Final Project Report of UGC Minor Research Project for the period from 01.02.2011 to 31.01.2013

"CO2 sequestration from fuel gas and it's mitigation by microalgal growth"

F. No. 39-671\2010 (SR)

Dated: 11-01-2011

By

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UNIVERSITY GRANTS COMMISSION
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Final Report of the work done on the Minor Research Project (2011-2013)

1. Project report No.	:	Final Report
2. UGC Reference No.	:	File No 39-671/2010 (SR)dt. 11.01.11
3. Period of report	:	From 01.02.2011 to 31.01.2013
4. Title of research project	:	CO2 sequestration from fuel gas and it's mitigation by microalgal growth
5. (a) Name of the Principal Investigator	:	Dr.A. David Ravindran, Professor
(b) Department and University/ College where work has progressed	:	Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram-624 302. Tamil Nadu
6. Effective date of starting of the project	:	01.02.2011
7. Grant approved and Expenditure incurred during the period of the report		
(a) Total amount approved	:	Rs. 2,00,000/-
(b) Total expenditure	:	Rs. 1,32,165/-
(c) Report of the work done	:	Enclosed

i. Brief objective of the project : **Annexure (1)**

ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication:

Annexure (1)

iii. Has the progress been according to original plan of work and towards achieving the objective? If not, state reasons

The standardization of utilization of flue gas has not been completed only some work on the utilization of flue gas by microalgae has been done, other objectives are fulfilled.

iv. Please indicate the difficulties, if any, experienced in implementing the project:

The collection of flue gas and introducing it into the algal tank had been worked out, by eliminating the flue gas temperature has to be reduced. The toxic chemicals in flue gas had been neutralized before using for algal growth for which I have standard the method.

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet

vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission :

ENCLOSED

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output,

(a) Manpower trained: MSc Microbiology students given training in *Spirulina sp* cultivation and biodiesel production and phycocyanin extraction

(b) Ph. D. awarded ; Project did not support research scholar grant however one scholar under my guidance worked in this area of research

(c) Publication of results : The research findings were presented in various conferences and seminars and some of the findings were also published as papers in journals. The list is furnished here-below.

1. Prabakaran, P and A. David Ravindran. 2011. A comparative study on effective cell disruption methods for lipid extraction from microalgae, *Letters of Applied Microbiology*, **53**: 150-154**. (245 citations)**

2. Prabakaran, P and Ravindran, A.D. 2011. A study on effective lipid extraction methods from certain freshwater microalgae, *Proceedings of International conference on Biodiversity and Aquatic Toxicology*, **1**: 193-196.

3. Pandian Prabakarn and A. David Ravindran. 2012. *Scenedesmus* as a potential source of biodiesel among selected microalgae, *Current Science*,102(**4**), 616-620 **(84 citations)**

4. Prabakaran, P and Ravindran, A.D. 2012. Study increase lipid content from *Chlorococcum* microalgae, Proceedings of Biologically active molecules, Excel publishers, 394-396.

5. Prabakaran, P and Ravindran, A.D. 2012. Biodiversity of fresh water algae from selected waterbodies in and around Dindigul District, Tamil Nadu,

Proceedings of Biodiversity: Richness, Uses, Threats and conservation, Excel publishers, 23-25

6. Prabakaran, P and Ravindran, A.D. 2012. Influence of different Carbon and Nitrogen sources on growth and CO_2 fixation of microalgae, *Advances in Applied Science Research*, 2012, 3 (**3**):1714-1717 **(20 citations)**

7. Prabakaran, P and Ravindran, A.D. 2012. Standardization of growth and augmenting lipid production from *Chlorococcum* microalga International Research Journal of Basic and Applied Sciences, **1**: 157-S161.

Papers presented in National and International Conferences

1. Pandian Prabakaran and **David Ravindran, A**. 2013. Characterization of microalgal species isolated from fresh water bodies as a potential source for bioenergy feedstock, National conference on Microbial technology for sustainable development, organized by Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram.

2. Pandian Prabakaran, Mageswari,M., Ragavathy, N. and **David Ravindran, A**. 2013. Analysis of efficacy of extraction methods on phycocyanin from *Spirulina platensis*, National conference on Microbial technology for sustainable development, organized by Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram.

3. Pandian Prabakaran and **David Ravindran, A**. 2012. Influence of different carbon and nitrogen sources on growth and CO_2 fixation of microalgae,

International workshop on climate change in Agriculture: Adaptation and mitigation strategies, organized by Faculty of Agriculture and Animal Husbandry, Gandhigram Rural Institute-Deemed University, Gandhigram.

