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EVALUATION OF GROWTH OF *Chlorella ellipsoidea* IN DIFFERENT CULTURE MEDIA

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Abstract

An experiment was conducted to evaluate the growth of *Chlorella ellipsoidea* in three different media viz,. medium I (pulse bran), medium II (soil extract) and medium III (inorganic) under the natural environmental conditions. The alga, *C. ellipsoidea*, reached maximum cell density of 56.32 × 10^6 cells ml^{-1} in 10 days in medium I (pulse bran), maximum cell density of 102.99 × 10^6 cells ml^{-1} in 11 days in medium II (soil extract) and maximum cell density of 64.23 × 10^6 cells ml^{-1} in 12 days in medium III (inorganic medium). The ranges of water temperature, air temperature and light intensity were 22 to 32°C, 22 to 34°C and 2.11 to 4.31 (× 10^3) lux, respectively during the culture period. The average sunshine period was 7.65 ± 1.57 hours. Total alkalinity, free CO_2, pH, NO_3-N, PO_4-P of algal culture medium I, medium II and medium III were 220, 200 and 150 mg L^{-1} ; 26, 9 and 19 mg L^{-1}; 7.9, 7.6 and 7.5; 45, 45 and 133.33 mg L^{-1}; 10.9, 15.1 and 37.06 mg L^{-1}, respectively. Cell densities of cultures of *C. ellipsoidea* under three treatments I, II and III, it can be concluded that cell densities under 3 treatments are significantly different (F=39.78) and treatment II (soil extract medium) is the best for algal (*C. ellipsoidea*) culture among three treatments.

Keywords: Soil Extract, *Chlorella* , Culture Media, Envioronmental Factors, Chemical Quality of Media

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Introduction

Microalgae, such as *Chlorella* are rich in nutrients especially protein, lipid and minerals (Becker, 1994a & b; Soeder, 1980; Geldenhuys *et al.*, 1988). A dried sample of pure *Chlorella* has nutrients which contain moisture 4.5%, crude protein 51.8%, crude fat 13.6%, carbohydrate 25.2%, crude fibre 2.3%, crude ash 4.9%, and chlorophyll 1.52%. Available energy from *Chlorella* was 430 cal g^{-1} (Tang and Suter, 2011). *Chlorella* contains all other essential nutrients that are needed for growth and development of both aquatic and terrestrial animals. So, people of some countries such as China and Japan have been using seaweeds and certain other algae as a source of food. Having perceived the miraculous nutritional quality of *Chlorella*, Japanese manufactured *Chlorella* tablets in the late 1950s; and from that time, numerous endeavors have emerged at specialized industries worldwide with a view to producing health food, food additives, animal feed, biofertilizers, biofuel and an assortment of natural products (Sasson, 1997; Christi, 2007). Two algae, *Scenedesmus* sp. and *Chlorella* sp. are of commercial importance due to their commercial significance of *Chlorella* sp. (Rydlo, 1973) which are (i) food, protein supplement/fortification in diets for malnourished children and adults; (ii) feed, protein/vitamin supplement in feed for poultry, cattle, pigs, and bivalves; (iii) health food, *Chlorella* sp. powder as ingredients and supplement in health food recipes and products; (iv) therapeutics, β-carotene as possible anti skin-cancer treatment is used for variety of skin diseases; (v) pigments, β-carotene as food color and food supplement (provitamin A); and (vi) other uses, biofertilizers, soil conditioner, and waste treatment.

So, considering very high importance of microalgae culture, *Chlorella ellipsoidea,* a very important microalga, has been selected to culture in inexpensive culture media, especially soil extract, to reduce the cost of culture and to introduce easy technique of culture.

Materials and Methods

Preparation of three algal culture media

1. *Preparation of inexpensive culture medium using pulse bran (masakalai bran, Vigna mungo) according to the method of Rahman (2000):*

This medium was prepared by mixing 1 kg pulse bran (*Vigna mungo*) in 20-litre tap water. After a week, 15 g urea [$(NH_2)_2$ CO] was added into the mixture. After three weeks, pulse bran mixture

was filtered through thin markin cloth to discard solid materials and then after several days the clear supernatant was siphoned to another bucket. To clear the medium, lime (CaO) was added to the medium at the rate of 2 g per litre and after a week supernatant was siphoned to another bucket. After adding lime, pH of the medium increased to about 10. Then to lower the pH to about 7.9, conc. H_2SO_4 was added to the medium at the rate of 0.325 mlL^{-1} and after one week, the medium was ready to be used for algae culture.

