



## Mass Cultivation of Microalgae in Open Raceway Pond for Biomass and Biochemicals Production

D. Karthikeyan, M. Muthukumaran<sup>1\*</sup> and B.S. Balakumar<sup>\*\*</sup>

PG & Research Department of Botany,  
Ramakrishna Mission Vivekananda College (Autonomous), Chennai - 600 004, India.

<sup>1\*</sup>E.Mail: [muthukumaran.tni@gmail.com](mailto:muthukumaran.tni@gmail.com)

<sup>\*\*</sup>E.Mail: [bsbviveka@gmail.com](mailto:bsbviveka@gmail.com)

### Abstract

Microalgal biotechnology has wide commercial applications. Microalgae are also used as nutrient supplements for human consumption due to their high proteins, vitamins and polysaccharides content. Some microalgae species contain high levels of lipids which can be extracted and converted into biofuels. They also find use in pharmaceutical industries, as some species of microalgae produce bioactive compounds such as antioxidants, antibiotics and toxins. In the present study an attempt was made to cultivate *Chroococcus turgidus*, at a pilot scale using open race way pond with varied parameters such as pH, conductivity, cell count, content etc. In addition, the extraction and estimation of total proteins, total carbohydrates, total lipid, carotenoids, chlorophyll-a, - c and amino acids were analysed and estimated at 5 day intervals. The results were compared with the analysis of dried biomass.

**Keywords:** *Chroococcus turgidus*, Biomass and biochemical production.

### 1. Introduction

Microalgae are small photosynthetic micro-organisms adapted to almost all possible environments. Microalgae are currently cultivated commercially around the world in several small- to medium-scale systems for the production of human nutritional products. Estimates indicate that global commercial microalgal biomass production is about 10,000 tons per year. The main microalgal species currently cultivated for nutritional products are *Spirulina* sp., *Chlorella* sp., *Dunaliella* sp., and *Haematococcus* sp. Almost half of the microalgae production takes place in mainland China, and most of the rest happens in Japan, Taiwan, and USA. Whereas, India and Australia, are smaller producers of micro algal products. Microalgae are the rich source of proteins, carbohydrates, fatty acids and other nutrients, (Shaila Hiremath and Pratima Mathad, 2010). Microalgae

play an important role as primary producer to various consumers such as Rotifer, Copepods, Shrimps etc., which are in turn, fed to late larval and juvenile fish and crustaceans (Richmond, 1996). *Chlorella vulgaris* is one such green microalga found cosmopolitan in occurrence. The genus *Chlorella* spp., includes a varied range of species with high temperature tolerance and can grow autotrophically in an inorganic medium (Mohan *et al.*, 2009).

Microalgae have vast industrial and economic potential. They serve as a valuable source for pharmaceuticals. The history of the commercial use of algal cultures with various applications spans over 50 years (Borowitzka, 1999). Furthermore, they solve public health problems by means of biological purification of waste water in a fast developing

society. Latest developments have established the potentiality of algae for production of a variety of compounds such as polysaccharides, lipids, proteins, carotenoids, pigments, vitamins, sterols, enzymes, antibiotics, hydrocarbons and biofuels (Huesemann and Benemann, 2009).

Microalgae have been studied in the laboratory and in mass outdoor cultures for more than a century. Initial understanding of photosynthesis was unraveled in the laboratory of Otto Warburg using *Chlorella* as model organism (Nickelsen, 2007). As Grobbelaar (2010) pointed out, applied phycology and the mass production of microalgae, became a reality in the 1940's. Since then, microalgae have been grown for a variety of applications; such as the production of lipids for energy using flue-gasses, anti-microbial substances, proteins for human nutrition and for obtaining various bio-chemicals. At present the focus is on bioenergy (Tan and Amthor, 1988). However, their real success has been established in the treatment of wastewater.

### 1.1 Microalgal Biomass Production

Microalgae cultivation can be done in open culture systems such as lakes, ponds and in highly controlled, closed- culture systems called photobioreactors (PBRs) (Mata, 2010). The growth of microalgal biomass requires light, CO<sub>2</sub>, water, organic salts and temperature of 20-30°C. The production of microalgal biomass can be achieved by varying the cultivating methods.

### 1.2. Raceway ponds:

The ponds in which the alga is cultivated outdoors are called the raceway ponds. In these ponds, the algae, water and nutrients circulate around a race track. A raceway pond is made of a closed loop recirculation channel that is typically about 0.3m deep with a paddlewheel. The paddlewheel is used to mix or circulate the algal biomass and to prevent sedimentation (Chisti, 2007). Raceway channels are built in concrete or compacted earth. It can be of different lengths and diameters and generally lined with white plastic. During daylight, the culture is fed continuously in front of the paddle wheel where the flow begins (Ananadhi Padmanabhan 2012). Raceways are perceived to be less expensive than PBRs, because they cost effective to built and simple to operate. Economically, it is 10 times less costly compared to PBRs. Chrome sludge from the electroplating industry when treated with micro alga

*Desmococcus olivaceus* in open raceway pond, there was a considerable amount of sludge reduction and biomass production in open raceways amended with chrome sludge (Muthukumaran *et al.*, 2012). Raceway ponds for mass culture of microalgae have been used since the 1950s. The largest raceway based biomass production facility occupies an area of 440,000m<sup>4</sup>. This facility was owned by Earthwise Nutritionals, which produce cyanobacterial biomass for human food.

