

## Resolution of Lipid Content from Algal Growth in Carbon Sequestration Studies

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### **Abstract**

*Green microalgae species was collected from Bhavani Lake of Erode in Erode District, Tamilnadu State, India. The Morphological studies were examined by utilizing Fourier Transform Infrared Spectroscopy, Microscope studies, Scanning Electron Microscopic studies and isolated and identified by PCR studies and by these studies that the collected specimen is to be Chlorella sp. In this study to check growth behavior and tolerance of fresh water green algae chlorella sp, under different pH, Temperature, different concentrations of sodium bicarbonate salt, carbon dioxide gas and under different levels of sodium chloride salt. From the study it is clear that optimum levels of proper carbon source and salinity is very much essential for growing Chlorella sp., as they influences its growth rate, biomass and lipid productivity. The experiment aims to analyze the biomass productivity and total lipid content of the microalgae strain under various growth conditions. All the cultures were grown for a period of 10 days. For the growth study reveals that, pH range 4-7, Temperature 26-40, sodium bicarbonate salt concentrations 50-60 mg/l, sodium chloride concentration 0.02, maximum growth rate was observed, when CO<sub>2</sub> gas was supplied at 40% concentration, maximum growth and maximum lipid yield of 15 in %dry cell weight were recorded. A highest total lipid content of 20 % was obtained.*

**Keywords:** *Algae, Carbon dioxide, salinity, lipid content*

### **1. Introduction**

Global warming induced by increasing concentrations of greenhouse gases in the atmosphere is a matter of great environmental concern. Carbon dioxide is the principal greenhouse gas. Atmospheric CO<sub>2</sub> has increased from 280 to 368 ppm in the last 200 years and is responsible for about 50% enhancement in the greenhouse effect [10]. Annual anthropogenic emissions of CO<sub>2</sub> are estimated to be  $2 \times 10^{10}$  tons, primarily from combustion of fossil fuels in association with an increasing population and industrialization [3-5]. Recently, many attempts have been made to reduce atmospheric CO<sub>2</sub>. Physical and chemical treatments have been used to separate and recover CO<sub>2</sub>.

Utilization of CO<sub>2</sub> by microalgae is among the most productive biological methods of treating industrial waste emissions, and the yield of biomass per acre is three to fivefold greater than from typical crops [7 and 14]. Direct use of flue gas reduces the cost of

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pretreatment, but the high concentration of CO<sub>2</sub> and the presence of SO<sub>x</sub> and NO<sub>x</sub> inhibit the growth of cyanobacteria and microalgae [12].

Algae are a large and diverse group of simple aquatic organisms ranging from unicellular to multi cellular forms, they mainly grow based on the photosynthesis mechanism, just like the plants, they capture the light energy to fuel the manufacture of sugars. They are simple creatures living in the marine and fresh water biological systems [8]. Many aquatic creatures such as fish and shrimp take the algae as their main food.

Algae play an important role in the global ecosystem. With the arising of global warming and GHG emission issue, algae are also studied to capture the carbon dioxide [1&2]. The potential ability of microalgae is positive, through the related theoretic calculations, the result is that per kilogram microalgae could capture nearly 1.83 kg CO<sub>2</sub> [7].

The microalgae have much higher growth rate than the most land-based plant due their higher photosynthesis efficiency. Algae have much shorter growth cycle, the weight double time is about three to five days and some species can have two harvest seasons in one day. The algae yield weight per year is nearly several times or even hundred times of food crop yield.

The goal of this study is to isolate microalgae in Coimbatore which can tolerate high CO<sub>2</sub> concentrations and high temperatures in order to sequester carbon dioxide and discover the optimal conditions for biomass production.

## 2. Materials and Methods

### 2.1. Sources of Algae Sample

Microalgae were isolated from several samples taken from rivers, lakes, ponds, of Erode and Coimbatore, KCT Hostel waste water was used as culture media for algae growth.