(d) Other impact, if any:

The project could develop a replicable model for CO_2 sequestration from flue gas, a low cost photobioreactor was developed which could be patented. Two products standardized for extraction from flue gas cultivated algae was biodiesel and phycocyanin. The publications based on this minor UGC project has around 380 citations.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

REGISTRAR

Annexure (1)

C. Report of the work done

i. Objective of the project and its status

		Status
1.	To study the biological aspect of microalgae, including screening and collecting a variety of algal species to access their potential for growth, CO_2 utilization and biodiesel production.	Done
2.	To standardized the factors influencing the microalgal growth such as CO_2 conc., pH, culture media, temperature and light.	Done
3.	To develop the further application for algal species and work out the cost/benefit ratio.	Done
4.	To study the algal mass production in open and closed system	Done
5.	To study the algal mass production using flue gas.	Initial work

ii. Work done so far, results achieved and publications

1. Isolation and Identification of microalgae

Water samples for microalgae isolation were collected from different sites (in and around Dindigul district, Tamilnadu, India.) that appeared to contain algal growth in fresh water bodies. All samples were collected at about the same time between 0800 to 1100 hrs. Surface water and water at a depth of 0.50 meter were collected at each location. Water samples were taken from the sites to laboratories in bottles cooled in ice. Ten ml of water samples were transferred to a 500 ml conical flask containing 200 ml of sterilized Bold's Basal Medium (BBM) and then incubated on a rotary shaker at 27°C and 100 rpm under continuous illumination using white fluorescent light (maximum 2500 lux) for three weeks. BBM was composed of (mg/L) $NaNO_3$, 250; K_2HPO_4, 75; KH_2PO_4, 175; $CaCl_2$, 25; NaCl, 25; $MgSO_4$, 75; $FeCl_3$, 0.3; $MnSO_4$. $7H_2O$, 0.3; $ZnSO_4$. $7H_2O$, 0.2; H_3BO_3, 0.2; $CuSO_4$. $5H_2O$, 0.06. Every two days, the flasks were examined for algal growth using microscope, with serial dilutions being made in BBM from flasks showing growth. Subcultures were made by inoculation 50 µl culture solution onto petri plates containing BBM solidified with 1.5% (w/v) of bacteriological agar. These procedures were repeated for each of the original flasks. Petri plates were incubated at 27°C under continuous illumination for two weeks. The purity of the cultures was confirmed by repeated plating and by regular

observation under a microscope. The freshwater microalgae were identified and authenticated based on the guidelines of the standard manual.

2. Growth analysis with different parameters.

2.1 Effect of pH, Light intensity and Sodium nitrate concentration on microalgae

At first, cells of identified microalgae were cultivated in 2 L flask using BBM and incubated batch wisely at 24°C. for 27 days. The culture was bubbled with a sterile air and illuminated at different light intensity, pH and sodium nitrate concentrations. Light intensity was measured using a Lux meter. Four light intensities were used: 1000 lux, 3300 lux, 3850 lux, 6000 lux. Growth conditions such as pH (at 5.0, 6.0, 7.0, 8.0 and 9.0), Sodium nitrate concentration (0.5, 1.0, 2.0 and 3.0 percent) were maintained. Every three days the algal growth was determined by measuring the Optical Density at 530 nm using a Elico SL 177i spectrophotometer.

2.2 Effect of nitrogen and carbon sources on growth of microalgae

The growth performance of microalgae were evaluated by varying the concentration (0.02%, 0.04%, and 0.06%) of different nitrogen sources (urea and ammonium nitrate) and carbon sources (glucose and mannose) were supplemented

to Bristol medium. To the treatments, 10% v/v of inoculum was introduced and triplicates were maintained. The growth of the algal species in the treatments was determined by spectrophotometric method at 560 nm at a regular interval of three days.

2.3 Estimation of chlorophyll content in different treatments

Algal cells were homogenized at 1,000 rpm for 1 min, using 100% acetone (50 ml for each g sample). The homogenates was filtered through two layer cheese cloths and was centrifuged at 2,500 rpm for 10 min. The supernatant was separated and the absorbance was read at 662 and 645 for Chl. A and Chl.b, according to the method described by Dere et al., (1998). The amount of these pigments was calculated according to the formulas of Lichtenthaler and Wellburn (1985):

$$\text{Chl.a} = 11.75\ A_{662} - 2.350\ A_{645}$$

$$\text{Chl.b} = 18.61\ A_{645} - 3.960\ A_{662}$$

2.4 Estimation of nitrogen content in different treatments

Nitrogen was estimated by microkjeldhal method was described by Jones(1991). Dried algal samples (50 mg) were taken and 10 ml of sulphuric acid was added and heated in heating mantle. 3 gm of catalyst mixture was added to the mixture. The solution was digested until it turned clear solution. Same procedure was followed for a blank without sample. To the cooled digested preparation 15 ml of distilled water and 15 ml of NaOH was added and the solution was transferred

to the distillation chamber. 20 ml of boric acid mixture was taken in a conical flask, kept at recovery end of the distillation apparatus prior to the start of the distillation process. When the colour of boric acid turned green and its volume made up to 30 ml, the distillation was stopped. The boric acid solution was titrated against 0.02 N sulphuric acid until the colour changed to pink. The total nitrogen was calculated by the following formula and results were tabulated

$$\% \text{ of Nitrogen} = \frac{14 \times 0.02 \times (\text{titrated value} - \text{Blank value})}{\text{Weight of the sample (gm)}}$$

2.5 Estimation of carbon content and carbon dioxide fixation rate

Dried algal (0.2 mg) samples were placed in 500 ml conical flask and 10 ml of 1N potassium dichromate and 20 ml of conc. H_2SO_4 mixture was diluted with 200 ml of distilled water and 10 ml of H_3PO_4 and 1 ml of diphenyl amine was added. Finally, titrated against 4 N ferrous ammonium Sulphate (FAS). The end point was brilliant green colour appeared. The carbon content was estimated using the following formula

$$a = \frac{3.951}{g}\left(1 \times \frac{T}{S}\right)$$

where a is carbon content, g is weight of the sample, T is FAS with blank (ml) and S is FAS with sample (ml). And the amount of carbon dioxide fixation rate was estimated using the formula, (Yun et al. 1997)

$$R_{CO_2} = C_c \times \mu_L \left(M_{CO_2} / M_C\right)$$

Where R_{CO2} and μ_L are the CO_2 fixation rate (g CO_2 m^{-3} h^{-1}) and the volumetric growth rate (g dry weight m^{-3} h^{-1}), respectively, in the linear growth phase. M_{CO2} and M_C represented the molecular weights of CO_2 and elemental carbon, respectively, C_C is average carbon content (algal dry weight [g]).