### 1.3 Chemical composition of microalgae

Microalgae are able to enhance the nutritional value of conventional food preparations and hence positively affect the health of humans and animals. The high protein content of various microalgal species is one of the main reasons to consider them as an unconventional source of protein (Cornet, 1998; Soletto, 2005). As algae cells are capable of synthesizing all amino acids, they can provide the essential amino acids ones to humans and animals. (Guil-Guerrero *et al.*, 2004). Carbohydrates in microalgae are found in the form of starch, glucose, sugars and other polysaccharides. Their overall digestibility is high, which is why there is no limitation to use dried microalgae in foods or feeds (Becker, 2004). The average lipid content of algal cells varies between 1% and 70% but can reach 90% of dry weight under certain conditions. Metting (1996) found that the algal lipids are composed of glycerol, sugars or bases esterified to saturated or unsaturated fatty acids. Among all the fatty acids in microalgae, some fatty acids of the 3 and 6 families are of particular interest (Borowitzka, 1999). Microalgae also represent a valuable source of nearly all essential vitamins (e.g., A, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, C, E, nicotinate, biotin, folic acid and pantothenic acid) (Becker, 2004). Microalgae are also rich in pigments like chlorophyll (0.5% to 1% of dry weight), carotenoids (0.1% to 0.2% of dry weight on average and up to 14% of dry weight for  $\beta$ -carotene in *Dunaliella sp*) and phycobiliproteins. These molecules have a wide range of commercial applications. Thus, their composition gives microalgae wide use in human and animal nutrition. However, prior to commercialization, algal products must be analyzed for the presence of toxic compounds to prove their safety.

### 1.4 Pigments

One of the most obvious and arresting characteristics of the algae is their color. In general, each phylum or

group has its own particular combination of pigments and individual colour. Aside chlorophylls, the primary photosynthetic pigment, microalgae also have various accessory or secondary pigments, such as phycobilin proteins and a wide range of carotenoids. These natural pigments function as antioxidants in plants as well as Humans. Therefore, microalgae are recognized as an excellent source of natural colorants and nutraceuticals (Daniel Chaumont, 1993).

### 1.5. Scope of the present investigation

In the present investigation an attempt was made to cultivate *Chroococcus turgidus*, at a pilot scale using open race way pond with varied parameters such as pH, conductivity, cell count, content of chlorophyll a & c and -carotene etc. The extraction and estimation of total protein, total carbohydrate, total lipid, carotenoids, chlorophyll-a, - c and amino acids were analysed and estimated at 5 day intervals. The results were compared with the analysis of dried biomass.

## 3. Materials and Methods

### 2.1. Micro algae used for mass cultivation

The blue-green alga *Chroococcus turgidus*, obtained from the culture collection of the Department of Botany, RKM Vivekananda College (Autonomous), Chennai – 600 004, was used for mass cultivation in an open raceway pond.

### 2.2. Preparation of algal inoculum

The inoculums of the algal cultures to be used for the outdoor mass cultivation have been prepared under laboratory conditions. *Chroococcus turgidus*, was grown in CFTRI medium (Venkataraman and Becker, 1985). The cultures were grown at  $24\pm 1^{\circ}\text{C}$  in a thermo-statically controlled room illuminated with cool white florescence lamps (Philips 40W, Cool daylight 6500K) at an intensity of 2500 lux in a 12 hrs light and dark regime.

### 2.3. Out-door cultivation of microalgae

#### 2.3.1. Pond construction

An outdoor algal raceway pond was constructed with the wall thickness of 25cm. The inner dimensions of the pond were such that the length was 4.26 m and the width was 1.8 m. The depth of the pond was kept at 0.44 m keeping in mind the penetration of sufficient light for the growth of algae. A partition wall was constructed in the middle of the pond with a length of 2.46 m and width of 11cm. The floor was constructed with a slight slope on either side of the partition in the opposite direction to enable proper mixing of the culture. The inside of the pond was covered with ceramic tile lining to avoid seepage of the medium into the wall. The tank was provided with a tap water connection for preparation of the medium and two outlets were provided to enable cleaning of the tank (Plate.1).



Plate1: Open raceway pond.

#### 2.3.2. Algal cultivation

*Chroococcus turgidus* was grown in improvised CFTRI medium (Venkataraman and Becker, 1985) with the incubation temperatures ranging from 30 to  $40^{\circ}$  with natural day night cycle (45 to 60 Klux). 1 KL

of the medium was prepared using tap water and the depth of the standing medium was maintained 15 cm. The algal inoculum was added into the medium and grown with daily stirring and harvesting of samples at every 5 day intervals (Plate.2 & 3).



A) Cultures inoculated



B) Cultures ready for harvest

Plate 2: Mass cultivation of *Chroococcus turgidus* in an open raceway pond

### 2.3.3. Microscopic examination

The microalgal cultures were microscopically examined using Olympus (HB) microscope under (10 × 40) magnifications.

### 2.3.4. Growth measurement

Growth of algal biomass was measured by counting cells using a Haemocytometer (Neubauer, improved) and the results were plotted in a semi-logarithmic graph. Growth rate (divisions/day) was arrived at using the formula,

$$\text{Log} = \frac{\text{Log}N - \text{Log}N_0}{\text{Log} 2 \times t}$$

Where,

N - No of cells per ml at the end of log phase or mg weight/l

N<sub>0</sub>. Initial count of cells ml or mg weight/l

t - Days of log phase

For dry weight method, the algal cultures were pelleted by centrifugation at 7500 rpm (Remi cooling microfuge) for 15 minutes. Cells were washed with glass- distilled water, again centrifuged and dried in an oven for 24 hours or until constant weight is reached.

The growth properties of algae can be determined by measuring the cell number per unit volume of cell suspension. Thus, the number of cells in the algal suspension is counted to calculate the concentration of cells in culture by using a haemocytometer.

### 2.3.5. pH Measurement

For all the trials pH was measured using digital pH meter (Elico L ± 120). pH is another important growth factor for algae. Different algal species have different favorite pH ranges and the growth is affected outside the optimal range resulting in slower growth rates. Algae can also change the pH of the medium during cultivation. When using CO<sub>2</sub> as carbon source, rapid growth of algae can cause the pH to rise due to photosynthetic uptake of inorganic carbon.

### 2.3.6. Conductivity study

Conductivity is measured using digital conductivity meter (Equiptronics EQ-660A).