They were explored in terms of growth study in air, different pH, different concentration of sodium bicarbonate salt (NaHCO<sub>3</sub>), different concentration of carbon dioxide gas, different concentrations of Sodium chloride salt, Further, from the experimental finding it is observed that the strain can efficiently utilize CO<sub>2</sub> gas and bicarbonate, as carbon source, but CO<sub>2</sub> gas has a poor dissolving capacity and most of it tend to lost in the air so it is convenient to use bicarbonate form instead of CO<sub>2</sub> gas, as microalgae cells have the machinery to convert bicarbonate salt into CO<sub>2</sub> with the help of the enzyme carbonic anhydrase so that there is efficient fixation of CO<sub>2</sub> occurs with the help of another enzyme rubisco (ribulose 1,5 bis phosphate).

The pH of the cultures was elevated 2 to 10. Culture flasks each having different concentration of bicarbonate salts, gaseous CO<sub>2</sub> and sodium chloride salts were prepared separately for each of the experiment. For bicarbonate growth study of the aforementioned strain, NaHCO<sub>3</sub> salt in the concentration ranges of 20, 40, 60, 80 and 100 mg/L (1mg/L=1ppm) were freshly weighed and added to each of the flasks. For salinity tolerance NaCl salt in different concentrations 0.02, 0.04, 0.06, 0.08 & 0.10 Molar were added.

Gaseous CO<sub>2</sub> is supplied to cultures in the concentrations of 10%, 20%, 30%, 40%, 50%. Then CO<sub>2</sub> gas is supplied till the pH falls high to low, so that mentioned amount of CO<sub>2</sub> concentration can be reached. Because of poor solubility of CO<sub>2</sub> gas, there is periodical (every after 6 hrs) addition of CO<sub>2</sub> gas by checking and maintaining the pH of the cultures. A constant supply of carbon dioxide gas at 120 ml/min was maintained by using a digital gas flow meter (Digital Gas Flow Meter, Model: DFM-01 / 02 / 03). Biomass increase per day (mg/L/day), cell no increase per day and Optical Densities were taken daily for growth characteristics of the strain. The strain was checked for 9 days of growth period in varying amount of carbon dioxide gas.

## 2.2. Analytical Method

Light microscopic cell count by was performed using optical microscope (Ample). Optical densities of microalgae cultures were measured at a regular interval of time (24Hrs) by checking absorbance at 680nm with the help of spectrophotometer (Thermo Evolution-201) in three replicates and average value was recorded. The spectrophotometer was blanked every time with each medium respectively. At the end of the experiment all the culture flasks were centrifuged & filtered and dry weights of pellets were measured (80.C for 3 hrs) to study the increase in biomass, cell count and lipid content and further studies.

## 3. Result and Discussion

### 3.1. Effect of carbon dioxide emission

India is famous for the largest population, now India is entering a brand new era with the rapid economic development. Unfortunately, the main energy source of India is still coal, oil, natural gas such fossil fuels. Besides, a tremendously large scale of infrastructure construction is under going, the manufacturing industry is booming, more and more vehicles are showing on the road, the people's living standard and welfare is promoting as the same. The country is consuming more and more fossil energy. However, as a result, the carbon dioxide emission amount is rocketing with the increasing energy demand. Due to these reasons, the increasing carbon dioxide emission in India is shown Figure 1.

