed source for aquaculture. *World Journal of Microbiology and Biotechnology*, 27(8): 1737-1746. DOI:10.1007/s11274-010-0632-z

Illman, A.M., Scragg, A.H. & Shales, S.W. (2000). Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. *Enzyme and Microbial Technology*, 27(8): 631-635. https://DOI:10.1016/S0141-0229(00)00266-0

James, C.M., Al-Khars, A.M. & Chorbani, P. (1988). pH dependent growth of *Chlorella* in a continuous culture system. *Journal of the World Aquaculture Society*, 19(2): 27-35. DOI:10.1111/j.1749-7345.1988.tb01042.x

Jusoh, M., Kasan, N.A., Hashim, F.S., Haris, N., Zakaria, M.F., Mohamed, N.N., Rasdi, N.W., Abd, W.M.E., Katayama, T. & Takahashi, K. (2020). Isolation of freshwater and marine indigenous microalgae species from Terengganu water bodies for potential uses as live feeds in aquaculture industry. *International Aquatic Research*, 12(1): 74-83. DOI:10.22034/iar(20).2020.671730.

Katina, K., Juvonen, R., Laitila, A., Flander, L., Nordlund, E., Kariluoto, S., Vieno, P. & Poutanen, K. (2012). Fermented wheat bran as a functional ingredient in baking. *Cereal Chemistry*, 89(2): 126-134. DOI:10.1094/ CCHEM-08-11-0106.

Khan, S., Arakawa, O. & Onoue, Y. (1996). Neurotoxin production by a chloromonad *Fibrocapsa japonica* (Raphidophyceae). *Journal of the World Aquaculture Society*, 27(3): 254-263. DOI:10.1111/j.1749-7345.1996.tb0060 7.x

Khan, M.I., Shin, J.H. & Kim, J.D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories*, 17(1): 1-21. DOI:10.1186/s12934-018-0879-x

Khatoon, H., Haris, H., Rahman, N.A., Zakaria, M.N., Begum, H. & Mian, S. (2018). Growth, proximate composition and pigment production of *Tetraselmis chuii* cultured with aquaculture wastewater. *Journal of Ocean University of China*, 17(3): 641-646. DOI:10.1007/s11802-018-3428-7

Kim, K.W., Bai, S.C., Koo, J.W., Wang, X. & Kim, S.K. (2002). Effects of dietary *Chlorella ellipsoidea* supplementation on growth, blood characteristics, and whole-body composition in juvenile Japanese flounder *Paralichthys olivaceus*. *Journal of the World Aquaculture Society*, 33(4): 425-431. DOI:10.1111/j.1749-7345.2002.tb00021.x

Kumar, M.S., Miao, Z.H. & Wyatt, S.K. (2010). Influence of nutrient loads, feeding frequency and inoculum source on growth of *Chlorella vulgaris* in digested piggery effluent culture medium. *Bioresource Technology*, 101(15): 6012-6018. DOI:10.1016/j.biortech.2010.02. 080

Kumaran, K., Lam, M.K., Tan, X.B., Uemura, Y., Lim, J.W., Khoo, C.G. & Lee, K.T. (2016). Cultivation of *Chlorella vulgaris* using plant-based and animal waste-based compost: a comparison study. *Procedia Engineering*, 148: 679-686.

Machado, A., Pereira, H., Costa, M., Santos, T., Carvalho, B., Soares, M., Quelhas, P. & Silva, J. (2020). Development of an organic culture medium for autotrophic production of *Chlorella vulgaris* biomass. *Applied Sciences*, 10(6), 2156. DOI:10.3390/app10062156

Mayo, A.W. (1997). Effects of temperature and pH on the kinetic growth of unialga *Chlorella vulgaris* cultures containing bacteria. *Water Environment Research*, 69(1): 64-72. DOI:10. 2175/106143097X125191

Mohshina, M.M., Shahjahan, M., Chowdhury, P. & Rahman, M.S. (2017). Culture of *Chlorella ellipsoidea* in different culture media. *International Journal of Agricultural Research, Innovation and Technology*, 7(1): 51-57. DOI:10.3329/ijarit.v7i1.33322

Mondal, B., Rahman, M.R., Alam, M.J., Tarafder, A.R., Habib, M.A.A. & Khaleque, M.A. (2005). A study on the culture of *Chlorella ellipsoidea* in various concentrations of unripe tomato juice media. *Pakistan Journal of Biological Sciences* 8(6): 823-828.