### 2.4. Pigments estimation

#### 2.4.1. Extraction and estimation of chlorophyll a & c Extraction

20 ml of algal culture was pelleted by centrifugation at 2000 rpm for 15 minutes. To the pellet, 6 ml of 90% acetone was added and sonicated for 15 minutes using ultrasonicator. After complete extraction, it was again centrifuged at 2000 rpm for 15 minutes and the clear supernatant was used for spectrophotometric estimation of chlorophyll pigments using a double beam UV- visible spectrophotometer.

#### Estimation

Estimation was done using Jeffrey and Humphrey's (1975) Trichromatic Equations - The absorbance value at 750 nm was subtracted from the absorbance values at 664, 647 and 630 nm and the concentrations (mg/L) of chl *a*, *b*, and *c* + 1 *c* in the extract solution was calculated by inserting the 750 nm-corrected 2, absorbance values into the following equations:

$$C = 11.85 (\text{Abs } 664) - 1.54 (\text{Abs } 647) - .08 (\text{Abs } 630) E,a$$

$$C = 21.03 (\text{Abs } 647) - 5.43 (\text{Abs } 664) - 2.66 (\text{Abs } 630) E,b$$

$$C = 24.52 (\text{Abs } 630) - 7.60 (\text{Abs } 647) - 1.67 (\text{Abs } 664) E,c$$

where:

C = concentration (mg/L) of chlorophyll *a* in the E,a extraction solution analyzed,

C = concentration (mg/L) of chlorophyll *b* in the extract E,b solution.

C = concentration (mg/L) of chlorophyll *c* + *c* in the E,c 1 2 extract solution analyzed.

#### 2.4.2. Extraction and estimation of -Carotene

Extraction and estimation of -carotene was done according to the method of Shaish *et al.*, (1992).

#### Extraction

Known quantity of algal culture was centrifuged at 2000 rpm for 5 minutes and 3ml of (2:1) ethanol: hexane mixture was added to the pellet ,Then 2ml of Double distilled water and 4ml of hexane was added, mixed well and centrifuged for 5 minutes. The hexane

layer was aspirated and the absorbance was measured at 450nm in a spectrophotometer.

$$\text{-Carotene } (\mu\text{g/ml}): A_{450\text{nm}} \times 25.2 \text{ (Green algae)}$$

### 2.5. Biochemicals

#### 2.5.1. Extraction and estimation of total carbohydrates

Extraction and estimation of total carbohydrates was done according to the method of Pons *et al.*, (1981)

#### Extraction

40 ml of algal culture was taken and centrifuged at 2000 rpm and the supernatants were discarded. The samples (pellets) were hydrolyzed with 5ml of 2.5 N-Hydrochloric acid by keeping it in a boiling water bath for three hours and then cooled to room temperature. After cooling, the samples were neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 10ml and centrifuged. The collected supernatant was taken and 0.5 and 1ml aliquots were used for analysis.

#### 2.5.2. Extraction and estimation of total protein

Extraction and estimation of total protein was done according to the method of Lowry *et al.*, (1951).

#### Extraction

40 ml of algal cells was centrifuged and pellets were washed twice with tris HCl buffer (pH-7.0) and re-suspended in 5ml of the same buffer. The suspension was sonicated in an Ultra-sonicator for 15 minutes, centrifuged at 2000 rpm for 15 minutes. The supernatant was treated with 10% Trichloro Acetic Acid (TCA) and the precipitate is obtained by centrifugation at 5000rpm in a cooling microfuge for 15 minutes. Then the precipitate is neutralized in known quantity of 2N NaOH and analyzed for protein.

#### 2.5.3. Estimation of lipid by Bligh and Dyer method

Estimation of lipid was done according to the method of Bligh and Dyer, (1959).

40 ml of algal cultures were pelleted by centrifugation at 2000 rpm for 15 min. The pellets were suspended in 10 ml of double distilled water and sonicated for 30 min at 200 V using an ultrasonicator. Then, 2:1 Chloroform-Methanol was added and kept overnight

at room temperature preferably in the dark condition. After the 12 hr standing time, 20 ml of Chloroform and 20 ml of double distilled water were added and mixed well, centrifuged at 2000 rpm for 15 min. A clear lower layer of chloroform containing all the lipid was obtained, which was aspirated carefully using a Pasteur pipette. After drying the solvent, the dry weight was measured using a digital balance and the % dry weight of total lipids was calculated.

#### 2.5.4. Extraction and estimation of total amino acid

Extraction and estimation of total amino acid was done according to method of Blackburn, (1978) and Walker, (1996).

A 0.3 g test sample was prepared for hydrolysis (0.3 g/ 10 ml of water) according to Blackburn (1978) and Walker (1996) prior to the determination of amino acids. Amino acid analyses were carried out using an

Eppendorf/Biotronik amino acid analyzer using an H 125 × type column.

### 3. Results

#### 3.1. Outdoor cultivation of *Chroococcus turgidus*

1000L of improvised CFTRI medium was prepared in an outdoor algal pond and 1L of actively grown culture *Chroococcus turgidus* was inoculated, it was cultured for 20 days and incubated. Microalgae cultivated in mass culture is shown in plate 2 & 3.

#### 3.2. Growth measurement of *Chroococcus turgidus*

The culture was sampled daily, the cell numbers were measured using a haemocytometer (Nebauer, improved). The growth pattern showed gradual increase in the cell number up to 2<sup>nd</sup> day and thereafter the increase was steep from 3<sup>rd</sup> day to 7<sup>th</sup> day. Then 8<sup>th</sup> day to 14<sup>th</sup> day and 15<sup>th</sup> day to 20<sup>th</sup> day cell numbers decrease. The results are given in table 1.