2. Preparation of soil extract medium

Soil (textural class, silty clay loam, pH, 7.3; organic carbon (%), 0.95; organic matter (%), 0.64; available phosphorous (mg L^{-1}), 17.12; total nitrogen (%), 0.087 was dried for 2 weeks. After drying, soil was crushed into powder to facilitate sieving. Soil was sieved through a small mesh sieve usually used to sieve rice powder for making cake. Then 2 kg soil was mixed with 5L tap water in a plastic bucket. Soil-water mixture was kept for 5 days and during this period, mixture was stirred everyday for half an hour. Then soil-water mixture was kept in this condition for several days untill the settling of soil particles at the bottom of the bucket. Then supernatant (soil extract) was siphoned with a plastic pipe to a plastic container. This supernatant was sterilized in an autoclave at 121°C temperature and 15 lb/inch² pressure for 20 minutes. Then soil extract was treated with commercial urea (5 g per litre) and T.S.P (2.5 g per litre) fertilizers, this soil extract were used as algal culture medium.

3. Preparation of inorganic medium

Inorganic medium was prepared with the inoculation of stock solutions of 8 major nutrients ($NaNO_3$, $MgSO_4.7H_2O$, K_2HPO_4, $CaCl_2.2H_2O$, Na_2CO_3, EDTA, Ferric ammonium citrate, Citric acid) and 6 minor nutrients (H_3BO_3, $MnCl_2.4H_2O$, $Na_2MoO_4.H_2O$, $ZnSO_4.7H_2O$ $CuSO_4.7H_2O$, $CaSO_4.7H_2O$). Ten liter distilled water was taken in a 30 litre plastic bucket and stock solutions were added and mixed well in the bucket and stored in a 15 litre plastic container. Stock solutions were prepared in distilled water using different chemical compounds as major nutrients and trace elements soil extract were used as algal culture medium.

Study of the environmental factors

Water temperature, air temperature was determined by thermometer and light intensity were determined by a lux-meter (LUX-101). Data of sunshine period and rainfall were collected from the "Weather Yard" of Bangladesh Agricultural University, Mymensingh.

Results

Cell densities of *C. ellipsoidea* in medium I (pulse bran)

The growth of *C. ellipsoidea* calculated in medium I (pulse bran) under treatment I in 4 replications in natural light and temperature conditions. Culture of these green algae, started with 0.152×10^6 cells ml^{-1} (inoculum was 5%) which attained the maximum cell density of 56.32×10^6 cells ml^{-1} in 10 days. The average cell density of *C. ellipsoidea* was 28.31×10^6 cells ml^{-1} after the culture period of 17 days (Table 1, Fig. 1).

Table 1. Daily variation of mean cell density of *C. ellipsoidea* (mean of 4 replications under the treatment) cultured in medium-I (pulse bran), medium-II (soil extract), medium-III (inorganic) and environmental conditions

Culture time (days)	Cell density ($\times 10^6$, cells ml^{-1})			Water temp. (°C) (Av.)	Air temp. (°C) (Av.)	Light intensity ($\times 10^3$ lux)	Sunshine Period* (hrs.)	Rainfall* (mm)
	Treatment I	Treatment II	Treatment III					
1	3.68	6.4	2.56	27.00	30.00	2.50	6.8	00.0
2	8.89	13.21	10.87	27.75	27.00	2.11	9.3	00.0
3	15.09	18.99	18.32	27.50	32.50	2.93	9.2	00.0
4	29.16	44.19	23.44	28.50	31.00	2.90	8.8	00.0
5	32.86	57.76	27.50	27.50	29.50	2.53	8.1	00.0
6	33.22	58.97	30.71	27.00	26.75	2.30	7.5	00.0
7	41.7	63.06	35.87	26.75	27.50	4.31	6.5	0.60
8	43.99	80.26	40.22	26.50	29.50	3.93	8.3	00.0
9	46.29	85.02	46.28	25.00	28.50	2.90	9.3	00.0
10	**56.32**	90.54	48.60	25.75	28.75	2.26	7.4	00.0
11	41.29	**102.99**	50.41	25.50	29.00	3.00	6.8	00.0
12	35.19	98.00	**64.23**	25.50	28.50	2.58	8.6	00.0
13	20.79	92.40	60.23	26.50	29.00	2.63	5.9	00.0
14	19.77	83.78	55.03	25.50	29.00	3.52	5.8	00.0
15	19.33	75.78	44.98	24.35	28.00	3.80	3.7	11.0
16	18.26	39.20	38.78	24.00	27.00	3.50	8.8	00.0
17	15.31	22.44	33.89	24.50	27.50	2.45	9.2	00.0
Mean± SD	28.31±15.08	60.76±30.56	36.98±15.65	26.18±1.31	28.76±1.49	2.95±0.64	7.65±1.57	0.68±0.97