Munoz, R. & Guieysse, B. (2006). Algal–bacterial processes for the treatment of hazardous contaminants: a review. *Water Research*, 40(15): 2799-2815. https://doi.org/10.1016/j.watres. 2006.06.011

Pavithra, K.G., Kumar, P.S., Jaikumar, V., Vardhan, K.H. & SundarRajan, P. (2020). Microalgae for biofuel production and removal of heavy metals: a review. *Environmental Chemistry Letters*, 18(6): 1905-1923. DOI:0.1007/s10311-020-01046-1

Posadas, E., Alcántara, C., García-Encina, P.A., Gouveia, L., Guieysse, B., Norvill, Z., Acién, F.G., Markou, G., Congestri, R., Koreiviene, J. & Muñoz, R. (2017). Microalgae cultivation in wastewater. In Gonzalez-Fernandez, C. and Munoz, R. (Eds). *Microalgae-based biofuels and bioproducts* (pp. 67-91). Woodhead Publishing. DOI:10.1016/B978-0-08-101023-5.00003-0

Prueckler, M., Siebenhandl-Ehn, S., Apprich, S., Hoeltinger, S., Haas, C., Schmid, E. & Kneifel, W. (2014). Wheat bran-based biorefinery 1: composition of wheat bran and strategies of functionalization. *LWT-Food Science and Technology*, 56(2): 211-221. DOI:10.1016/j.lwt. 2013.12.004

Pruska-Kedzior, A., Kedzior, Z. & Klockiewicz-Kaminska, E. (2008). Comparison of viscoelastic properties of gluten from spelt and common wheat. *European Food Research and Technology*, 227(1): 199-207. DOI:10.1007/ s00217-007-0710-0

Rahman, M.S., Hossain, M.A., Fatema, S. & Hossain, M.A. (2005). Culture of green algae *Chlorella ellipsoidea* in inexpensive media. *Bangladesh Journal of Fisheries Resources*. 9(2): 185-190.

Rizal, M.A, Yeasmin, M.F, Hossain, M.A, Akter, T. & Rahman, M.M (2017). Replacement of sodium nitrate in Kosaric medium with urea for culture of *Spirulina platensis*. *International Journal of Fisheries and Aquatic Studies*. 5(3): 403-408.

Roy, S.S. & Pal, R. (2015). Microalgae in aquaculture: a review with special references to nutritional value and fish dietetics. *Proceedings of the Zoological Society*, 68: 1-8. DOI:10.1007/ s12595-013-0089-9

Scragg, A.H., Illman, A.M., Carden, A. & Shales, S.W. (2002). Growth of microalgae with increased calorific values in a tubular bioreactor. *Biomass and Bioenergy*, 23(1): 67-73. DOI:10.1016/S0961-9534(02)00028-4

Shibata, S., Natori, Y., Ishihara N.T, Tomisaka, K., Matsumoto, K., Sansawa, H. & Nguyen, V.C. (2003). Antioxidant and anti-cataract effects of *Chlorella* on rats with streptozotocin induced diabetes. *Journal of Nutrition Science and Vitaminology*, 49(5): 334-339.

Singh, S., Gamlath, S. & Wakeling, L. (2007). Nutritional aspects of food extrusion: a review. *International Journal of Food Science and Technology*, 42(8): 916-929. DOI:10.1111/j. 1365-2621.2006.01309.x

Spolaore, P., Joannis-Cassan, C., Duran, E. & Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, 101(2): 87-96. DOI:10.1263/jbb. 101.87

Sun, X., Cao, Y., Xu, H., Liu, Y., Sun, J., Qiao, D., & Cao, Y. (2014). Effect of nitrogen-starvation, light intensity and iron on triacylglyceride/carbohydrate production and fatty acid profile of *Neochloris oleoabundans* HK-129 by a two-stage process. *Bioresource*

Technology, 155*:* 204-212. DOI:10.1016/j. biortech.2013.12.109

Toyub, M.A., Ahmed, S.R., Miah, M.I. & Habib, M.A.B. (2007). Growth performance and nutritional value of *Chlorella ellipsoidea* in fertilizer factory effluent media. *Asian Fisheries Science*, 20(1/2): 65-79. DOI:10.33997/j.afs. 2007.20.1.006