**Table 1: Total cell count of *Chroococcus turgidus* cultured in open raceway pond**

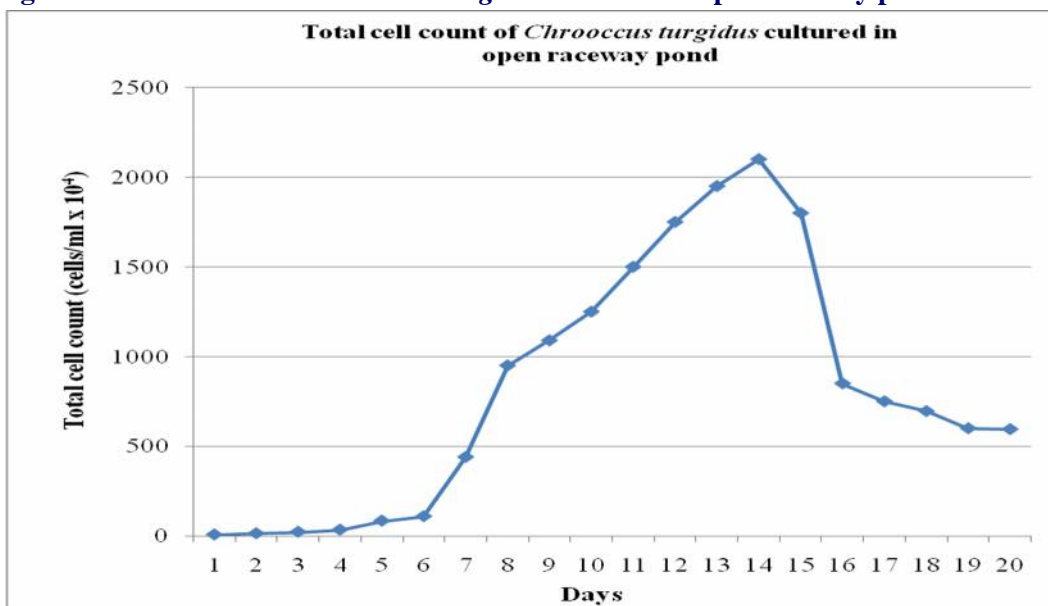
Days	Cell count (Cells/ml × 10 <sup>4</sup> )
01	08
02	15
03	23
04	35
05	85
06	110
07	440
08	950
09	1090
10	1250
11	1500
12	1750
13	1950
14	2100
15	1800
16	850
17	750
18	696
19	600
20	595

#### 3.3. Measurement of pH and conductivity

The pond requires constant stirring, mixing and recirculation of the culture. Stirring system provides homogenous light to extreme microalgae. Both pH and conductivity were measured daily and the results

indicate that the pH level rose from day to day. The initial pH is 9.30. Regarding the electrical conductivity, it was almost uniform ranging between (2.4 and 2.8 mmhos/ cm). The results are given in table 2.

**Fig 1: Total cell count of *Chroococcus turgidus* cultured in open raceway pond**



**Table 2: Changes in pH and conductivity in open raceway pond cultures of *Chroococcus turgidus***

Days	pH	Electrical conductivity (mmhos/cm)
01	8.39	2.83
02	8.40	2.81
03	8.49	2.80
04	8.56	2.80
05	8.72	2.79
06	8.82	2.79
07	8.91	2.76
08	9.02	2.79
09	9.12	2.64
10	9.19	2.71
11	9.25	2.54
12	9.28	2.38
13	9.34	2.38
14	9.44	2.47
15	9.34	2.46
16	9.35	2.41
17	9.32	2.51
18	9.30	2.56
19	9.29	2.45
20	9.30	2.46

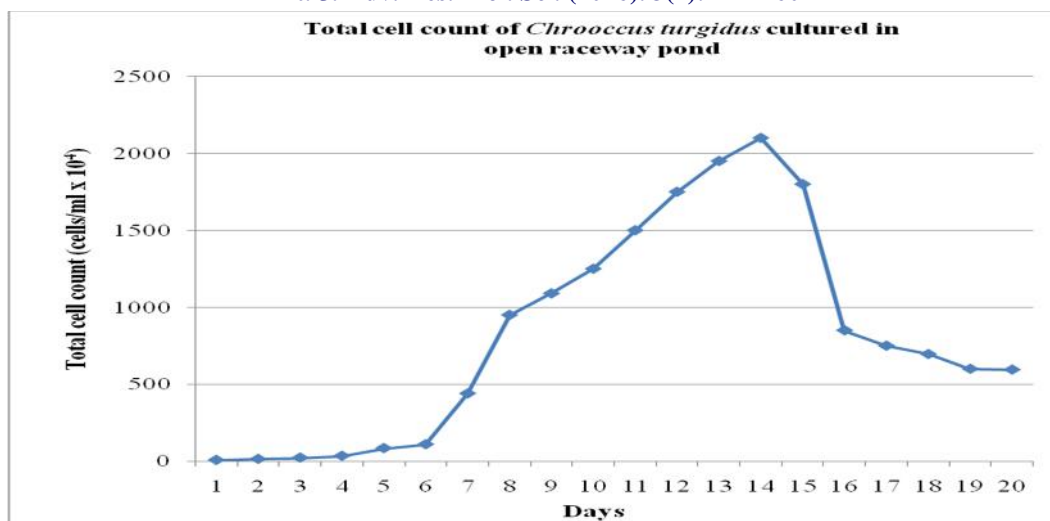


Fig 2: Changes in pH and conductivity in open raceway pond cultures of *Chroococcus turgidus*

### 3.4. Estimation of pigment level

#### Chlorophyll

Chlorophyll was estimated spectrophotometrically for *Chroococcus turgidus*. Culture was sampled from an outdoor algal pond daily, to measure the chlorophyll content. Among the measures the pigment content was found to be highest on the 20<sup>th</sup> day; Chlorophyll a 4.35mg/ml and Chlorophyll b 1.17mg/ml. The results are given table 3.

#### -Carotene

-carotene was estimated spectrophotometrically for *Chroococcus turgidus*. Culture was sampled daily from outdoor algal pond, for measurement of -carotene content. Among the readings the pigment content was high on the 20<sup>th</sup> day registering 0.171mg/ml -carotene. The results are given in table 3.

