ISSN: 2224-0616
Int. J. Agril. Res. Innov. & Tech. 7 (1): 51-57, June, 2017 Available online at http://www.ijarit.webs.com

CULTURE OF *Chlorella ellipsoidea* IN DIFFERENT CULTURE MEDIA

M.M. Mohshina[1], M. Shahjahan[2], P. Chowdhury[3]* and M.S. Rahman[4]

Received 30 April 2017, Revised 31 May 2017, Accepted 26 June 2017, Published online 30 June 2017

Abstract

An experiment of algal culture was conducted in natural light and temperature conditions at a balcony of a room at the 2nd floor of Fisheries Faculty Building facing the north. The experiment was done to evaluate the growth of *Chlorella ellipsoidea* in four different media, viz, medium I (inorganic), medium II (organic, whole pulse powder extract), medium III (organic, whole lentil powder extract) and medium IV (organic, whole gram powder extract) under natural environment conditions during January-June, 2015. Growth rates of the algal species in four different media were found not significantly different. The alga, *C. ellipsoidea* attained maximum cell density of 28.89×10^6 cell ml^{-1} in the 15th day in medium I, of 30.69×10^6 cell ml^{-1} in the 13th day in medium II, of 26.18×10^6 cell ml^{-1} in the 15th day in medium III and of 21.12×10^6 cell ml^{-1} in the 13th day in medium IV. The ranges of air temperature, water temperature and light intensity were 21°C to 38°C, 23°C to 36°C and $2.28\times10^3$to 9.60×10^3 Lux respectively during the culture period. The average sunshine period was 5.87±2.82 hrs. Total alkalinity, free CO_2, pH , NO_3-N and PO_4-P of algal culture media I, II, III and IV were 128, 540, 554 and 322 mgL^{-1}; 32, 162, 102, 70 mgL^{-1}; 7.4, 8, 7.9 and 7.9; 180, 36.6, 62.4 and 150 mgL^{-1}, and 25.2, 48.2, 42.4 and 45.6 mgL^{-1}, respectively. According to ANOVA of cell densities of cultures of *C. ellipsoidea* under treatments are not significantly different (F=1.441077). It is clear that differences between them are not significant i.e. mean algal cell densities are more or less same as differences between treatments are less than 20%.

Keywords: *Chlorella ellipsoidea*, Algal Culture, Media, Water Quality

[1]Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[2]Professor, Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[3]Scientific Officer, Bangladesh Fisheries Research Institute, Mymensingh-2201, Bangladesh.
[4]Professor, Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

*Corresponding author's email: nitoldhk@gmail.com (P. Chowdhury)

Introduction

Following global fears of an uncontrollable human population boom during the late 1940s and the early 1950s, *Chlorella* was seen as a new and promising primary food source and as a possible solution to the then-current world hunger crisis. Many people during that time thought hunger would be an overwhelming problem and saw *Chlorella* as a way to end the crisis by providing large amounts of high-quality food for a relatively low cost (Belasco, 1997).

Microalgae play an important role in aquaculture as live food for larval stage of many species of crustacean and fish as well as for all stages of bivalves and as food for the zooplankton. However, microalgae are eventually fed to late larval and juvenile fish and crustaceans in hatcheries (Renaud *et al.*, 1991). Addition of various microalgae to the water during early first feeding of marine fish larvae frequently has resulted in improved growth and survival (Howell, 1979). It is observed that nursery cultivation of bivalve molluses required a consistent supply of suitable algae culture to maintain growth (Claus, 1981). Actually, successful fish culture practices primarily depend on the maintenance of healthy aquatic environment and the production of sufficient fish food organisms.

Microalgae are an essential component of the diet of marine bivalve mollusk (e.g. Oysters, Clams, Scallop and Mussels), the larvae of some marine gastropods (abalone), larva of salt water shrimp, fish species (e.g. Tilapia, Silver carp, Milk fish) and zooplankton. To some extent, microalgae are also used for rearing the larvae of freshwater prawn and larvae of some marine fish like sea bass (Fujimura and Okamoto, 1972).