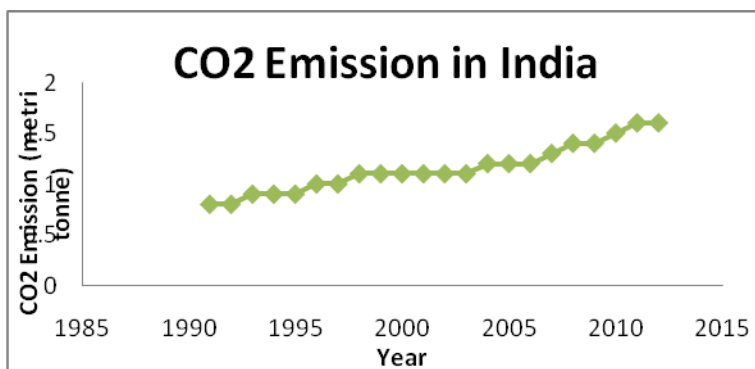


Figure 1. Carbon Dioxide Emmission in India

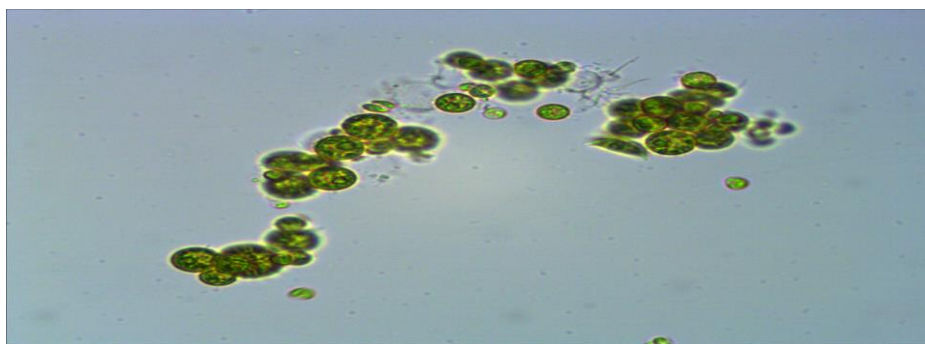
India needs to take actions to reduce the carbon dioxide emission, as one important role in the world's sustainable development, India must takes relative international responsibility, and the Chinese government has promised that the carbon dioxide emission per capita must be reduced by 50%-60% in 2020 compared with 2005. It is a big challenge for India.

Table 1. The Rank of Carbon Dioxide Emission in the World [CARMA, 2011]			
Nation	Area	TONS CO <sub>2</sub>	RANK
China	Asia	3120000000	1
Us	North America	2820000000	2
India	Asia	638000000	3
Russia	Europe	478000000	4

Germany	Europe	429000000	5
<b>Structure of Energy Consumption in India</b>			
	Industry	41%	
	Transport	18%	
	Residential	14%	
	Commercial	2%	
	Other energy uses	8%	
	Non-energy uses	9%	
	Agriculture	8	

### 3.2. Identification of Isolated Microalgae

Some morphological and chemical characteristics of this isolates, unicellular green micro with a diameter of 3-6  $\mu\text{m}$ . Each cell had one cup-shaped chloroplast with a distinctive pyrenoid, Figure 2.



**Figure 2. light microscopic image of chlorella sp**

### 3.4. Effect of Ph

The linear growth rate of *Chlorella* sp. was 10 g/l at an initial pH of 2 to 10, increased gradually to 24 mg/L at a pH of 6.0, and remained constant at a pH of 7.0. The cell growth of *Chlorella* sp. was inhibited at an initial pH below 3.0. [11-12] also reported that the growth of *Chlorella* was not affected by culture pH when the value was higher than pH 4.0, and the growth rate was inhibited drastically at pH 3.0. The culture was color changed. In this study, *Chlorella* sp. was able to grow at pH 4.0 to 7.0 shown in Figure 3. This characteristic is very important and suitable for stack gases using the cultivation of *Chlorella* for biomass production.

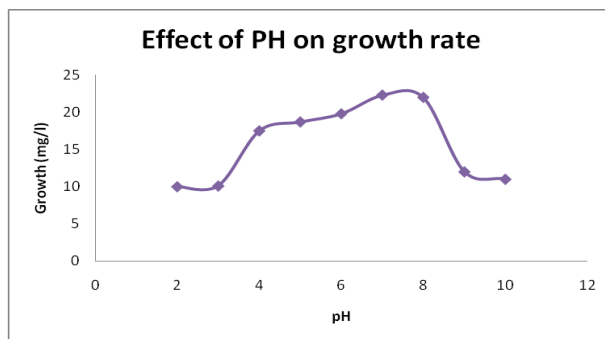


Figure 3. Effect of p H on growth rate

### 3.5. Effect of Cultivation Temperature

The growth rates of the *Chlorella* isolates showed in Figure 4 significant inhibition at incubation temperatures 25°C to 40°C. *Chlorella* sp. had a high growth rate at 35 and 40°C while *Chlorella* sp. had a high value at 30°C ( $A_{680\text{ nm}} = 1.51$ ). The optical density was only 0.05 to 0.20 when they were cultivated at 25, 30, 35 and 40°C.