Table 3: Chlorophyll -a & -c and - carotenoid levels in *Chroococcus turgidus* cultured in open raceway pond

Days	Chlorophyll a (mg/ml)	Chlorophyll c (mg/ml)	-Carotene (mg/ml)
1	0.06	0.04	0.022
2	0.05	0.05	0.027
3	0.18	0.27	0.032
4	0.04	0.06	0.037
5	0.14	0.06	0.004
6	0.19	0.06	0.045
7	0.05	0.03	0.045
8	0.44	0.02	0.047
9	1.5	0.19	0.047
10	2.3	0.28	0.052
11	2.4	0.44	0.088
12	2.63	1.96	0.118
13	2.65	0.22	0.098
14	4.32	0.45	0.095
15	4.18	0.33	0.163
16	1.38	0.16	0.123
17	2.33	0.21	0.138
18	2.06	0.17	0.012
19	4.34	0.43	0.158
20	4.35	0.52	0.171



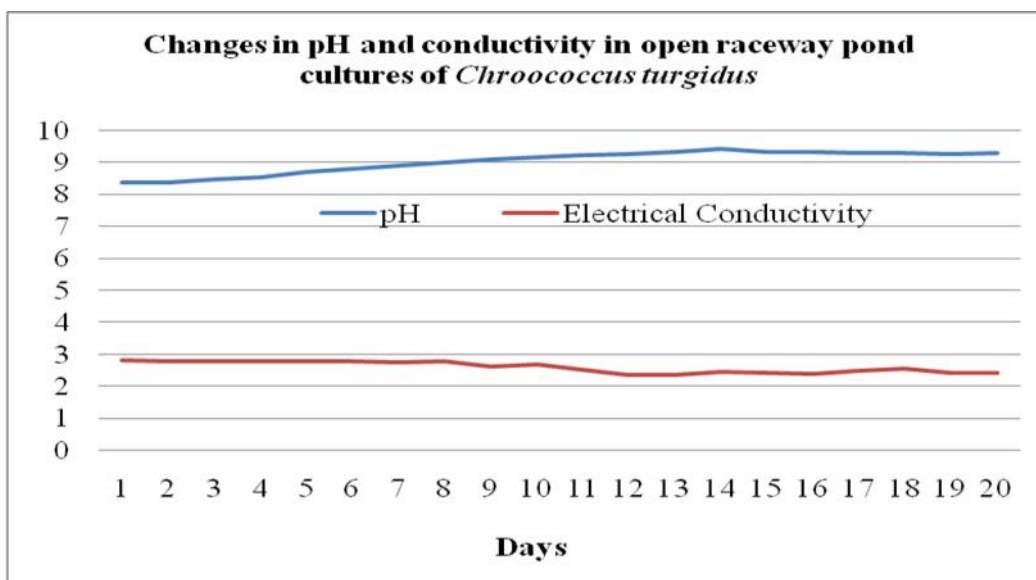


Fig 3: Chlorophyll -a & -c and -Carotenoid levels in *Chroococcus*

**3.5. Analysis of total protein, carbohydrate and lipid content of *Chroococcus turgidus***

**Protein**

Algal biomass was collected from outdoor algal pond on 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup> and 20<sup>th</sup> day, for analysis of total protein content and it was estimated by Lowry’s (1951) method. The results showed that the total protein content was 117mg/gram on the 5<sup>th</sup> day, 137mg/gram on the 10<sup>th</sup> day, 182mg/gram on the 15<sup>th</sup> day and 83mg/gram on the 20<sup>th</sup> day. From the above results on the 15<sup>th</sup> day total protein level recorded high level and thereafter, there was a slight reduction in the protein content. The results are given in table 4.

**Carbohydrate**

Algal biomass was collected from the algal pond on 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup> and 20<sup>th</sup> day, for the analysis of total carbohydrate content and it was estimated by anthrone

method, in 5 day interval. The results showed that the total carbohydrate content 204mg/gram on the 5<sup>th</sup> day, 313mg/gram on the 10<sup>th</sup> day, 519mg/gram on the 15<sup>th</sup> day and 449mg/gram on the 20<sup>th</sup> day. The highest carbohydrate content was recorded on the 15<sup>th</sup> day. The total carbohydrate level, like the protein content, exhibited a slight reduction after 15<sup>th</sup> day of culture. The results are given in table 4.

**Lipid**

Algal biomass was collected from the algal pond on 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup> and 20<sup>th</sup> day for the analysis of lipid. Total lipid content was estimated by Bligh and Dyar, (1959) method; the results showed that the total lipid content was found to be: 5<sup>th</sup> day 19.4mg/gram, on the 10<sup>th</sup> day 28mg/gram, on the 15<sup>th</sup> day 17.4mg/gram and on the 20<sup>th</sup> day 20.7 mg/gram. The highest lipid content was on the 10<sup>th</sup> day. The result are given table 4.

**Table 4: Biochemicals of *Chroococcus turgidus* cultured in open raceway pond**

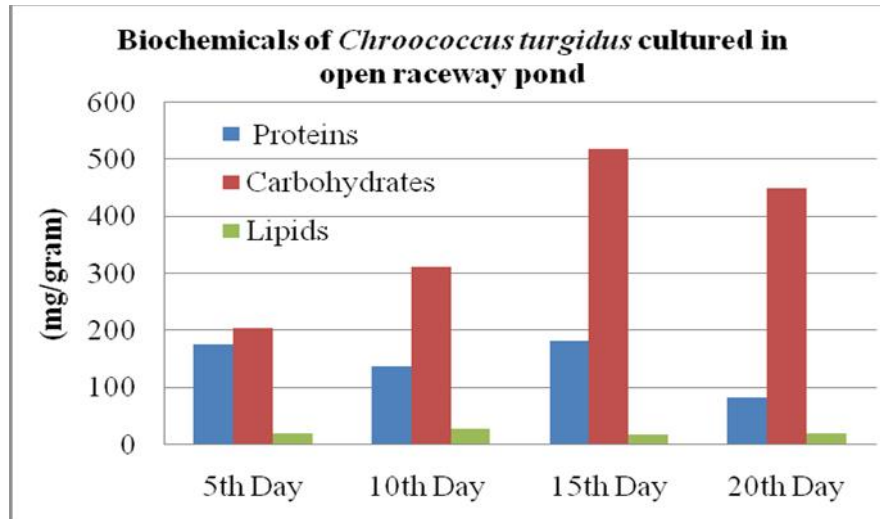
Biochemicals	Days (mg/gram)			
	5 <sup>th</sup>	10 <sup>th</sup>	15 <sup>th</sup>	20 <sup>th</sup>
Protein	177	137	182	83
Carbohydrates	204	313	519	449
Lipid	19.40	28	17.42	20.71