Microalgae are used as a human food source for over-populated countries (Barlew, 1953) and for space travel (Haldane, 1951). There are several suggested advantages of microalgae. If algae are grown under suitable environment conditions, the protein yield from it may be quite better (Spoehr and Milner, 1949). In China and Japan,

seaweeds and certain other algae Porphyra, Ulva, Alaria and Chlorella are most commonly used. These not only consist of an important ingredient of soups but also are used for flavoring meat. Different vitamins such as B, C, Folic acid and Niacin are also found in them (Kumar and Singh, 1976). In Japan, Korea, Myanmar, China, America, Mexico and many countries of Europe the marine algae are very popular food, especially it is used as salad. Algae are also used as poultry food in different farms and it can be suggested the addition of algae in animal feeding (Becker, 1994).

It has been demonstrated that algae can also be used as fertilizer. The blue-green algae, which are rich in nitrogen and phosphorous, are excellent fertilizer. A suitably, blended mixture of seaweed and cyanophycean manures (i.e. bloom of *Microcystis* sp.) may serve as an ideal fertilizer and this can relieve the acute shortage of fertilizers in developing countries in tropical region; bottom-mud of dried up ponds is regularly used as manure in crop cultivation, the manurical value is mostly due to the high content of blue-green algae (Kumar and Singh, 1976).

Chlorella is a genus of single-cell green algae of the phylum of Chlorophyta. Its shape is spherical, about 2 to 10 micron in diameter, and has no flagella. The green photosynthetic pigments chlorophyll-a, chlorophyll-b are found in chloroplast of Chlorella. Requiring only carbon dioxide, water, sunlight, and a small amount of minerals to reproduce, it multiplies rapidly through photosynthesis.

So, considering very high importance of culture of microalgae, *C. ellipsoidea*, a very important microalgae, has been selected for culture in different media to evaluate the best culture media.

Materials and Methods

Preparation of organic media

Collection of pulse powder, lentil powder and gram powder

For preparation of organic medium, pulse powder (*Vigna mungo*), lentil powder (*Lens culinaris*) and gram powder (*Cicer arientium*) were collected from Choto Bazaar, Mymensingh City.

Preparation of inexpensive culture medium using whole pulse powder (Vigna mungo) according to the method of Rahman (2000).

This medium was prepared by mixing 1 kg pulse powder (*Vigna mungo*) in 30-litre tap water. After a week, 15 g urea was added into mixture. After three weeks, pulse powder mixture was filtered through thin marking cloth to discard solid materials and then after several days the clear supernatant was siphoned to another bucket. After adding lime for making medium clear, pH of the medium increased to about 10. Then to lower the pH to 7, conc. $H_2SO_4$was added to the medium at the rate of 0.325 ml per litre and after one week, the medium was ready for use as algal culture medium.

Preparation of inexpensive organic culture medium using whole lentil powder (*Lens culinaris*) and gram powders (*Cicer arientium*) are more or less similar to the pulse powder preparation.

Preparation of inorganic medium

Inorganic medium was prepared with the inoculation of stock solutions of nutrients. Ten litre distilled water was taken in a 15 litre plastic container and stock solutions were added and mixed well.

Table 1. Composition of inorganic algal growth medium (modified GBII algal growth medium of Stanier *et al*., 1971).

a) Major nutrients

Ingredients (compounds)	Concentration in stock solution	Inoculation in growth medium
$NaNO_3$	75.0 g 500 ml^{-1}	100 ml 10 L^{-1}
$MgSO_4.7H_2O$	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
K_2HPO_4	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
$CaCl.2H_2O$	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
Na_2CO_3	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
EDTA	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
Ferric ammonium citrate	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
Citric acid	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}

b) Trace elements

Ingredients (compounds)	Concentration in stock solution	Inoculation in growth medium
H_3BO_3	2.680 g L^{-1}	10 ml 10 L^{-1}
$MnCL_2.4H_2O$	1.810 g L^{-1}	10 ml 10 L^{-1}
$Na_2MoO_4.2H_2O$	0.390 g L^{-1}	10 ml 10 L^{-1}
$ZnSO_4.7H_2O$	0.220 g L^{-1}	10 ml 10 L^{-1}
$CuSO_4.7H_2O$	0.079 g L^{-1}	10 ml 10 L^{-1}
$CaSO_4.7H_20$	0.049 g L^{-1}	10 ml 10 L^{-1}

Experimental layout

Culture of *C. ellipsoidea* was done in four types of medium. Seeds of *C. ellipsoidea* were collected from previous cultures, which were done by the researcher Professor Md. Shahidur Rahman of the Department of Fisheries Management, BAU, Mymensingh.

