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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

A Study on the Culture of *Chlorella ellipsoidea* in Various Concentrations of Unripe Tomato Juice Media

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Abstract: The experiment was conducted to evaluate the performance of organic nutrients of Unripe Tomato Juice (URTJ) added with 0.2 g L⁻¹ urea and Bold Basal Medium (BBM) as standard, for growing of *C. ellipsoidea* in laboratory condition for three months. *C. ellipsoidea* was cultured four different concentrations such as 0.7, 1.4, 2.8 and 4.15 g L⁻¹ URTJ with added 0.2 g L⁻¹ urea and Bold Basal Medium for a period of 16 days. The initial cell density of *C. ellipsoidea* was 2.90×10⁵ mL⁻¹, which attained a maximum cell density of 108.03×10⁵ mL⁻¹ in BBM followed by 75.24×10⁵, 69.67×10⁵, 54.80×10⁵ and 27.35×10⁵ mL⁻¹ in 2.8, 1.4, 0.7 and 4.15 g L⁻¹ of URTJ media with added 0.2 g L⁻¹ urea, respectively on the 12th day of culture. Similar trend was observed in case of optical density of *C. ellipsoidea*. The proximate composition of *C. ellipsoidea* cultured in URTJ media was analyzed and found that the ranges of crude protein, crude lipid, ash and moisture ranged from 22.39-39.91%, 3.66-5.19%, 10.10-11.11% and 8.15-9.45%, respectively. Results showed that growth of *C. ellipsoidea* was significantly (p<0.05) higher in the concentrations of 2.8 g L⁻¹ of URTJ with added 0.2 g L⁻¹ urea than other concentrations (0.7, 1.4 and 4.15 g L⁻¹) of URTJ with added urea at the temperature of 29.04°C, dissolved oxygen of 5.71 mg L⁻¹, pH of 8.08 and light intensity of 1583 luxm⁻²s⁻¹

Key words: *C. ellipsoidea*, unripe tomato juice, bold basal medium, urea

INTRODUCTION

Chlorella sp. is one kind of alga, contain long chain Polyunsaturated Fatty Acids (PUFA), which make it valuable for marine invertebrates and fish^[1]. It was shown that the presence of *Chlorella* sp. improved the growth and survival of 40 species of fish studied^[2]. To some extent, microalgae are also used for rearing the larvae of freshwater prawn and some marine fish like sea bass^[3]. The protein content of *Chlorella* sp. is appreciably higher (50% of dry weight) than that of the best vegetable sources used in animal feed^[4]. According to Yap *et al.*^[5] a still higher proportion of protein (33%) can be replaced by *Spirulina* sp. or *Chlorella* sp. without negative symptoms.

Recently, in some countries *Chlorella* sp. health foods are available in the form of tables, granules and drinks. These *Chlorella* sp. foods came into market in 1964 and met with increased sales during the 1970s. More than 70 companies now have their *Chlorella* sp. health foods registered at Nihon Kenko Eiyou Shokhin Kyokai

(Japan Health Food Association) and their annual sales are estimated to be above 40 billion yen^[6]. This rapid spread may be due to the fact that various health-promoting effect of *Chlorella* have been clarified.

Algae act as ideal waste remover in nature^[7]. It acts not only on agroindustrial but animal wastes as well by converting them into food materials. Algae can also play an important role in the gradual degradation and metabolizing of pesticides into simple inorganic forms (Recalcitrant compounds) from the aquatic bodies to make the environment free from hazardous materials^[7].

Live food is considered to be the best food for fishes. Due to the shortage of live food, the tropical fish exporting industry is being threatened^[8]. Thus, to accelerate the development of aquaculture industry, it is very important to culture microalgae. It is also expected to enhance our understanding of knowledge of biological production in the natural aquatic ecosystem to support our life by supplying lower cost protein rich food. Ultimate objective of the experiment was to develop low cost media for large scale production of microalgae.

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MATERIALS AND METHODS

The experiment was conducted in the live food culture laboratory of Department of Aquaculture, Bangladesh Agricultural University (BAU), Mymensingh, for a period of three months from February to April, 2003.

Culture of microalgae:

Collection of unripe tomatoes and preparation its juice: For the preparation of unripe tomato juice medium, unripe tomatoes were collected and was made unripe tomato juice.