**Amino acid**

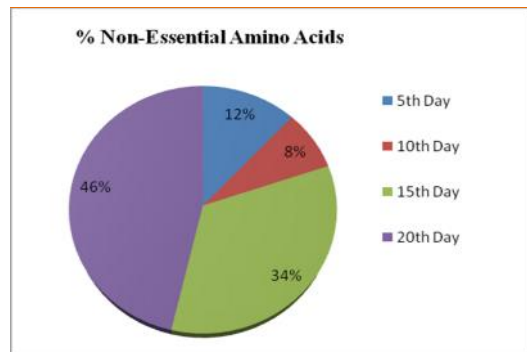
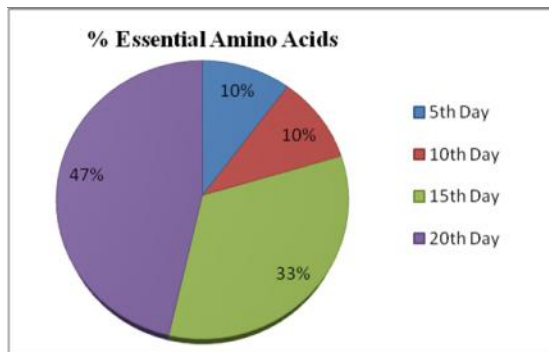
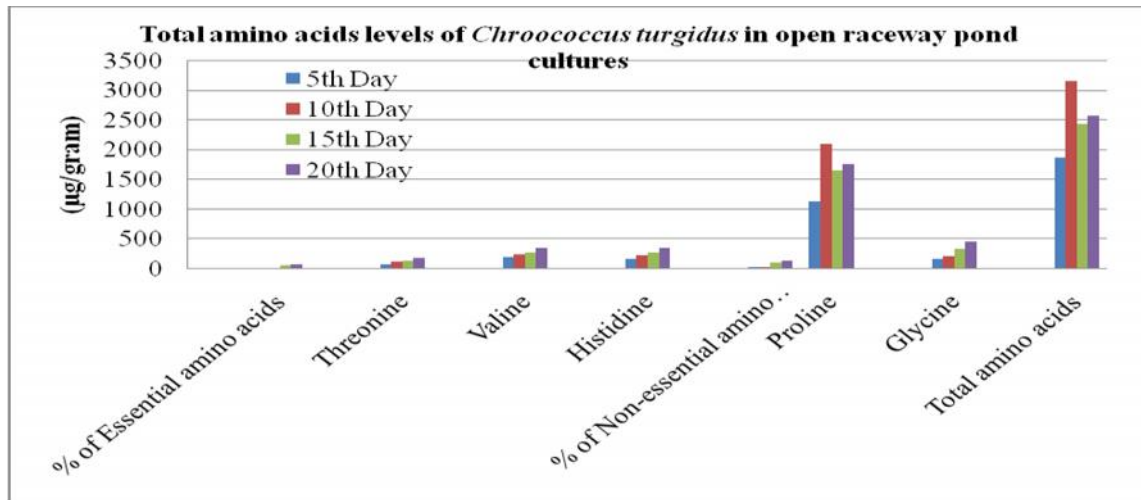
Algal biomass was collected from the algal pond on 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup> and 20<sup>th</sup> day for the analysis of essential amino acids (Threonine, Valine and Histidine) and non-essential amino acids (Proline and Glycine). Total amino acids content was estimated by Blackburn,(1978) and Walker,(1996) methods. The results showed that the total amino acids content was

1870.41mg/gram on the 5<sup>th</sup> day, 3166.68mg/gram on the 10<sup>th</sup>day, 2443.96mg/gram on the 15<sup>th</sup>day and 2579.46mg/gram on the 20<sup>th</sup> day. The 15<sup>th</sup> day samples had highest content of total amino acid. The total estimation of essential amino acids was maximum in the 20<sup>th</sup> day (78.02 %) samples. The non-essential amino acids was maximum in the 20<sup>th</sup> day (138.196 %) samples. The results are given in table 5.

**Fig 4: Biochemicals of *Chroococcus turgidus* cultured in open raceway pond**



**Fig 5: Total amino acids levels of *Chroococcus turgidus* in open raceway pond cultures**



**Table 5: Total amino acids levels of *Chroococcus turgidus* cultured in open raceway pond**

Parameters	Days			
	5 <sup>th</sup>	10 <sup>th</sup>	15 <sup>th</sup>	20 <sup>th</sup>
% of Essential amino acids	17.15	17.22	55.72	78.02
Threonine	79.97	127.75	138.71	177.18
Valine	206.79	243.69	279.11	349.93
Histidine	169.51	228.30	279.11	349.93
% of Non-essential amino acids	35.52	23.41	101.20	138.196
Proline	1135.14	2103.94	1656.43	1759.59
Glycine	170.06	212.06	338.11	457.44
Total amino acid in µg/g of tissue	1870.41	3166.68	2443.96	2579.46

#### 4. Discussion

Microalgae are of increasing economic importance as sources of biomass for health food supplement, aquaculture, useful biochemicals, new drugs and bioactive molecule and in processes such as wastewater treatment. The microalgae have a potential to produce a wide range of products due to its high quantity proteins, lipids, carbohydrates, vitamins, pigments and enzymes content. The ultimate production system of microalgae includes cultivation and dewatering process. This could not be established so far due to unsuccessful commercial-scale operation. Although various commodities are produced from microalgae, extensive studies need to be carried out on upstream and downstream processes in order to compete with the existing demand for algae based products. Besides, microalgae also have great potential to be used in environmental applications such as removal of excess organic/inorganic nutrients and heavy metals. Further, algae is also currently gaining attention for being capable of significantly reducing concentration of greenhouse gases, thus providing a solution to global warming. Overall, the production of microalgae promotes global prospects and may provide a sustainable economic and environmental development in future.