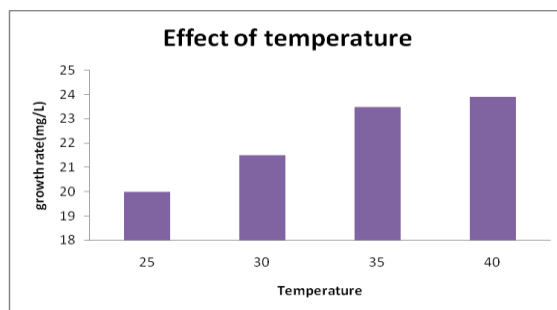


Figure 4. Effect of Temperature on Growth Rate

### 3.6. Effect of Light Intensity

Light condition, especially light intensity, is an important factor because the light energy drives Photosynthesis. Typical light intensity requirements of microalgae are relatively low in comparison to higher plants. For example, saturating light intensity of *Chlorella* sp. is approximately 200 mol/sec/m<sup>2</sup> [9]. Microalgae often exhibit photo inhibition under excess light conditions. Photo inhibition is often suspected as the major cause of reducing algal productivity. The use of a photo bioreactor with a solar collector device for the CO<sub>2</sub> mitigation has been explored. Maximum light intensity of 15.7 Wm<sup>-2</sup> could be attained using the system, and the culture of *Chlorella* sp. could be maintained. The efficiency of light collection and transmission to the algal cells was 8 -10% (. Recently, improvements are being made to the solar collecting devices.

The system can utilize infrared heat as well as visible light. In addition, artificial lighting is combined so that lighting is possible when there is no natural sunlight. The use of such novel solar collecting and distributing devices would improve CO<sub>2</sub> sequestration efficiency. Low light intensity reduced the growth rate and biomass production. Culture not grown on dark cycle.

The effect of light intensity on the photosynthetic oxygen evolution of *Chlorella* sp. increased with the increasing of light intensity. Therefore, the growth rate of *Chlorella* sp.

might be improved if the cultures were grown under high light intensity and a sufficient nutrient supply.

### 3.7. Effect of CO<sub>2</sub> Concentration on Cell Growth

To investigate the effect of CO<sub>2</sub> concentration on the growth of the isolated microalgae, were incubated at 30°C under aeration with different concentrations of CO<sub>2</sub> at 0.15 vvm for 6 to 10 days. It was shown that microalgae grew slowly in air aeration condition. When these isolates were incubated under aeration with 10 % - 40% CO<sub>2</sub>, isolates had an optical density at 680 nm higher than 2.0, tested isolates over 10 day's cultivation.

Flue gas contains 13 to 15% CO<sub>2</sub>, meaning these isolated microalgae were incubated at 30°C and 589 μmol m<sup>-2</sup> s<sup>-1</sup> under aeration with 10 % CO<sub>2</sub>. Cell dry weight increased on incubation. Isolate had a cell dry weight higher than 1.6 g l<sup>-1</sup>, and the maximal growth rate was more than 0.18 g l<sup>-1</sup> d<sup>-1</sup>.

The specific growth rate of this isolate was between 0.177 and 0.271 d<sup>-1</sup> during 2 to 8 days incubation. Isolates had high cell biomass and appropriate specific growth rate during 2 to 5 days cultivation.