N.B Starting date of the algal culture was 3.10.11. Initial cell density of culture on zero day was 0.152×10^6 cells ml^{-1}

* Data of sunshine period and rainfall were collected from "Weather Yard", Bangladesh Agricultural University, Mymensingh.

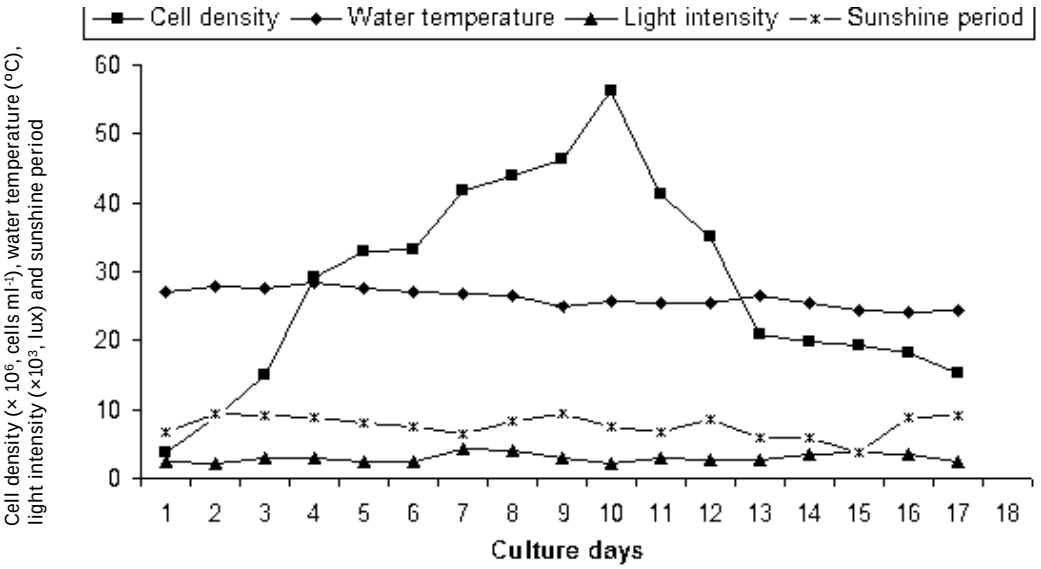

Fig. 1. Daily variations of mean cell density (×10^6, cells ml^{-1}) of *C. ellipsoidea* cultured in medium-I (pulse bran extract, treatment-I) and average water temperature (°C), light intensity (×10^3, lux) and sunshine period (hrs.)

Cell densities of *C. ellipsoidea* in medium II (soil extract medium)

The growth of *C. ellipsoidea* cultured in medium II (soil extract) attained the maximum cell density of 102.99 × 10^6 cells ml^{-1} in 11 days. The average density of *C. ellipsoidea* was 60.76 × 10^6 cells ml^{-1} after the culture period of 17 days (Table 1, Fig. 2).