Analysis of proximate composition of unripe tomato juice and cultured *C. ellipsoidea*: The proximate composition (Crude protein, Crude lipid, Moisture and Ash) of Unripe Tomato Juice (URTJ) was analyzed in the Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, following standard methods^[9]. For the analysis of proximate composition of cultured *C. ellipsoidea*, at first *C. ellipsoidea* were inoculated into 2.0 L bottle containing various concentrations of unripe tomato juice media to produce a culture containing 10% *C. ellipsoidea* suspension (Optical density at 620 nm = 0.20)^[10]. Then all the bottles were kept under fluorescent light (TFG FL-40SD/38 Day light, Taiwan) in light: dark (12:12) conditions in the laboratory. These bottles were continuously aerated using electric aerator. *C. ellipsoidea* were attained at peak stage up to stationary position, the *Chlorella* cells were collected from the culture bottles by centrifuge machine (Denley, BS 400) at 3000 rpm. Then the collected samples of *C. ellipsoidea* were dried at 50°C over night to get complete drying. After that the proximate composition (protein, lipid, moisture and ash) of cultured *C. ellipsoidea* was analyzed in nutrition laboratory following the procedure used to analyze the proximate composition of unripe tomato juice.

Collection and Maintenance of pure stock culture of *C. ellipsoidea*: Microalage, *Chlorella ellipsoidae* was cultured in the Bold Basal Media (BBM) and collected from the laboratory of Department of Aquaculture, Bangladesh Agricultural University, Mymensingh and pure stock culture of collected *C. ellipsoidea* samples was maintained in the laboratory in Bold Basal Medium (BBM). Growth of *C. ellipsoidea* was monitored daily and was checked under microscope to confirm its purity.

Preparation of unripe tomato juice media and Bold Basal Medium (BBM) for *C. ellipsoidea*: Four different concentrations, from Unripe Tomato Juice (URTJ) and one

from BBM^[11] were prepared for *C. ellipsoidea* culture. Unripe tomato juice was made from unripe tomatoes with the help of blender. At first the collected tomatoes were weighed and then smashed into juicy form through the blender for 10 minutes. Then four different amount of blended tomato juice (0.7, 1.4, 2.8 and 4.15 g L⁻¹) were measured, taken in four conical flask, filled with distilled water upto the mark and kept for fermentation for 15 days. These fermented mixtures were filtered through 30 µm plankton net and divided into 12 conical flasks of 1.0 L capacity. Distilled water and 0.2 g urea were added to make the volume 400 mL in each flask if necessary.

Stock solutions of different chemical ingredients of BBM were prepared with distilled water as mentioned in Table 1. Then to prepare final solution of BBM, 10 mL each from No. 1 to 6 and 1 mL each from No. 7 to ten were pipetted in a 1.0 L conical flask, mixed and volume was made up to 1.0 L with distilled water.

Neutralization and sterilization of culture media: To naturalize all the culture media, 0.1 N HCl and 0.1 NaOH were used. After measuring the pH of the media, either acid or alkali were added by dropper and mixed thoroughly. When the media were acidic, NaOH was added and when the media were alkaline HCl was added. This process was continued to make pH of media at 7.0±0.01 and to sterilize the culture media, the flasks containing tomato juice media and BBM were sterilized at 126°C for 15 min, with moist heat in an autoclave (Express equipment, Dixon's Surgical Instrument LTD). After autoclaved, the media were kept for over night to confirm if there any contamination.

Culture of *C. ellipsoidea* in unripe tomato juice media and BBM: Five treatments with 3 replicates for each, four from URTJ media with added 0.2 g L⁻¹ urea in different concentrations (0.7, 1.4, 2.8 and 4.15 g L⁻¹) and 1 from Bold Basal Media (BBM) were used to grow microalgae, *C. ellipsoidea* in 1.0 L conical flask. It was continued for 16 days. *C. ellipsoidea* were inoculated into each culture flask from pure culture containing 10% *C. ellipsoidea* suspension (Optical density at 620 nm = 0.20)^[10]. Eighty milliliter of *C. ellipsoidea* suspension was required for getting the required density. All the flasks were kept under fluorescent lights (TFC, FL-40SD/38 Day light, Taiwan) in light: dark = 12:12 h conditions in the laboratory. These culture flasks were continuously aerated using electric aerator (Daivo Pump, Aquarium Pump NS-8200). Nine sub-samplings were taken at every alternative day from to observe *C. ellipsoidea* cell density, optical density, physical and chemical properties of culture media.