Type of medium	Algal species cultured	Glassware used for culture	Amount of medium	Amount of seeds used	Duration of culture
Inorganic Medium (Treatment-I)	*Chlorella ellipsoidea*	1 litre conical flasks	500 ml	5% or 25 ml	21 days
whole Pulse powder extract ((Treatment-II)	-d0-	-d0-	-d0-	-d0-	-d0-
whole Lentil powder extract (Treatment-III)	-d0-	-d0-	-d0-	-d0-	-d0-
whole Gram powder extract (Treatment-IV)	-do-	-d0-	-d0-	-d0-	-d0-

N.B. Media were sterilized. The culture was done in natural light and temperature conditions on a steel framed glass shelf in a balcony of a room at 2nd floor of the Fisheries Faculty Building, BAU, Mymensingh.

Study of the environment factors

Water temperature (°C)

Maximum and minimum water temperature data were taken by a maximum-minimum thermometer daily.

Air temperature (°C)

Maximum and minimum air temperature data were taken by a maximum-minimum thermometer daily.

Light

Light intensity (Lux) at the place of algal culture was measured by a digital Lux-Meter (LX-1010B) daily during the culture period.

Sunshine period and rainfall

Data of sunshine period and rainfall were collected from the "Weather Yard" under the Department of Irrigation and Water Management of Bangladesh Agricultural University, Mymensingh.

Determination of chemical status of culture media

pH: pH of the culture media were measured by an electronic digital pH meter (Hanna Instruments Co.) before starting culture experiment.

Free CO_2: Free CO_2 of culture media were determined by titrimetric method (APHA, 1971).

Total Alkalinity: Total alkalinity of culture media was determined by methyl orange indicator method (APHA, 1971).

Nitrate-nitrogen (NO_3-N): For determining nitrate-nitrogen, samples were filtered through high glass microfiber filter paper with the help of vacuum pressure bottle. Then nitrate-nitrogen of the culture media was determined by a Nitrate Meter (Hanna Instruments Co.).

Phosphate-phosphorus (PO_4-P): For Phosphate-phosphorus determination samples were filtered in the same way of nitrate-nitrogen and Phosphate-phosphorus of the culture media were determined by phosphate Meter (Hanna Instruments Co.).

Estimation of cell density (cells ml^{-1}) of Chlorella ellipsoidea culture by a haemacytometer

The calculation of the cell density of algal culture (cells ml^{-1}) was done by the following formula (modified from Rahman, 1992)

$N = A \times 1000$

Where,

N = No. of plankton cells per ml of culture medium.
A = Average no. plankton cells in 0.1 cubic mm × 10
= Average no. plankton cells in 1 cubic mm

Statistical analysis

A computer through the pregame SPSS having three replications did ANOVA of cell densities of *C. ellipsoidea*, cultured in four different media (four treatments) each.

Results

Table 2. Daily variation of mean cell density of culture of *Chlorella ellipsoidea* cultured in four different media for a period of 21 days (Treatments 4, Replications 3).