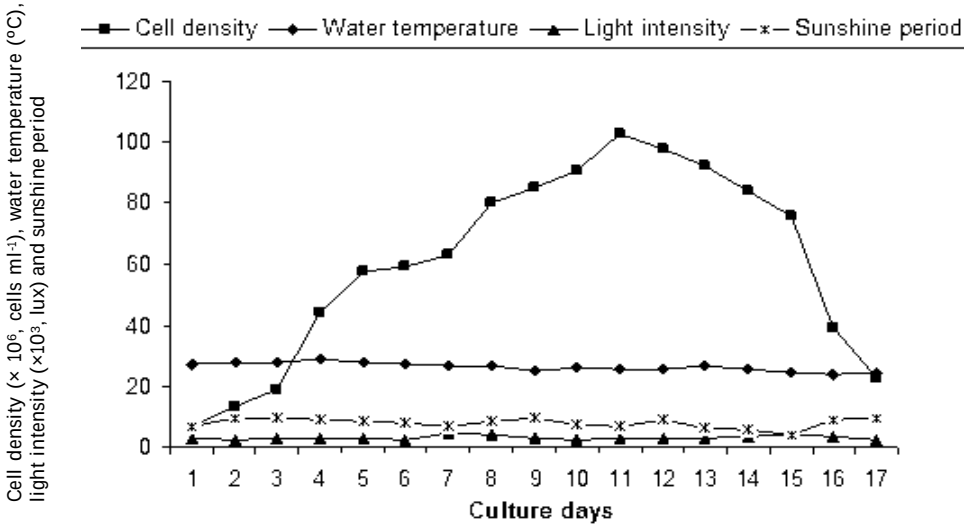

Fig. 2. Daily variation of mean cell density (×10^6, cells ml^{-1}) of *C. ellipsoidea* cultured in medium-II (soil extract, treatment-II) and average water temperature (°C), light intensity (×10^3, lux) and sunshine period (hrs.)

Cell densities of *C. ellipsoidea* in medium III (inorganic medium)

The growth of *C. ellipsoidea* cultured in medium III (inorganic) attained the maximum cell density of 64.23×10^6 cells ml^{-1} in 17 days. The average cell density of *C. ellipsoidea* was 37.17×10^6 cells ml^{-1} after the culture period of 17 days. (Table 1, Fig. 3). Comparison of daily variations of mean cell density (x10^6 cell ml^{-1}) *of C. ellipsoidea* of media I , II, and III have been presented in Fig. 4.

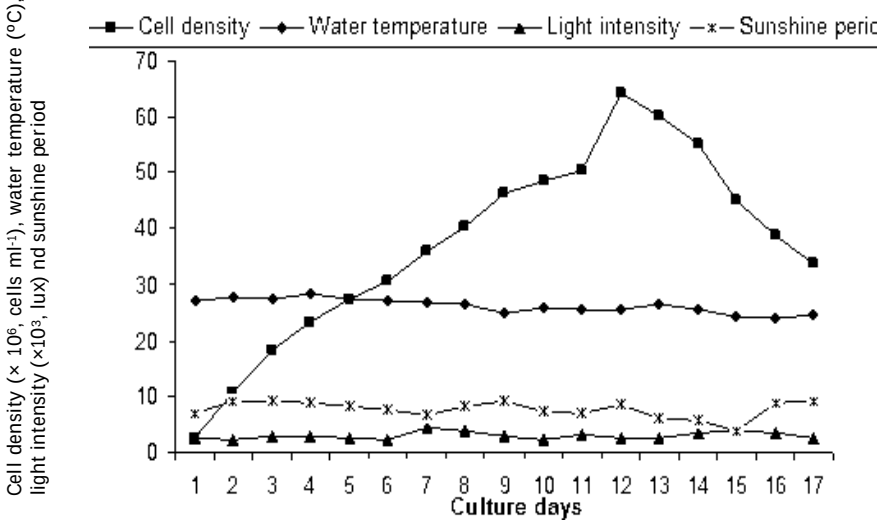

Fig. 3. Daily variation of mean cell density (×10^6 cells ml^{-1}) of *C. ellipsoidea* cultured in medium-III (Inorganic, treatment-III) and average water temperature (°C), light intensity (×10^3, lux) and sunshine period (hrs.)