2.6 Effect of Nitrate, CO_2 and Temperature

For the treatments viz., temperature (26 and 30ºC), CO_2 (without or with supplementation: 0.04 and 5% [v/v], respectively), and nitrate (with or without), and all combinations of these variables. The culture was continuously illuminated with six fluorescent lamps at an irradiance level of 2500 lux for 21 days. Growth was evaluated over time in terms of dry weight (DW).

3. Fabrication of Photobioreactors

Constructed two photobioreactors one is 15 L capacity and another one is 30L capacity. The photobioreactors having on/off timer for six artificial florescent light and aeration with regulator. Mass production of microalgae cultivated in this photobioreactors.

4. Phycocyanin extraction from *Spirulina platensis*

Phycocyanin was extracted from the wet biomass of *Spirulina platensis* using the following methods.

Method 1 Water extraction: *Spirulina* biomass was suspended in distilled water and the phycocyanin leached out was estimated spectrophotometrically (Siegelman and Kycia, 1978).

Method 2 Homogenisation of cells in a mortar and pestle: Biomass was homogenised in a mortar and pestle in the presence of acid washed neutral sand using 50 mM sodium phosphate buffer pH 6.8. The extract was centrifuged and the supernatant contained phycocyanin. The pellet was re-extracted with buffer to ensure complete recovery of phycocyanin.

Method 3 Freezing and thawing: Phycocyanin was extracted by repeated freezing and thawing of cells in 50 mM phosphate buffer pH 6.8.

Method 4 Acid extraction: The wet biomass was treated with different concentrations of hydrochloric acid (2, 4, 6, 8 and 10 N) at room temperature. At different time intervals (2, 4, 24 h) samples were centrifuged and supernatants taken for phycocyanin and estimated by the method of Sigelman and Kycia (1978) and the amount of phycocyanin was calculated as mg phycocyanin per ml using the equation O.D at 615 nm–0.474 (O.D at 652 nm)/5.34. The same method was followed for estimation of phycocyanin extracted by other procedures.

Experimental work and findings

Isolation and identification of microalgae

Microalgae are present in all existing earth ecosystems, not just aquatic, but also terrestrial representing a large variety of species living in a wide range of environmental conditions (Sydney, 2010). In this study, a total of 21 microalgal cultures were isolated from six different water bodies. Only seven microalgae (*Chlorella, Chlorococcum, Spirulina, Scenedesmus, Desmococcus, Sytonema* and *Tolypothrix*) were selected for extraction of lipids based on their purity (Table 1).

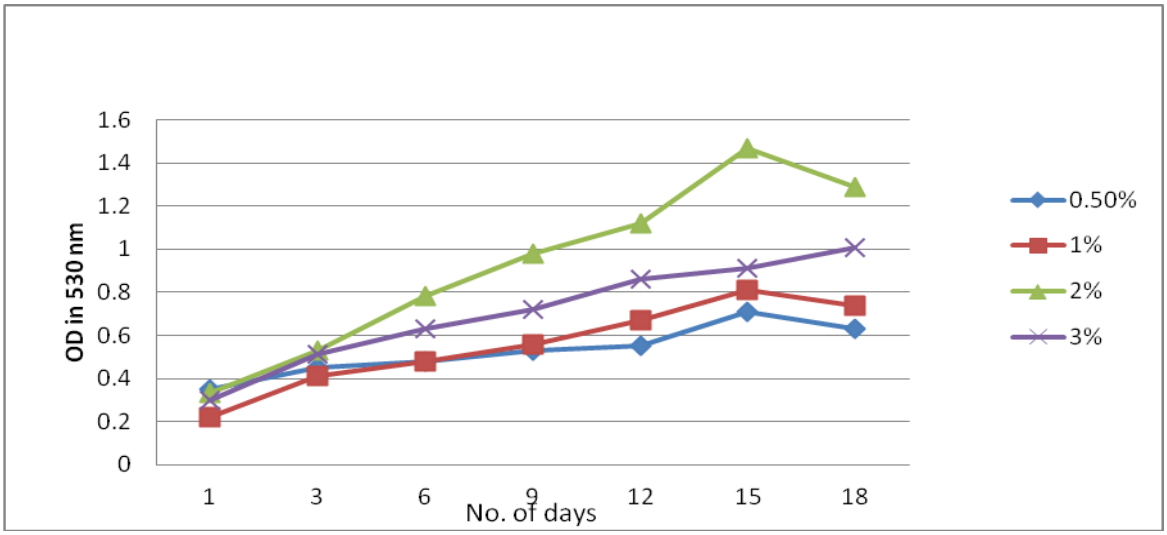
Table 1. Isolation of microalgae from different water bodies in and around Dindigul district

S. No	Location	Latitude	Longitude	Name of the microalgae
1	Kamarajar dam	10°17’43.44” N	77°48’44.06” E	*Chlorella* sp.
2	Palar dam	10°24’30.61” N	77°29’38.39” E	*Chlorococcum* sp.
3	Palani pond	10°26’12.59” N	77°30’52.27” E	*Spirulina* sp., *Scenedesmus* sp.
4	Manjalar dam	10°11’37.15” N	77°37’55.86” E	*Desmococcus* sp.
5	Nerhu pond	10°16’39.12” N	77°56’04.75” E	*Sytonema* sp.
6	Anaippatti dam	10°05’20.15” N	77°51’10.28” E	*Tolypothrix* sp.