Culture time (days)	Sampling Date	Cell density (×10⁶ cells ml⁻¹)±S.D			
		Treatment-I (Medium-I, inorganic)	Treatment-II (Medium-II , organic, whole pulse powder extract)	Treatment-III (Medium-III, organic, whole lentil powder extract)	Treatment-IV (Medium-IV , organic, whole gram powder extract)
0	26.04.16	2.05±0.00	2.05±0.00	2.05±0.00	2.05±0.00
1	27.04.16	5.42±0.20	3.92±0.22	4.75±0.48	5.26±0.31
2	28.04.16	8.69±1.13	5.27±0.30	5.89±0.68	9.93±0.52
3	29.04.16	9.60±0.88	7.15±0.40	6.57±0.48	10.83±0.57
4	30.04.16	10.80±1.23	9.03±0.51	7.14±1.14	12.00±0.51
5	01.05.16	11.56±0.46	10.15±0.57	7.61±1.49	12.50±0.65
6	02.05.16	14.15±2.55	12.63±0.71	9.10±2.05	13.51±0.70
7	03.05.16	16.85±2.84	13.53±0.76	9.53±2.31	14.16±0.74
8	04.05.16	16.95±0.67	14.88±0.84	11.83±1.85	16.02±0.84
9	05.05.16	17.35±0.69	16.85±0.95	13.92±1.83	16.95±0.89
10	06.05.16	18.23±0.72	17.55±0.99	15.15±1.61	17.25±0.90
11	07.05.16	20.05±0.80	18.25±1.03	15.62±1.72	17.56±0.92
12	08.05.16	22.80±2.28	21.51±1.21	16.60±2.92	19.50±1.02
13	09.05.16	24.60±1.75	30.69±1.73	17.31±7.54	21.12±1.10
14	10.05.16	25.60±1.01	27.84±1.57	18.04±5.77	18.03±1.38
15	11.05.16	28.89±1.14	26.27±1.48	26.18±1.27	16.15±2.10
16	12.05.16	24.48±0.97	19.98±1.13	20.90±1.08	14.95±1.38
17	13.05.16	22.11±0.88	18.97±1.07	20.28±1.15	13.50±1.04
18	14.05.16	20.60±3.57	17.94±1.01	18.90±1.40	12.65±1.63
19	15.05.16	18.50±0.00	14.10±0.79	16.95±1.83	12.12±1.67
20	15.05.16	15.83±0.63	13.87±0.78	13.10±0.80	11.23±0.47
Mean±SD		16.91±6.98	15.35±7.63	13.21±6.24	13.68±4.48

**There were three replications under each treatment.*

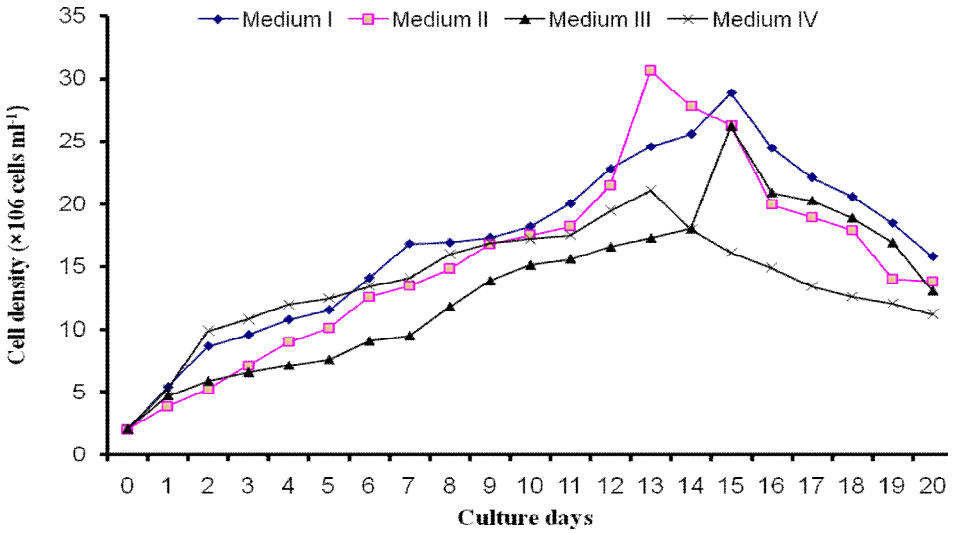

Fig. 1. Composition of daily variation of mean cell density (× 10⁶ cell ml⁻¹) of *C. ellipsoidea* cultured in medium I (inorganic), medium II (organic, whole pulse powder extract), medium III (organic, whole lentil powder extract) and medium IV (organic, whole gram powder extract).