The effect of CO<sub>2</sub> concentration on the cell growth of *Chlorella* sp. is shown in Figure 1. Aeration with addition of CO<sub>2</sub> stimulated cell growth, and strain had a maximal growth at 10% CO<sub>2</sub>, which decreased gradually with increasing CO<sub>2</sub> concentration. A long lag period was observed under aeration with 40% CO<sub>2</sub>, and the cell growth decreased significantly. Strain had a maximal linear growth rate under aeration with 40 % CO<sub>2</sub> (between 0.28 and 0.31 g l<sup>-1</sup> d<sup>-1</sup>). This rate decreased slightly under aeration with 20 to 30% CO<sub>2</sub> (from 0.21 to 0.27 g l<sup>-1</sup> d<sup>-1</sup>), fell moderately under aeration with 10% CO<sub>2</sub> (between 0.15 and 0.18 g l<sup>-1</sup> d<sup>-1</sup>), and plunged under aeration with 60% CO<sub>2</sub> (between 0.06 and 0.07 g l<sup>-1</sup> d<sup>-1</sup>) [9] indicated that CO<sub>2</sub>-tolerant microalgae, *Chlorella* sp., had growth rates between 0.15 and 0.18 g l<sup>-1</sup> d<sup>-1</sup> at 10 to 40% CO<sub>2</sub>, and they could not grow at 60% CO<sub>2</sub>. The growth rate of isolates of *Chlorella* sp. might be improved by the adaptation of carbon dioxide enrichment and by the adjustment of culture conditions.

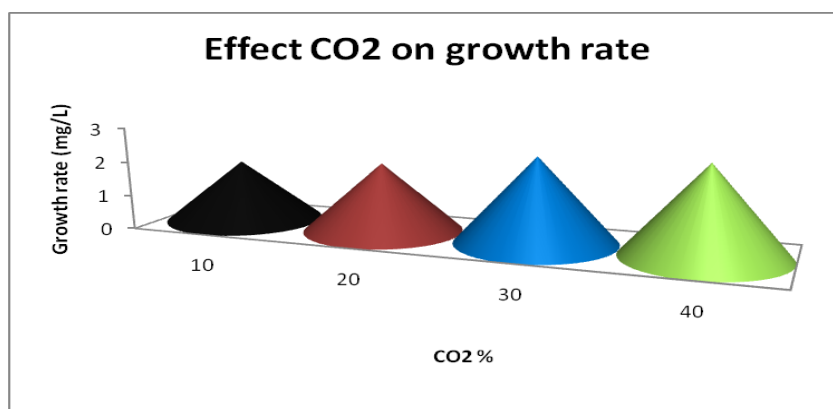
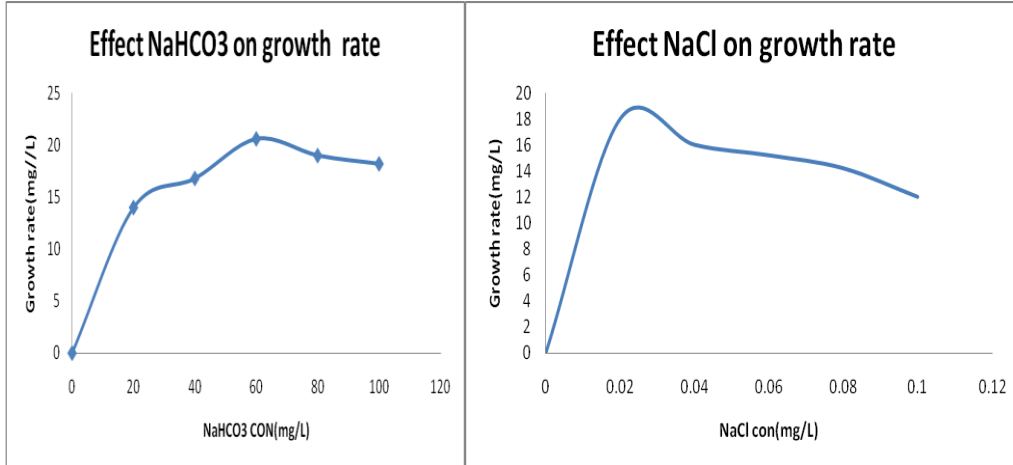