Growth analysis of microalgae with different parameters

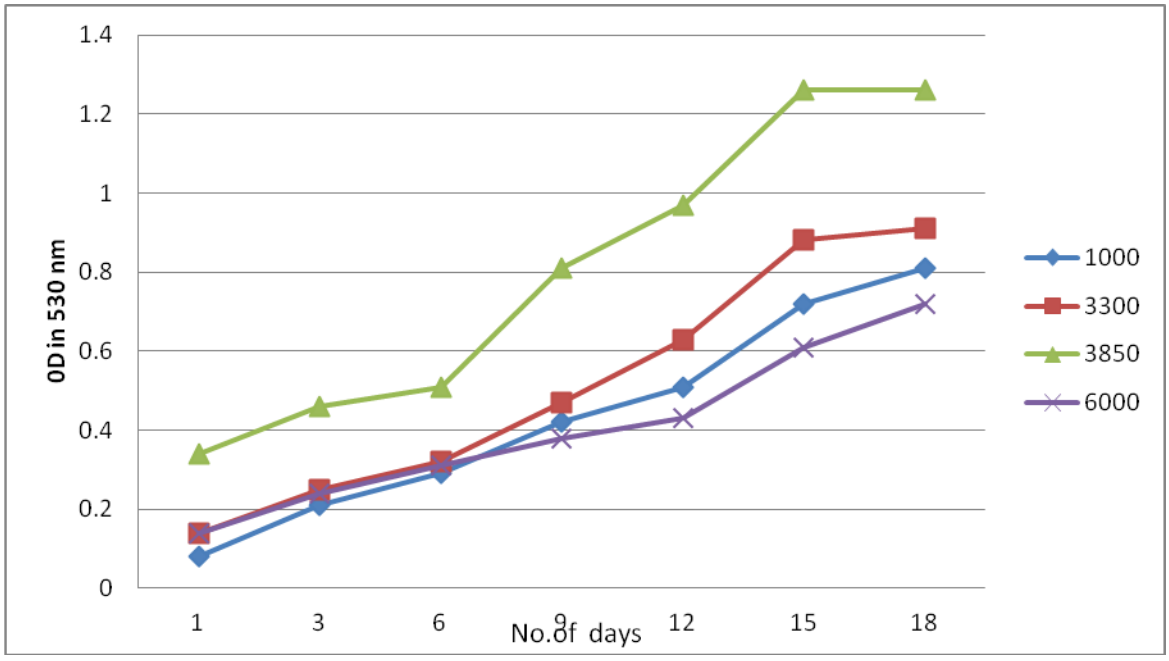
In the present study, *Chlorococcum* sp. was selected based on the growth and purity. Under suitable conditions and sufficient nutrients, microalgae can grow profusely (Chisti, 2007). Fig.1. shows that the growth rate of *Chlorococcum* sp. with different concentrations of $NaNo_3$. The growth curve indicating enhanced growth rate corresponding with the incubation time of *Chlorococcum* sp. The growth recorded after peak growth 15 days of incubation. Maximum growth was observed in 2% of $NaNO_3$. Anitha *et al*. (2009) reveals that at decreasing concentration of nitrogen sources there was decreased growth, chlorophyll and biomass. Nitrogen starvation also triggered a rapid decline in nitrogen containing compound such as photosynthetic pigments.

Fig. 1. Growth analysis of *Chlorococcum* sp. with different concentrations of $NaNo_3$



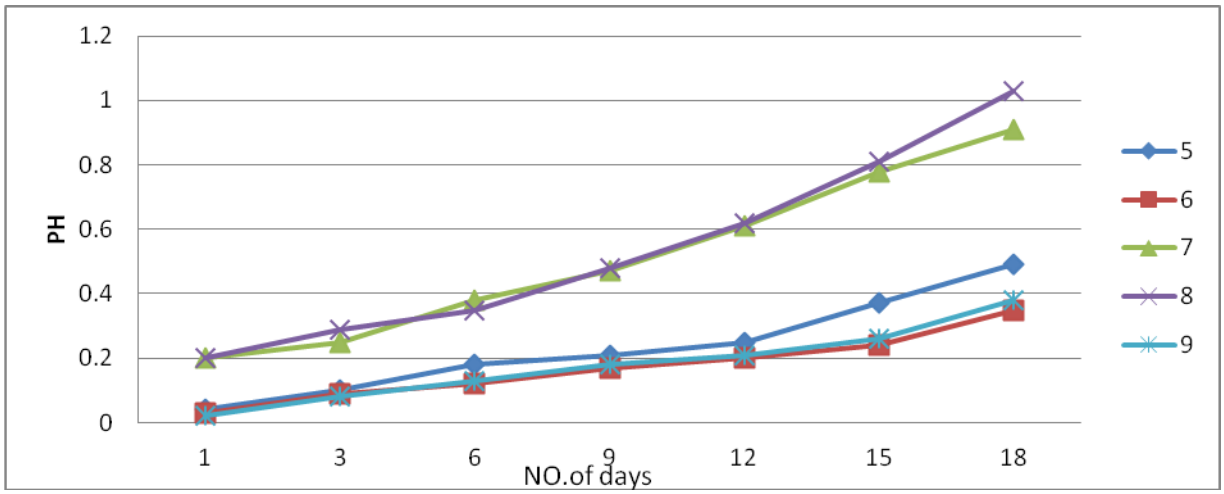
Similar work was done by Dayanantha *et al*. (2005) with *Botryococcus braunii* using different substrates such as potassium, calcium and sodium nitrates compared with urea and ammonium nitrate. In the absence of nitrates, the growth was poor and the cells were bleached turning brown. Among the nitrates tested, potassium nitrates appear to be more effective compared to calcium and sodium nitrate. Growth analyses of *Chlorococcum* sp. with different light intensity the results are recorded in fig. 2. The growth reached a maximum at 3850 lux and minimum was recorded 6000 lux on 15 days of incubation. The main factor which influences the growth of photoautotrophic microorganisms is light and temperature.