Table 3. Comparison of culture of *Chlorella ellipsoidea* between medium-I (inorganic), medium-II (organic, whole pulse powder extract), medium-III (organic, whole lentil powder extract) and medium-IV (organic, whole gram powder extract).

Parameters	Medium-I (inorganic)	Medium-II (organic, whole pulse powder extract)	Medium-III (organic, whole lentil powder extract)	Medium-IV (organic, whole gram powder extract)
Duration of experiment	21 days	21 days	21 days	21 days
Range of water temperature (°C)	23 to 36	23 to 36	23 to 36	23 to 36
Average water temperature (°C)	27.98±1.35	27.98±1.35	27.98±1.35	27.98±1.35
Range of air temperature (°C)	21 to 38	21 to 38	21 to 38	21 to 38
Average air temperature (°C)	29.50±1.55	29.50±1.55	29.50±1.55	29.50±1.55
Range of light intensity (×10^3, lux)	2.28 to 9.60	2.28 to 9.60	2.28 to 9.60	2.28 to 9.60
Average of light intensity (×10^3, lux)	6.48±2.89	6.48±2.89	6.48±2.89	6.48±2.89
Average sunshine period (hrs.)	5.87±2.82	5.87±2.82	5.87±2.82	5.87±2.82
Average rainfall (mm)	10.91±16.44	10.91±16.44	10.91±16.44	10.91±16.44
Mean cell density (×10^6 , cells ml^{-1})	16.91±6.98	15.35±7.63	13.21±6.25	13.68±4.48
Maximum cell density (×10^6 , cells ml^{-1})	28.89±1.12	30.69±1.73	26.18±0.75	21.12±0.94
Day of maximum cell density	On 15th day	On 13th day	On 15th day	On 13th day

Table 4. Chemical status of the culture media.

Chemical medium (Treatment)	pH	Free CO_2 (mgL^{-1})	Total alkalinity (mgL^{-1})	NO_3-N (mgL^{-1})	PO_4-P (mgL^{-1})
Inorganic medium (modified GBII of Stainer *et al.*, 1971) (Treatment I)	7.4	32	128	180	25.2
Organic medium (whole pulse bran extract) (Treatment II)	8.00	162	540	36.6	48.2
Organic medium (whole lentil powder extract) (Treatment III)	7.9	102	554	62.4	42.4
Organic medium (whole gram powder extract) (Treatment IV)	7.9	70	322	150	45.6

The result of analysis of ANOVA of mean cell densities of *C. elliposoidea* of three replications of treatment-I (inorganic medium), treatment-II (organic medium, whole pulse powder extract), Treatment-III (organic medium, whole lentil powder extract) and treatment-IV (organic medium, whole gram powder extract) have been presented in Table.

Table 5. Analysis of variance (ANOVA) of cell densities of cultures of *C. ellipsoidea* under treatment I, II, III and IV.

Source of variation	Degree of freedom (df)	Sum of squares	Mean square	F-value	Sig.
Between treatments	3	179.3691	59.78971	1.441077	0.23703^{NS}
Within treatment	80	3319.169	41.48962		
Total	83	3498.538			

NS means not significant

Discussion

Cell density in four algal culture media

Cell density of *C. ellipsoidea* in medium I (inorganic medium) ranged from 2.05 to 28.89 (×10^6) cell ml^{-1}, during the culture period. The average cell density was 16.91±6.98 (×10^6) cells ml^{-1}. During the culture period of *C. ellipsoidea* in medium I, exponential phase was attained up to 15th day from the starting of culture and after that from stationary phase cell density began to decline toward death phase.

During the culture period, the range of cell density of *C. ellipsoidea* in medium II (organic, whole pulse powder extract) ranged from 2.05 to 30.69 (×10^6) cells ml^{-1}. The average cell density was 15.35±7.63 (×10^6) cell ml^{-1}. During the culture period of *C. ellipsoidea* in medium II, exponential phase was attained up to 13th day from the starting of culture and after that from

stationary phase cell density began to decline toward death phase.