Table 1: Inorganic nutrients of Bold Basal Medium (BBM) for *C. ellipsoidea* culture

Chemicals/Compounds	Concentration in stock solution (g L ⁻¹)	Amount in culture medium (m L ⁻¹)
NaNO ₃	25.00	10.00
MgSO ₄ ·7H ₂ O	7.50	10.00
NaCl	2.50	10.00
K ₂ HPO ₄	7.50	10.00
KH ₂ PO ₄	17.50	10.00
CaCl ₂ ·2H ₂ O	2.50	10.00
Trace elements:		1.00
ZnSO ₄ ·7H ₂ O	8.82	--
MnCl ₂ ·4H ₂ O	1.44	--
MoO ₃	0.71	--
CuSO ₄ ·5H ₂ O	1.57	--
Co(NO ₃) ₂ ·6H ₂ O	0.94	--
H ₃ BO ₃	11.40	1.00
EDTA-KOH solution		1.00
EDT Na ₂		50.00
KOH	31.00	
FeSO ₄ ·H ₂ O	4.98	1.00
Cone. H ₂ SO ₄	1.0 mL/1 L	

Table 2: Maximum and minimum mean values of light intensity (luxm⁻²s⁻¹), Dissolved Oxygen (mg L⁻¹), temperature (°C) and pH of URTJ media and BBM contained *C. ellipsoidea*

Parameters	Mean value	Media	Day
Light	Minimum	1386.00	URTJ (0.7 g L ⁻¹) 14th
Intensity	Maximum	1760.00	BBM 6th
Dissolved Oxygen	Minimum	3.60	URTJ (4.15 g L ⁻¹) 1st
	Maximum	5.95	BBM 12th
Temperature	Minimum	28.25	URTJ (0.7 g L ⁻¹) 3rd
	Maximum	29.68	URTJ (0.7 g L ⁻¹) 1st
pH	Minimum	7.00	URTJ (all) and BBM 12th
	Maximum	8.34	BBM 12th

Estimation and calculation of *C. ellipsoidea* cell density and optical density: Cell density of *C. ellipsoidea* was estimated using an improved Neubauer ruling haemaytometer according to formula modified from Clesceri *et al.*^[12]. The cell mL⁻¹ were calculated by the following formula^[12]:

$$\text{Cell per mL} = \frac{\text{Cell Number per 16 big square}}{10^{-4}} \times \text{dillution factore}$$

Optical densities of the samples were determined at 620 nm, by using UV Spectrophotometer (Milton Roy, Spectonic 1001 Plus)^[12].

Study on the physical and chemical factors of culture media: During sampling time the physical parameters such as light intensity and temperature and chemical parameters such as Dissolved Oxygen (DO) and pH were measured.

Statistical analysis: One-way analysis of variance (ANOVA) was done to determine any significant difference among the treatment means in respect to cell number and optical density of *C. ellipsoidea* cultured in

different media. Same analysis was followed to find any significant difference among treatment means of dissolved oxygen, pH and optical density^[13].

RESULTS

Culture of *C. ellipsoidea*

Cell densities: Results of Table 4 showed that the initial cell density of *C. ellipsoidea* was 2.90×10⁵ mL⁻¹ which was attended to a maximum mean values of 75.24±6.11×10⁵ mL⁻¹ in medium III (2.8 g L⁻¹ URTJ) and 108.03±8.30×10⁵ in BBM. The highest cell number of *C. ellipsoidea* was found in medium III and BBM on 12th day of culture. A decreased trend in cell number was observed in all the media started from 14th day of culture.

Optical densities: The initial optical density (OD) of *C. ellipsoidea* was 0.036 at 620 nm which was attained to the highest mean values of optical density 0.921±0.04 when *C. ellipsoidea* was cultured in medium III (2.8 g L⁻¹ URTJ) followed by 1.219 cultured in BBM on 12th day (Table 5). Decreasing trend of optical density of *C. ellipsoidea* observed from 14th day of culture in all the media.