Fig. 2. Growth analysis of *Chlorococcum* sp. with different light intensity



Growth studies of *Chlorococcum* sp. in different pH results are presented in fig.3. According to the results, the *Chlorococcum* sp. recorded maximum growth at pH 8 and minimum in pH 6. In previous study, Patil *et al*. (2011) observed that stress induced formation of triacylglyceride by high irradiance under nitrogen-depleted conditions. It is enhanced with increasing salinity and light intensity, indicating the augmented photosynthetic carbon flow towards biosynthesis of storage lipids under combined stress.

Fig. 3. Growth analysis of *Chlorococcum* sp. in different pH concentration



In this study, two isolates of fresh water microalgae viz., *Scenedesmus sp*., and *Chlorococcum* sp. were selected. Samples were withdrawn from all the treatments during the initial and final day (21day). The chlorophyll content of the algal cultures was determined using Arnon method (1949). The results are shown

in Table 2 and 3. The chlorophyll content increased in all the treatments after 21 days of incubation. Maximum amount of chlorophyll content was recorded in 0.02% urea for *Scenedesmus* and *Chlorococcum*. In carbon source, the amount of chlorophyll was maximum in 0.06% glucose at *Scenedesmus* sp. and *Chlorococcum* in 0.02% glucose. Anitha *et al*. (2009) reveals that at decreasing concentration of nitrogen sources there was decreased growth, chlorophyll and biomass. Nitrogen starvation also triggered a rapid decline in nitrogen containing compound such as photosynthetic pigments causing complete loss of photosynthetic efficiency. Similar work was done by Dayanantha *et al*. (2005) with *Botryococcus braunii* using different substrates such as potassium, calcium and sodium nitrates compare with urea and ammonium nitrate. In the absence of nitrates, the growth was poor and the cells were bleached are turned brown. Among the nitrates tested, potassium nitrates appear to be more effective compared to calcium and sodium nitrate.

The nitrogen content of dried biomass was estimated using microkjeldhal method. The results are shown in table 1 and 2. In *Scenedesmus* sp. the amount of nitrogen were maximum in 0.04% glucose and 0.04% ammonium nitrate. In *Chlorococcum* sp. the amount of nitrogen were maximum in 0.04% mannose. The carbon content of the dried algal biomass was estimated by Walky and Black method. The carbon content is shown in table 1 and 2. The maximum amount of

carbon in *Scenedesmus* sp. was recorded at 0.02% glucose and *Chlorococcum* sp. was 0.06% ammonium nitrate. The carbon dioxide fixation rate of *Scenedesmus* and *Chlorococcum* are shown in table 1 and 2. The carbon dioxide fixation rate was maximum at 0.02% urea treatment in *Scenedesmus* and *Chlorococcum.* Ki-Don Sung *et al.* (1999) reported that *Chlorella* and *Scenedesmus* were highly CO_2 tolerant microalgae show excellent stabilities to high concentration of CO_2 and high temperature. From the present study it is evident that the growth and CO_2 fixation rate are influenced by the carbon and nitrogen present in the medium. Among the two nitrogen sources tested, best results were recorded in the treatments with 0.02% of urea. Among the carbon sources tested, glucose exhibited the maximum results. Therefore, urea and glucose can serve as potential nitrogen and carbon source for the selected microalgae

Table 2. Growth analysis and CO_2 fixation of *Scenedesmus* sp. with different carbon and nitrogen sources