The costs and energy consumption for biomass harvesting are of significant concerns that need to be addressed properly. Different technologies, including chemical flocculation (Knuckey *et al.*, 2006), biological flocculation (Divakaran and Pillai, 2002), filtration (Molina Grima *et al.*, 2003), centrifugation

(Olaizola, 2003), and ultrasonication (Bosma *et al.*, 2003) have been investigated for microalgal biomass harvesting. In general, chemical and biological flocculation require low operating costs. However, they have the disadvantage of requiring long processing period and base the risk of bioreactive product decomposition. On the other hand, filtration, centrifugation and ultrasonic flocculation are more efficient but costly, i.e. they demand more power supply. However, the methods used in this study, autoflocculation and autoflocculation, are not only efficient but also cost effective. Thus, the selection of cost-effective technologies for biomass harvesting and drying depends on the value of target products, the biomass concentration and the size of microalgal cells of interest.

In the present investigation technical feasibility of cultivating the microalgae *Chroococcus turgidus*, in an outdoor open raceway pond was evaluated. The growth rate, pH, conductivity, Biomass production, Chlorophyll -a & -c, -carotene were analyzed daily and a 5 day interval analysis was done. The growth of *Chroococcus turgidus* was found to be maximum on the 14<sup>th</sup> day.

In case of pH and conductivity, it showed a steady increase in pH reaching around 9.30 at the end of the study. Regarding the electrical conductivity there was a significant reduction from 2.83mmhos/cm to 2.46mm. The growth rate of *Chroococcus turgidus*, cultivated an outdoor algal pond culture growth rate was also measured, and the highest cell number was shown on the 14<sup>th</sup> day (2100 X 10<sup>4</sup> cells/ml).

Extraction and estimation of various pigments were done using the mass cultivation of the microalga *Chroococcus turgidus* in open race way pond cultures. The pigments were analyzed spectrophotometrically and the results showed that the higher pigment content was observed in case of chlorophyll-a (4.35mg/ml) 20<sup>th</sup> day, chlorophyll-c (1.96mg/ml) 12<sup>th</sup> day and -carotene (0.171mg/ml) 20<sup>th</sup> day. All above the pigments increased day by day depending on the increase in cell count. The protein and carbohydrate content was high on the 15<sup>th</sup> day (protein 182mg/gram) and (carbohydrate 519mg/gram). The lipid content was high on the 10<sup>th</sup> day (28 mg/gram). The lipid content indicates the amino acid profile, that on the 10<sup>th</sup> day highest level of essential amino acids and non-essential amino acids content were present. The total estimation of essential amino acids was maximum in the 20<sup>th</sup> day (78.02 %) and the non-essential amino acids was maximum on the 20<sup>th</sup> day (138.196 %).

In conclusion, efforts have been made to cultivate the microalgae in an open HRA (High Rate Algal) pond and the biomass was harvested, following cost-effective methods. Effort has also been made in this work to study comprehensively the pros and cons of the outdoor cultivation of microalgae. The technical data presented in this chapter are of relevance and offer suggestions and value, for further development and generate additional interest in the field of algal technology.

## 5. Conclusion

The present study deals with growth and utilization of *Chroococcus turgidus* to obtain various useful biochemicals. The green algae *Chroococcus turgidus*, obtained from the culture collection the Department of Botany, RKM Vivekananda College (Autonomous), Chennai – 600 004, was used in the mass cultivation in an open race way pond. The results suggest that the outdoor culture exhibit better growth rate compared to indoors. The extraction and estimation of various pigments and biochemicals from the *Chroococcus turgidus* were also carried out. In addition carbohydrate, protein, lipid and amino acid levels were studied during the, 20 days of the culture period.

and chlorophyll levels increased over the 20 day of culture. The protein and carbohydrate reached high level on the 15<sup>th</sup> day. Lipid was at a high level on the 10<sup>th</sup> day. Essential amino acids and Non-essential amino acids showed a maximum percentage on the 20<sup>th</sup> day. But total amino acid registered a maximum

on the 10<sup>th</sup> day. The present study established that the microalgae, *Chroococcus turgidus* can be cultivated in an open raceway pond for 10 to 15 days exhibiting good growth, chlorophyll content, -carotene, protein, carbohydrate, lipid and amino acid levels. Therefore, a 15-20 day period is suitable for harvesting the algal biomass to reach good, efficient economical realization.

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## References

- Ananadhi Padmanabhan M.R. and Shaleesha A. Stanley (2012). Microalgae as an Oil Producer for Biofuel Applications, *Res.J.Recent Sci.*, 1(3), 57-62.
- Ayehunie S, Belay A, Baba TW, Ruprecht RM. (1998). Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis* (*Arthrospira platensis*). *J Acquir Immune Defic Syndr Hum Retroviral* 18; 7–12.
- Blackburn S (1978). Sample preparation and hydrolytic methods, in amino acid determination methods and technique, Blackburn, S. Ed., Marcel Dekker, New York. p. 7.
- Becker, E.W. and Venkataraman, L.V (1984). Production and Utilization of blue-green algae *Spirulina* in India. *Biomass*. 4: 105-125.
- Bennett A and Bogorad L (1971). Properties of subunits and aggregates of blue green algal biliproteins. *Hlth. Lab.Sci.* 3: 90-100.
- Bligh, E.G. and Dyer, W.J. (1959). A rapid method for total lipid extraction and purification. *Can.J.Biochem.Physiol.* 37:911-917.
- Borowitzka, M. A (1999). Vitamins and fine chemicals from microalgae, In Borowitzka, M. A. and Borowitzka, L. J. (ed.), *Micro-algal biotechnology*. Cambridge.
- Bosma R, VanSpronsen W. A, Tramper J and Wijffels R. H (2003). Ultrasound, a new separation technique to harvest microalgae. *J. Appl. Phycol.*, 15 (2-3), 143-153.
- Chamorro G, Salazar M, Favila L, Bourges H. (1996). Pharmacology and toxicology of *Spirulina* alga. *Rev Invest Clin.* 48: 389–99
- Chanava G, Furnadzhieva S, MinkovaK, Lukavsky J. (2007). Effect of light and temperature on the