Physical and chemical parameters:

Physical parameters: The mean values of light intensities (luxm⁻²s⁻¹) of different concentrations URTJ media and BBM were measured during the experimental period of algal culture. More or less similar light intensities were observed during culture. The range of recorded light intensities were 1386 to 1760 luxm⁻²s⁻¹. The mean values of temperature in different culture media during the experiment were varied from 28.25 to 29.68°C. Temperature recorded during culture was more or less similar. The highest temperature was 29.68°C recorded in medium 0.7 g L⁻¹ URTJ on 12th day and lowest also was 28.25°C in the same medium on 3rd day of culture (Table 2).

Chemical parameters: The mean value of Dissolved Oxygen was 3.60 mg L⁻¹ recorded in URTJ (4.15 g L⁻¹) on the first day of culture where the maximum (5.95 mg L⁻¹) was recorded in BBM on 12th day of culture. The mean values of pH varied from 7.00 to 8.34. The highest pH value of 8.34 was recorded in BBM on 12th day of culture and lowest was 7.00 in all URTJ media on first day of culture (Table 2).

Proximate composition: The proximate composition of Unripe Tomato Juice (URTJ) media and *C. ellipsoidea* cultured in different concentration of URTJ media with added 0.2 g L⁻¹ urea are shown in Table 3-5, respectively.

Table 3: Mean±SD of proximate composition (Crude protein and crude lipid) of *C. ellipsoidea* grown (on 12th day) in various concentrations of Unripe Tomato Juice (URTJ) media with added urea 0.2 g L⁻¹

Unripe tomato juice media					
Components	Medium I (0.7 g L ⁻¹)	Medium II (1.4 g L ⁻¹)	Medium III (2.8 g L ⁻¹)	Medium IV (4.15 g L ⁻¹)	BBM
Moisture	8.13±0.02	8.16±0.02	9.43±0.03	8.31±0.03	8.25±0.02
Crude protein	27.92±0.62 ^d	30.68±0.99 ^e	32.11±0.57 ^b	22.39±1.07 ^e	39.91±1.17 ^a
Crude lipid	4.14±0.17 ^b	3.80±0.29 ^{cd}	3.83±0.42 ^e	5.19±0.95 ^a	3.66±0.16 ^d
Ash	10.23±0.02	10.64±0.03	10.21±0.01	10.69.01±0.02	11.15±0.02

Table 4: Mean±SD of cell (× 10⁵) mL⁻¹ of *C. ellipsoidea* grown in four different concentrations of Unripe Tomato Juice (URTJ) with added 0.2 g L⁻¹ urea and BBM from 2nd day to 16th day

Original order (day)								
Treatments (medium)	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	16th day
I	5.32±0.20 ^d	9.08±0.6 ^d	11.62±0.36 ^d	25.19±3.22 ^d	38.02±8.11	54.80± 8.72	49.75± 10.88 ^d	37.38±5.00 ^d
II	6.30±0.10 ^e	13.29±0.64 ^e	19.56±2.04 ^e	38.83±2.57 ^e	56.89±9.17	69.67± 2.34	64.88±2.17 ^e	43.96±1.10 ^e
III	8.39±0.34 ^f	14.12±0.44 ^f	24.94±4.92 ^f	39.84±5.07 ^f	60.88±10.11	75.24± 6.11	68.48±4.71 ^f	45.14±3.00 ^f
IV	4.43±0.50 ^f	6.86± 0.57 ^e	4.94± 0.70 ^e	12.97±2.61 ^e	17.98±2.81	27.35± 3.38	24.88±3.76 ^e	17.30±2.48 ^e
V (Control as BBM)	9.39±0.13 ^a	18.87±0.78 ^a	26.99±7.03 ^a	51.01±8.70 ^a	83.17±6.56	108.03± 8.30	99.80±11.62 ^a	87.09±6.82 ^a

Table 5: Mean±SD of optical density at 620 nm of *C. ellipsoidea* grown in four different concentrations of Unripe Tomato Juice (URTJ) with added 0.2 g L⁻¹ urea and BBM from 2nd day to 16th day