Microalgae	**Nutrients**	**Concentration**	**Chlorophyll**		**Nitrogen**		**Carbon**	**CO_2 fixation rate(g/ml/day)**
			Initial value	Final value	Initial value	Final value	21^{th} day (g/dry weight)	
		Control	3.51±0.01	5.80±0.1	0.173±0.001	0.15±0.001	20.1±0.5	0.66
	Urea	0.02	3.42±0.1	18.60±0.1	0.171±0.01	0.652±0.004	28.8±0.5	1.37
		0.04	3.48±0.1	10.50±0.1	0.172±0.001	0.8±0.002	24.3±0.5	1.33
***Scenedesmus* sp.**		0.06	3.50±0.1	15.50±0.1	0.17±0.001	0.4±0.003	20.5±0.5	1.33
	Ammonium nitrate	0.02	3.49±0.1	11.20±0.1	0.17±0.001	0.5±0.004	32.4±0.4	0.82
		0.04	3.43±0.1	10.10±0.1	0.17±0.01	0.83±0.002	22±0.5	1.30
		0.06	3.50±0.1	9.32±0.1	0.17±0.01	1.51±0.01	33±0.4	1.04
	Glucose	0.02	3.72±0.1	12.60±0.1	0.25±0.02	0.6±0.04	36±0.4	0.79
		0.04	3.80±0.1	10.10±0.1	0.24±0.02	0.85±0.02	25±0.5	0.57
		0.06	3.80±0.1	17.90±0.1	0.25±0.02	0.35±0.03	23±0.5	0.58
	Mannose	0.02	3.80±0.1	15.60±0.1	0.25±0.02	0.65±0.04	35±0.4	0.76
		0.04	3.70±0.1	14.50±0.1	0.24±0.03	0.7±0.05	26.5±0.5	0.77
		0.06	3.80±0.1	13.00±0.1	0.25±0.02	0.68±0.04	16.5±0.4	0.42

Table 3. Growth analysis and CO_2 fixation of *Chlorococcum* sp. with different carbon and nitrogen so

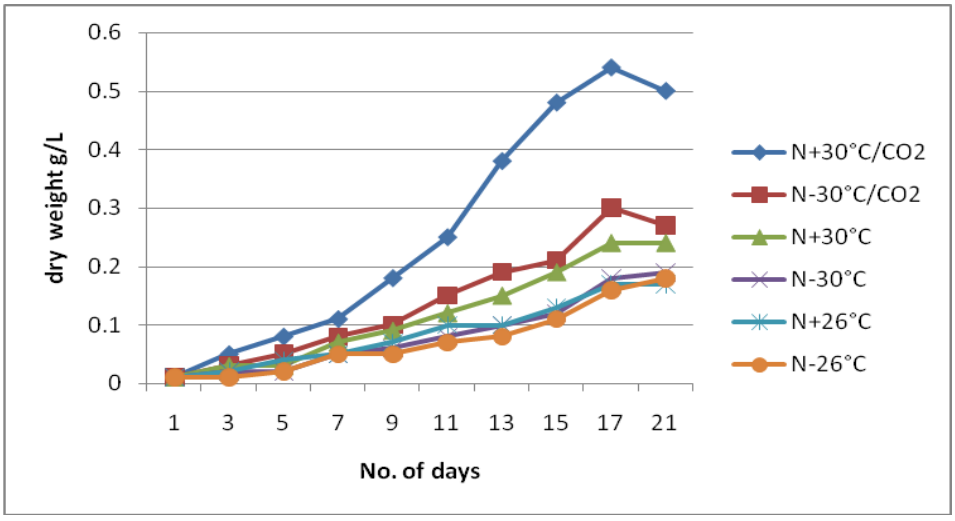
Microalgae	**Nutrients**	**Concentration**	**Chlorophyll**		**Nitrogen**		**Carbon**	**CO_2 fixation rate(g/ml/day)**
			Initial value	Final value	Initial value	Final value	21^{th} day (g/dry weight)	
		Control	2.5±0.1	3.5±0.3	0.03±0.002	0.25±0.03	13.9±0.3	0.15
	Urea	0.02	2.5±0.1	4.6±0.1	0.03±0.002	0.31±0.03	20.5±0.5	0.75
		0.04	2.5±0.1	4.2±0.1	0.03±0.002	0.23±0.03	17±0.5	0.62
		0.06	2.4±0.1	3.9±0.1	0.03±0.002	0.28±0.03	18±0.5	0.65
	Ammonium nitrate	0.02	2.5±0.1	2.9±0.1	0.03±0.002	0.3±0.03	13.5±0.3	0.49
		0.04	2.5±0.1	2.5±0.1	0.03±0.002	0.35±0.03	10.5±0.3	0.38
		0.06	2.5±0.1	2.3±0.1	0.03±0.002	0.33±0.03	30±0.4	0.12
	Glucose	0.02	2.7±0.1	3.7±0.1	0.17±0.02	0.21±0.03	14.5±0.4	0.53
		0.04	2.8±0.1	3.5±0.1	0.17±0.02	0.32±0.03	12.5±0.4	0.43
		0.06	2.8±0.1	3.2±0.1	0.17±0.02	0.35±0.03	13±0.4	0.61
	Mannose	0.02	2.8±0.1	3±0.1	0.17±0.02	0.45±0.02	16±0.3	0.08
		0.04	2.7±0.1	2.5±0.1	0.17±0.02	0.85±0.04	7.5±0.3	0.27
		0.06	2.5±0.1	2.2±0.1	0.17±0.02	0.6±0.02	6.5±0.3	0.3