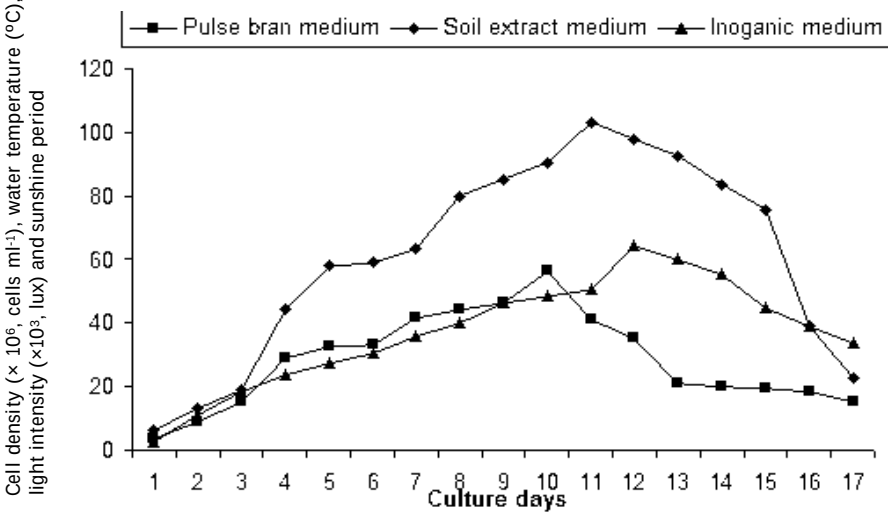

Fig. 4. Comparison of daily variation of mean cell density (×10^6 cells ml^{-1}) of *C. ellipsoidea* cultured in medium-I (pulse bran extract, treatment-I), medium-II (soil extract, treatment-II) and medium-III (inorganic, treatment-III)

Chemical properties of the three cultural media have been presented in Table 2 .

Table 2. Chemical properties of the culture media

Culture medium	pH	Free CO_2 (ppm)	Total alkalinity (ppm)	NO_3-N (ppm)	PO_4-P (ppm)
Pulse bran medium	7.9	26	220	45	10.9
Soil extract medium	7.6	9	200	45	15.1
Inorganic medium	7.5	19	150	133.33	37.06

Discussion

The range of cell density of *C. ellipsoidea* in medium I (pulse bran extract medium) was 3.68 to 56.32 × 10^6 cells ml^{-1} during the culture period. The average cell density was 28.31 ± 15.084 × 10^6 cells ml^{-1}. During the culture period of *C. ellipsoidea* in medium I exponential phase was up to 10th day from the beginning and then from stationary phase cell density started to decline towards death phase.

The range of cell density of *C. ellipsoidea* in medium II (soil extract medium) was 6.4 to 102.99 $\times 10^6$ during the culture period. The average cell density was 60.76 ± 30.56 × 10^6 cells ml^{-1}. During the culture period of *C. ellipsoidea* in medium II, exponential phase was found up to 11th day from the beginning and then from stationary phase cell density started to decline towards death phase.

The range of cell density of *C. ellipsoidea* in medium III (inorganic medium) was 2.56 to 64.23×10^6 cells ml^{-1} during the culture period. The average cell density was 37.17 ± 16.43 × 10^6 cells ml^{-1}. During the culture period of *C. ellipsoidea* in medium III, exponential phase was found up to 12th day from the beginning and then from stationary phase cell density started to decline towards death phase.

Wongsnansilp *et al.* (2007) found, in an experiment of culture of an alga, *Chlorosarcinopsis* sp. (PSU/CHL20), the highest cell density of 14.8 × 10^6 cells ml^{-1} at 30°C on 14 day of culture which have little similarities with those of the present experiment but the highest densities found in the present experiment are much higher than those of Wongsnansilp *et al.* (2007). Singha (2001) performed an experiment on growth performance of *C. vulgaris* in different concentrations of sugarcane mill effluent medium (SMEM), press mud medium (PMM) and bold basal medium (BBM). The highest standing crop of 159.00 × 10^5 cell ml^{-1} resulted from the treatment of 50% SMEM, 163.25 × 10^5 cell ml^{-1} from PMM (1.0 g L^{-1}) and 213.043 × 10^5 cell ml^{-1} for all the media. The results of growth performance revealed that the growth of *C. vulgaris* was significantly higher ($P \leq 0.01$) in PPM at the concentration of 1.0 g L^{-1} than other concentrations of PPM and SMEM.

According to ANOVA and DMRT of cell densities of cultures of *C. ellipsoidea* under treatments I, II and III, it can be concluded that cell densities under 3 treatments (i.e. 3 media) are significantly different (F=39.78) and among these 3 treatments, treatment II (soil extract medium) is the best for algal culture than treatment I and treatment III. Preparations of soil extract medium and pulse bran medium are simple and the materials are inexpensive and easily available in Bangladesh. So, the use of inexpensive soil extract and pulse bran as algal culture medium might be used commercially and economically to culture algae, especially *Chlorella* sp., which can be used as feed for fish fry, poultry, livestock and as human food as live feed or as dried powder form.

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