Original order (day)								
Treatments (medium)	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	16th day
I	0.079±0.01 ^{cd}	0.158±0.02 ^c	0.214±0.01 ^d	0.385±0.05 ^c	0.548±0.09 ^e	0.727±0.10 ^d	0.686±0.13 ^c	0.546±0.05 ^e
II	0.094±0.00 ^f	0.215±0.03 ^{bc}	0.308±0.04 ^e	0.530±0.04 ^{bc}	0.747±0.11 ^{bc}	0.892±0.02 ^c	0.855±0.02 ^{bc}	0.677±0.01 ^{bc}
III	0.132±0.00 ^f	0.221±0.01 ^b	0.393±0.05 ^b	0.567±0.11 ^b	0.786±0.10 ^b	0.921±0.04 ^b	0.888±0.04 ^b	0.682±0.02 ^b
IV	0.074±0.00 ^d	0.130±0.02 ^d	0.157±0.03 ^e	0.23±0.01 ^d	0.272±0.07 ^d	0.438±0.06 ^e	0.410±0.07 ^d	0.293±0.01 ^d
V (Control as BBM)	0.193±0.00 ^a	0.289±0.01 ^a	0.397±0.07 ^a	0.660±0.08 ^a	0.986±0.09 ^a	1.219±0.13 ^a	1.123±0.14 ^a	0.990±0.07 ^a

Figures in the same column having same superscripts are not significantly difference (p>0.05)

Where, Medium, I = 0.7 g L⁻¹ of unripe tomato juice with added 0.2 g L⁻¹ urea Medium, II = 1.4 g L⁻¹ of unripe tomato juice with added 0.2 g L⁻¹ urea Medium, III = 2.8 g L⁻¹ of unripe tomato juice with added 0.2 g L⁻¹ urea Medium, VI = 4.15 g L⁻¹ of unripe tomato juice with added 0.2 g L⁻¹ urea Medium, V = BBM as control

The mean values of crude protein of *C. ellipsoidea* cultured in various concentrations of URTJ and BBM on 12th day were highest (39.91%) when grown in BBM and lowest (22.39%) when grown in 4.15 g L⁻¹ URTJ. The highest crude lipid (5.19%) of algae was observed when cultured in 4.15 g L⁻¹ URTJ and lowest (3.66%) grown in BBM. Moisture (%) and ash (%) content in cultured *C. ellipsoidea* were more or less similar.

Statistical analysis: Statistical analysis for cell number and optical density of cultured *C. ellipsoidea* were done by Zar^[14] and the differences among the treatments were performed following the Duncan's Multiple Range Test^[15]. The cell (× 10⁵) mL⁻¹ of *C. ellipsoidea* cultured in 2.8 g L⁻¹ URTJ with added 0.2 g L⁻¹ urea was significantly (p<0.05) higher than other concentrations (0.7, 1.4 and 4.15 g L⁻¹) of URTJ media with added 0.2 g L⁻¹ urea. From statistical analysis, it was also found that the cell (× 10⁵) mL⁻¹ and optical density was significantly higher in BBM than those of *C. ellipsoidea* grown in four different media with added 0.2 g L⁻¹ urea (Table 4 and 5).

DISCUSSION

C. ellipsoidea was cultured in different concentration of Unripe Tomato Juice (URTJ) media with added 0.2 g L⁻¹

urea and Bold Basal Medium (BBM). The initial cell number was 2.90×10⁵ in each medium of URTJ and BBM. But this was attained a maximum cell density of 108.03×10⁵ mL⁻¹ BBM, 75.24×10⁵ in 2.8 g L⁻¹ URTJ with added 0.2 g L⁻¹ urea. Though the culture was started with the same inoculum, the cell growth varied in different media (URTJ media and BBM). This variation might be due to the different nutrient composition of different media. The growth rate of *C. ellipsoidea* was higher in 2.8 g L⁻¹ URTJ with added 0.2 g L⁻¹ than another concentration of URTJ (0.7, 1.4 and 4.15 g L⁻¹) with added urea. It might have happened due to the suitable amount of nutrients in 2.8 g L⁻¹ URTJ with added urea than another media of URTJ. Karmakar^[6] found the maximum cell densities of *C. ellipsoidea* were 68.28 ×10⁵ mL⁻¹ in Ripe Bean Seed Powder (RBSP) medium, 3.36×10⁵ mL⁻¹ in Unripe Bean Seed Powder (URBSP) medium and 93.73×10⁵ mL⁻¹ in BBM on 8th day which were lower than the present results because of different nutrient composition.