***Chlorococcum* sp.**

Effect of Nitrate, CO_2 and Temperature

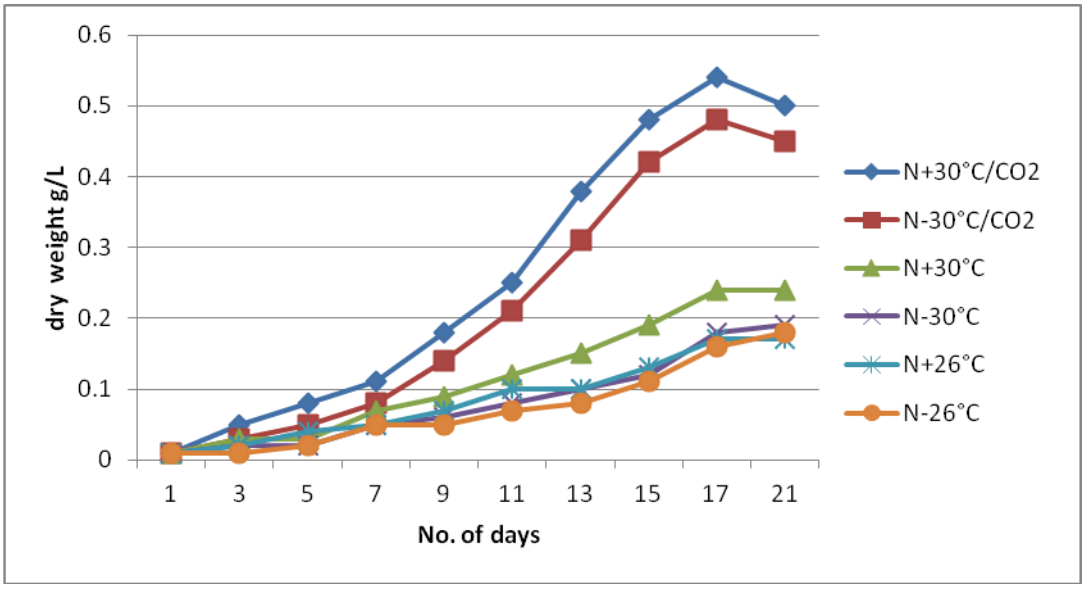
Microalgal biomass growth data of *Chlorococcum* sp. and *Scenedesmus* sp. under the six experimental conditions are depicted in Fig. 4 and 5 Culture curves showed the fastest growth for N+/30ºC/CO_2 (nitrate sufficient medium with CO_2 enrichment at 30ºC). Comparing this culture with N-/30ºC/CO_2 culture (nitrogen free medium with CO_2 enrichment at 30ºC), it can be observed that the former attained a 2-fold higher growth after 15 days. The CO_2 enrichment in the inlet air flow enhanced algal growth as expected, which can be seen by comparing the culture curves for N+/30ºC/CO_2 with N+/30ºC and N-/30ºC/CO_2 with N-/30ºC, showing that cultures under air bubbling were C-limited. In terms of temperature influence on *Chlorococcum* sp. biomass productivity, Fig. 4 curves showed that *Chlorococcum* sp. grew faster at 30 ºC than 26ºC, irrespective of supplemented CO_2.

Fig. 4. Average dry weights of *Chlorococcum* sp. under different growth conditions over time



Culture curves showed the fastest growth for the treatment N+/30ºC/CO_2 (nitrate sufficient medium with CO_2 enrichment at 30ºC). Comparing this culture with N-/30ºC/CO_2 culture (nitrogen free medium with CO_2 enrichment at 30ºC), it can be observed that the former treatment recorded higher growth. The CO_2 enrichment in the inlet air flow enhanced algal growth as expected, which can be seen by comparing the culture curves for N+/30ºC/CO_2 with N+/30ºC and N-/30ºC/CO_2 with N-/30ºC, showing that cultures under air bubbling were C-limited. In terms of temperature influenceing on *Scenedesmus* sp. biomass productivity (Fig. 5). The curves showed that *Scenedesmus* sp. grew faster at 30 ºC as compared with 26ºC, irrespective of supplemented CO_2.

Fig. 5. Average dry weights of *Scenedesmus* sp. under different growth conditions over time



Fabrication of Photobioreactors

Fig.6. Photobioreactor for algae cultivation (15L capacity)