During the culture period, the range of cell density of *C. ellipsoidea* in medium III (organic, whole lentil powder extract) ranged from 2.05 to 26.18 ($\times 10^6$) cell ml^{-1}. The average cell density was 13.21±6.24 ($\times 10^6$) cell ml^{-1}. During the culture period of *C. ellipsoidea* in medium III, exponential phase was attained up to 15th day from the starting of culture and after that from stationary phase cell density began to decline toward death phase.

Cell density of *C. ellipsoidea* in medium IV (organic, whole gram powder extract) ranged from 2.05 to 21.12 ($\times 10^6$) cells ml^{-1}, during the culture period. The average cell density was 13.68±4.48 ($\times 10^6$) cells ml^{-1}. During the culture period of *C. ellipsoidea* in medium IV, exponential phase was attained up to 13th day from the starting of culture and after that from stationary phase cell density began to decline toward death phase.

Jahan (2011), in an experiment of culture of *Chlorella* sp. in three culture media (pulse bran extract medium, soil extract medium and inorganic medium), observed maximum ell densities in pulse bran extract medium (56.32×10^6 cells ml^{-1}), in soil extract medium (102.99×10^6 cell ml^{-1}) and in inorganic medium (64.23×10^6 cells ml^{-1}). These results of cell densities are much higher in comparison to those of the present experiment.

Ara (2010), in an experiment of culture of *Chlorella* sp. in three culture media (treated eutrophic-pond-water medium, inorganic medium and organic medium), found maximum cell densities in eutrophic-pond-water medium (161.69×10^6 cells ml^{-1}),in inorganic medium (170.03×10^6 cells ml^{-1}), and in organic medium (165.03×10^6 cells ml^{-1}). These results of cell densities are much higher in comparison to those of the present experiment.

Sultana (2009), in an experiment of culture of *Chlorella sp.* in three culture media (organic, inorganic and eutrophic pond water media), recorded maximum cell densities in organic medium (35×10^6 cells ml^{-1}), in inorganic medium (45.05×10^6 cells ml^{-1}) and in eutrophic pond water medium (24.05×10^6 cells ml^{-1}). These results are more or less similar to those of the present experiment.

Mishu (2008), in an experiment of culture of green alga, *Scenedesmus* sp. in two culture media, medium I (organic) and medium II (inorganic), observed that the maximum cell densities of 17.03×10 cells ml^{-1} in 9 days in medium I, maximum densities of 17.41×10^6 cells ml^{-1} in medium II in 8 days, which are much lower than those of present experiment.

Wongsnansilp *et al.* (2007) did an experiment on the culture of an alga, *Chlorosarcinopsis* sp. (PSU/CHL20). He found the highest cell density of 14.8×10^6 cells ml^{-1} in 30°C on 14 days of culture, which are much lower than those of than the present study.

Hossain (1996), in an experiment of algal culture of *C. ellipsoidea* in five different media, viz, medium I (inorganic medium), medium II (medium of whole pulse powder), medium III (medium of pulsed bran), medium IV (mixed medium = 50% inorganic + 50% whole pulse powder medium) and medium V (mixed medium = 50% inorganic + 50% pulse bran medium), found that the ranges of cell densities were 0.08 to 0.62 ($\times 10^6$) cells ml^{-1} in medium I, 0.02 to 4.02 ($\times 10^6$) cells ml^{-1} in medium II, 0.18 to 4.38 ($\times 10^6$) cell ml^{-1} in medium IV, 0.07 to 4.38 ($\times 10^6$) cells ml^{-1} in medium V. These results of cell densities of C. *ellipsoidea* were much lower than those of the present study.