Similar type of work was performed by Alam^[17] who found that the green alga, *Chlorella* sp. attained a maximum cell density of 282.77×10⁵ mL⁻¹ in modified Nichols medium (MNM), 215 ×10⁵ in NPK medium on 10th day of culture. It was observed that the cell densities of *C. ellipsoidea* grown in different media of URTJ with

added 0.2 g L⁻¹ urea and BBM were lower than the findings of Alam^[17]. It might be due to the different nutrient compositions of media used in culture and the size of cultured species. His culture species was different from the present culture species. The size of *C. ellipsoidea* comparatively larger than other *Chlorella* spp. From the findings of Hossain^[18], it was recorded that the green alga, *C. ellipsoidea* attained a maximum cell density of 4.38×10⁵ mL⁻¹ on 11th day in medium IV (mixed medium 50% inorganic medium +50% whole pulse powder medium) which was lower than the present findings. The reports made by Vass and Bhanu^[4], Martinez Jeronimo and Espinosa-cakez^[19], Khan^[20] on the cell densities of freshwater microalgae, *Chlorella* sp. *Ankistrodesmus falcatus* and *Scenedesmus incrassatulus*, were lower than present findings which might be due to adequate nutrient composition, appropriate cell size and optimum temperature. Among the various concentrations (0.7, 1.4, 2.8 and 4.15 g L⁻¹) of URTJ media with added urea (0.2 g L⁻¹), the maximum optical density (0.921 at 620 nm) of *C. ellipsoidea* was found in 2.8 g L⁻¹ URTJ medium followed by optical density of 1.219 in BBM. It might have happened due to appropriate nutrient composition, which was more suitable in 2.8 g L⁻¹ URTJ medium with added 0.2 g L⁻¹ urea compared to other concentrations of URTJ media.

The maximum cell density of *Chlorella* sp. cultured in 2.8 g L⁻¹ in URTJ medium with added 0.2 g L⁻¹ urea was found at lower light intensity (1653 luxm⁻²s⁻¹) during the present study among the light intensity (2080 to 3000 luxm⁻²s⁻¹) used by Haq^[21] and Karmakar^[16] which might be due to optimum light intensity.

Mayo^[22] reported that the maximum growth rate of *Chlorella vulgaris* was found at pH of about 6.13 to 6.48, which is not similar with the present results. According to the findings of Alam^[17], Rahman^[23] and Mayo^[22], maximum growth of different microalgae observed at the pH range from 6.84 to 8.38. James *et al.*^[24] observed maximum cell density at pH of 6.5. Habib^[10] found the maximum growth of *Chlorella* species at pH 6.5. Thus the range (7.00 to 8.24) of pH observed in the present study is suitable for microalgae culture. Dissolved Oxygen (DO) is another important chemical parameter responsible for normal living of microalgae. The Dissolved Oxygen values in the experimental period were found between 3.60 to 5.95 mg L⁻¹. Fluctuation in DO value might be due to alteration of photosynthetic rate in the culture media. Alam^[17] and Rahman^[23] found maximum DO 5.46 mg L⁻¹ and 4.49 mg L⁻¹, respectively when *Chlorella* sp. cultured in MNM, BBM and NPK. The present value of DO is more or less similar of other findings. Temperature of the media was recorded from 28.25°C to 29.68°C during the study,

which was more or less similar to that of Kamakar^[16], Alam^[17], Rahman^[23] and Khan^[20]. The highest temperature recorded during culture period was 31.9°C. Mayo^[22] observed the maximum growth rate of microalgae *Chlorella vulgaris* at the optimum temperature of 32.4°C. Khan^[20] found maximum cell density of cultured *Chlorella antiqua* at temperature 25°C. Lewin and Macks^[25] recorded that *Chlorella armatum* reached at its optimum level at 20°C while *Asterionella socialis* had an optimum growth at 10°C. From the findings and review, it is concluded that the optimum growth temperature varies from one species to another.

On the basis of results obtained in the present study, it may be concluded that the highest growth of *C. ellipsoidea* among URTJ media was at a concentration of 2.8 g L⁻¹ with added urea. But the highest growth among all of the media was found in BBM. Thus, the concentration 2.8 g L⁻¹ with added 0.2 g L⁻¹ urea is suitable for culture *C. ellipsoidea*. So, the culture of *C. ellipsoidea* in URTJ at a concentration of 2.8 g L⁻¹ may be recommended for further microalgae culture.

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