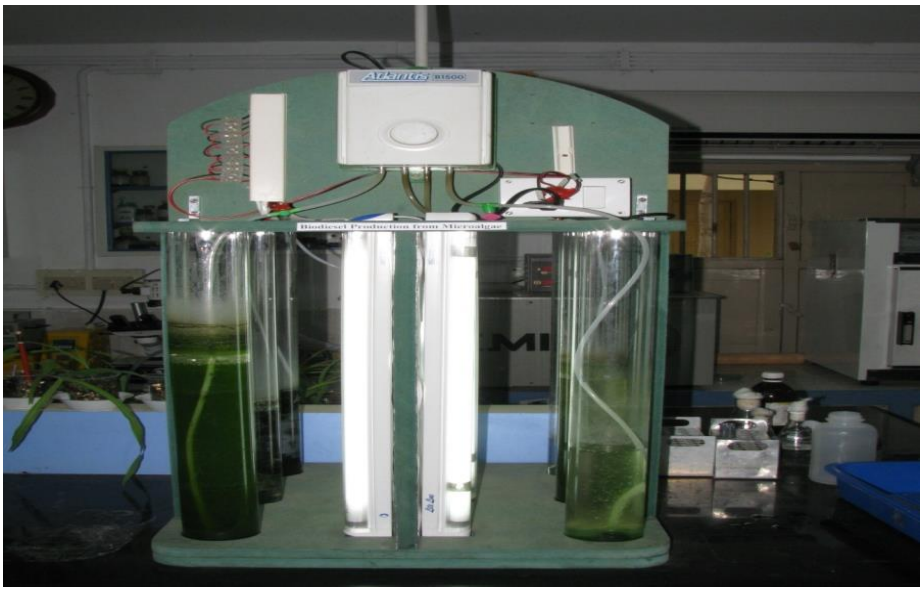
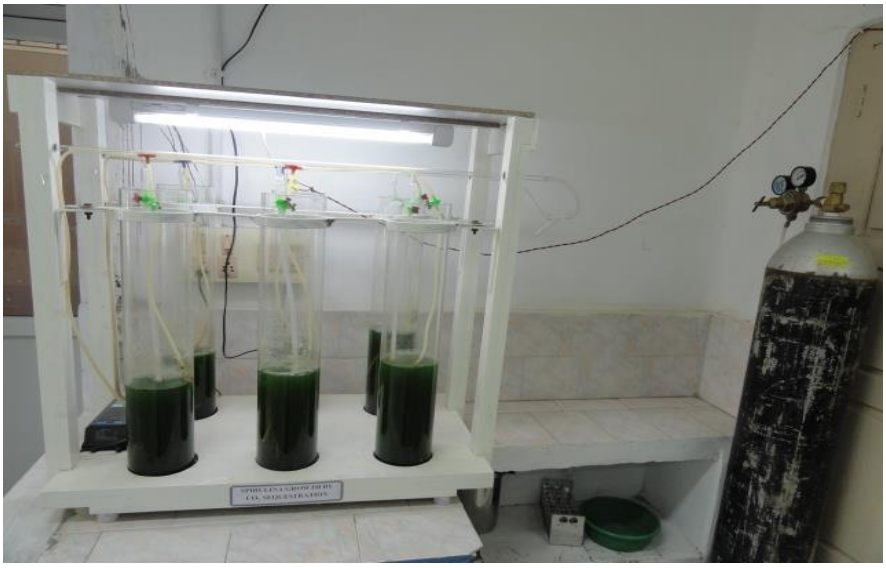


Fig.7. Photobioreactor for algae cultivation (30L capacity)



Extraction of Phycocyanin from *Spirulina platensis*

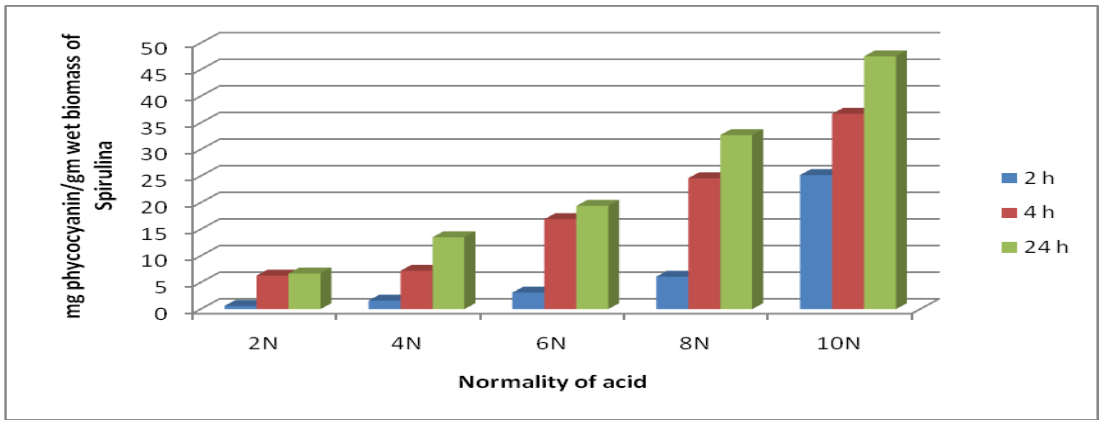
The phycocyanin content extracted from fresh biomass was considered as 100%. In oven dried, the phycocyanin content was 67% and sun dried 82%. There was considerable loss of phycocyanin in dried samples. The significant loss of phycocyanin in dried samples could be due to its peripheral position in phycobilisomes on the thylakoid membrane and attributable to its sensitivity to temperature (Gantt, 1981).

Table 4. Comparision of different methods for phycocyanin extraction from *Spirulina*

S. No	Methods	Phycocyanin (mg/100 mg)
1	Water extraction	17.25
2	Homogenization (mortar and pestle)	18.11
3	Freezing and thawing	17.68

In the extraction methods, water extraction method is the slowest and takes 2-3 days for comparable yields of phycocyanin while homogenization either by mortar and pestle or freezing and thawing takes 8-10 h. In Table 4 homogenization method showed higher extraction of phycocyanin than freezing and thawing method. In the water extraction process, phycocyanin leaching was very slow and observed only in cells harvested in late exponential phase.

Fig. 8. Phycocyanin extraction from *Spirulina platensis* wet biomass with different concentrations of HCl



The results obtained for the effect of increasing concentration of hydrochloric acid on extraction of phycocyanin with time are shown in Fig. 8. Higher amount of phycocyanin extraction was achieved only with 8–10 N hydrochloric acid. The microscopic observation of cells indicated complete disintegration of cells at these concentrations and also resulted in separation of the pigment phycocyanobilin from phycobiliprotein, phycocyanin (O'hEocha, 1963). Hydrochloric acid concentration of 2N, 4N and 6N of were not good enough for phycocyanin extraction.