Statistical analysis

According to ANOVA (Table 5) of cell densities of cultures of *C. ellipsoidea* under treatments I, II, III and IV, it can be concluded that cell densities under 4 treatments are not significantly different ($F=1.4441077$), that is the four algal culture media are more or less of similar quality. But the mean amount of cell densities fr0m higher to lower are 30.69±1.73 ($\times 10^6$) cells ml^{-1} (medium II, organic, whole-pulse-powder extract), 28.89±1.12 ($\times 10^6$) cells ml^{-1} (medium I, inorganic), 26.18±0.75 ($\times 10^6$) cells ml^{-1} (medium IV, organic, whole-gram-powder extract). So, highest suitable medium was medium II (organic, whole-pulse-powder extract) although the differences among media are not significant and medium I and medium II are almost of same quality.

References

APHA. 1971. Standard method for the examination of water and waste water. American Public Health Assoc. 1015 Eighteenth Street, Washington D.C. 874p.

Ara, I. 2010. Use of treated eutrophic pond water as a medium for culture of *Chlorella ellipsoidea*. MS Thesis, Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 22-24.

Becker, E.W. 1994. Production and utilization of the blue-green alga *Spirulina* in India. *Biomass.* 4: 105-125.

Belasco, W. 1997. Algae burgess for a Hungry World. The Rise and Fall of *Chlorella cuisine. Tech. Culture.* 38: 340-680.

Barlew, J.W. 1953. Algal culture from laboratory to pilot plant. Carnegie Institution, Washington D.C. 151p.

Claus, C. 1981. Trends in nursery rearing of bivalve mollusks. *In:* N de Pauw and E Jaspers (Eds), Nursery culturing of Bivalve Mollusks. European Mariculture Society Bredene, Belgium. pp. 1-33.

Fujimura, T. and Okamoto, H. 1972. Notes on progress made in developing a mass culturing technique for *Macrobrachium resenbergii* in Hawaii. *In:* TVR Pillay (Eds) Coastal Aquaculture in the Indo-pacific Region. Fishing News Book Limited Survey England. pp. 27-313.

Haldane, J.B.S. 1951. Biological problems of space flight. *J. British Interplant Soc.* 10: 1-54.

Hossain, M.A. 1996. Culture of microalgae (*Scenedesmus quadricauda* and *Chlorella ellipsoidea* in inexpensive medium. MS Thesis, Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 51-53.

Howell, B.R. 1979. Experiments on the rearing of larval turbot (*Scophthalrnus maxirnus*). *Aqua.* 18: 215-225.

Jahan, F. 2011. Uses of soil extract as a medium for culture of *Chlorella* sp. MS Thesis, Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 30-35.

Kumar, H.D. and Singh, H.N. 1976. A text book on algae. Affiliated East-West Press, New Delhi, India. 289p.

Mishu, L.A. 2008. Culture of green algae, *Scenedesmus* sp. in inexpensive medium. MS Thesis, Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 21-22.

Rahman, M.S. 1992. Water Quality Management in Aquaculture. BRAC Prokashana, Mohakhali, Dhaka. pp. 71-72.

Rahman, M.S. 2000. Mass culture of Phytoplankton in inexpensive Medium-BRAC, ARMP, IDA Credit 2815-BD. Bangladesh Agricultural Research Council, Farm Gate, Dhaka. 85p.

Renaud, S.M., Parry, D.L., Thinh, L.V., Kou, C., Padovan, A. and Sammy, N. 1991. Effect of light intensity on the proximate biochemical and fatty acid composition of *Isochrysis* sp. and *Nannochloropsis oculata* for use in tropical aquaculture. *J. Appl. Phycol.* 3: 43-53.

Spoehr, H.A. and Milner, H.W. 1949. The chemical composition of *Chlorella*: Effect of environmental condition. *Plant Physiol.* 24 (1): 120-149.

Stainer, R.Y., Kunisawa, R., Mandel, M. and Cohen-Bazire, G. 1971. Purification and properties of unicellular blue-green algae (Order: Chrococcales). *Bacteria Rev.* 35: 171-205.

Sultana, M. 2009. Algal culture in different inexpensive culture media, MS Thesis, Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 30-31.

Wongsnanslip, T., Tansakul, P. and Arunyanart, M. 2007. Factors affecting growth and beta-carotene content of *Chlorosarcinopsis* sp. (PSU/CHL20) in batch culture. *Kasetsart J. Nat. Sci.* 41: 153-157.