Figure 5. Effect of CO<sub>2</sub> on Growth Rate

### 3.8. Effect of Salt Concentration Growth Rate and Lipid Production

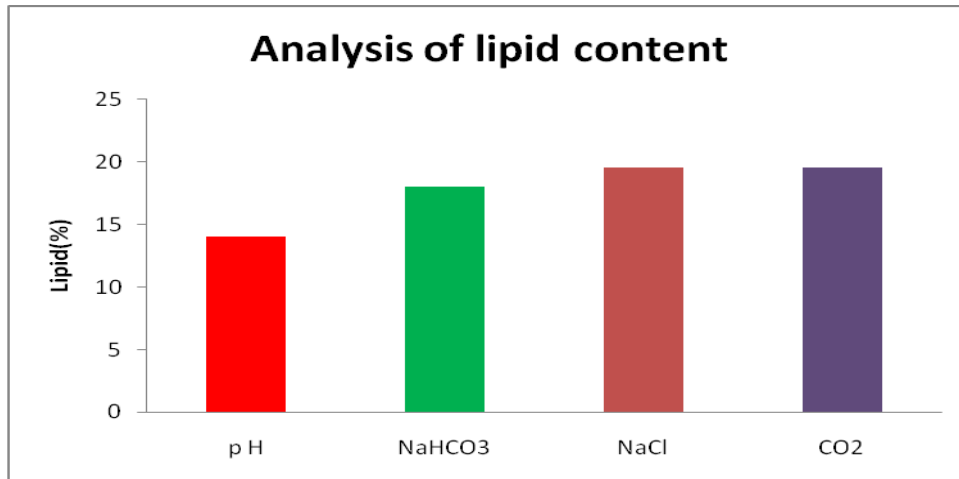
The aforementioned algae strain showed a good growth under different nutrition environments such as variable concentrations of bicarbonate, carbon dioxide gas and salinity. The growth behavior of the strains under different bicarbonate levels are shown in Figure 6,

the bicarbonate concentration at which maximum growth rate was obtained was at 60 mg/L (ppm). Jeong found that 15.3 mg/L bicarbonate salt is equivalent to 243 mg/L CO<sub>2</sub> gas in our strain showed its best growth at 952.9 mg/L CO<sub>2</sub> gas which is equivalent to 60 mg/L bicarbonate. The total lipid content in terms of % dry cell weight was 18.2%.



**Figure 6. Effect of Salt Concentration on Growth Rate**

The maximum total lipid was found to be 15% in terms of dry cell weight (Figure 6). It was noticed that a higher flow rate inhibited algal growth. Also a higher concentration of CO<sub>2</sub> in the media is lethal to algae strains because it results in decreasing pH as it leads to carbonic acid production. The alga can optimally grow in the pH range of 4-7. Highest lipid content was found in case of 0.02M salinity, 60 mg/L of bicarbonate salt concentration and 40% of CO<sub>2</sub>.



**Figure 7. Total Lipid Production in Different Condition**

#### 4. Conclusion

In conclusion, the local isolates *Chlorella* sp. grew well at high temperature, high cell density, high CO<sub>2</sub> concentration, and over a broad-range of pH values. They are suitable

strains for large-scale, dense cultivation with industrial discharge gases to fix CO<sub>2</sub> directly to reduce global warming and create a cell biomass for producing industrially valuable compounds.

The cultivation system for

- high photo efficiency,
- less space ,
- cheap cost,
- high productivity,
- easy management,
- high reliability,
- The cultivation system needs to be improved to be climate independence.
- Harvesting methods should be improved to consume less energy and higher efficiency.

## Acknowledgements

The authors wish to express the gratitude for the support and financial help given by Department of Science and Technology, New Delhi for their support under Net Work Programme of Bio-Algae Carbon Sequestration research under the title “Carbon Sequestration and cultivating algae for green bio-fuel Production”.

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