- cyanobacterium *Arthonema africanum* a prospective phycobiliprotein producing strain. *Journal of Applied Phycology*. DOI 10.1007/s10811-007-9167-6.
- Chisti Y. (2007). Biodiesel from microalgae: A Review, *Biotechnology Advances*; 25, 294-306.
- Cornet, J. F. (1998). Le technoscope: les photobioréacteurs. *Biofutur*, 176, 1–10.
- Divakaran R and Pillai V. N. S. (2002). Flocculation of algae using chitosan. *J.Appl.Phycol.* 14 (5), 419-422.
- Grobbelaar JU. (2010). Microalgal biomass production: challenges and realities. *Photosynth. Res.* 106: 135-144.
- Guil-Guerrero, J. L., Navarro-Juárez, R., López-Martínez, J. C., Campra-Madrid, P and Reboloso-Fuentes, M.M. (2004). Functionnal properties of the biomass of three microalgal species. *J. Food Eng.*, 65: 511–517.
- Huesemann MH and Benemann JR. (2009). Biofuels from Microalgae: review of products, processes and potential, with special focus on *Dunaliella* sp. In: Ben-Amotz A, Polle JEW, Subba Rao DV, editors. The alga *Dunaliella*: Biodiversity, physiology, genomics, and biotechnology. Enfield: Science Publishers.
- Jeffrey, S.W. and Humphrey, G.F. (1975). New spectrophotometric equation for determining chlorophylls a, b, c and c2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.*, 167: 191-194.
- Kim HM, Lee EH, Cho HH, Moon YH. (1998). Inhibitory effect of mast cell mediated immediate-type allergic reactions in rats by *Spirulina*. *Biochem Pharmacol.* 55:1071– 1076.
- Knuckey R, Brown M, Robert M. R, Frampton R, D.M.F. (2006). Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. *Aquacult. Eng.* 35(3), 300- 313.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. (1951). Protein measurement with the Folin phenol reagent. *J.Biol. Chem.*, 193: 265-275.
- Mata T.M., Martins A.A and Caetano N.S (2010). Microalgae for biodiesel production and other applications, *Renew. Sust. Energ. Rev.*, 14(1), 217-32.
- Metting, F. B. (1996). Biodiversity and application of microalgae. *J. Ind. Microbio.*, 17: 477-489.
- Muthukumar M, P. Thirupathi, K. Chinnu and V. Sivasubramanian. (2012) Phycoremediation efficiency and biomass production by micro alga *Desmococcus olivaceus* (Persoon et Acharius) J.R. Laundon treated on *chrome-sludge* from an electroplating industry-A open. *International Journal of Current Science* (Special Issue): 52-62.
- Miranda MS, Cintra RG, Barros SB, (1998). Mancini FJ Antioxidant activity of the micro alga *Spirulina maxima*. *Braz J Med Biol Res* 31:1075–1079.
- Mohan N, Hanumantha Rao P, Ranjith Kumar R and Sivasubramanian V. (2010). Mass Cultivation of *C. turgidus* and *Oscillatoria* sp. and Effective Harvesting of Biomass by Low-cost methods. *Nature Precedings* : doi:10.1038/npre.4331.1.
- Molina Grima E, Belarbi E. H, Acien Fernandez F. G, Robles Medina A and Chisti Y (2003). Recovery of microalgal biomass and metabolites: Process options and economics. *Biotechnol. Adv.* 20 (7-8), 491-515.
- Nickelsen K. (2007). Otto Warburg’s first approach to photosynthesis. *Photosynth. Res.* 92 109-120.
- Olaizola, M (2003). Commercial development of microalgal biotechnol- ogy: From the test tube to the market place. *Biomol. Eng.*, 20 (4-6), 459-466.
- Qureshi MA, Garlich JD and Kidd MT (1996). Dietary *Spirulina platensis* enhances humoral and cell-mediated immune functions in chickens. *Immunopharmacol Immunotoxicol* 18: 465–476.
- Pons, A., Rola, P., Agvilo, C., Garcia, F.J., Alemarry, M., Paloo, A., (1981). A method for the simultaneous determinations of total carbohydrate and glycerol in biological samples with the anthrone reagent. *J. Biochemical and Bio Physical methods*, 4 (3-4): pp. 227-231.
- Richmond, A (1996). Efficient utilization of high irradiance for production of photoautotrophic cell mass: a survey. *J. Appl. Phycol.* 8: 381-387.
- Shaila Hiremath and Pratima Mathad (2010). Impact of salinity On the Physiological and Biochemical traits of *Chlorella vulgaris* Beijerinck. *J.Algal Biomass Utln.*, 1(2):51-59.
- Shaish A, Ben-Amotz, A & Avron M. (1992). Biosynthesis of  $\beta$ -carotene in *Dunaliella*. *Methods Enzymol.* 213:439- 444.
- Schwartz J, Shklar G, Reid S, Trickler D. (1988). Prevention of experimental oral cancer by extracts of *Spirulina–Dunaliella* algae. *Nutr Cancer.* 11:127-134.
- Soletto, D., Binaghi, L., Lodi, A., Carvalho, J. C. M., and Converti, A. (2005). Batch and fed-batch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. *Aquaculture*, 243: 217–224.
- Tan DKY., Amthor JS. (1988). Bioenergy. In: Dubinsky, Z. (ed.) *This publication* University Press, Cambridge p. 153–196.

- Venkataraman L.V and Becker E.W (1985).  
*Biotechnology and Utilization of algae*. The Indian  
Experience. Department of Science and  
Technology, New Delhi, India.
- Walker JM (1996). The protein protocol handbook.  
Humana press, USA.

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