

Full Length Research Paper

# Studies on the growth behavior of *Chlorella*, *Haematococcus* and *Scenedesmus* sp. in culture media with different concentrations of sodium bicarbonate and carbon dioxide gas

Devgoswami, Ch.R.<sup>1\*</sup>, Kalita, M. C.<sup>1</sup>, Talukdar, J.<sup>1</sup>, Bora, R.<sup>2</sup> and Sharma, P.<sup>1</sup>

<sup>1</sup>Environmental Biotech Lab. Department of Biotechnology, Gauhati University, Guwahati Assam, India

<sup>2</sup>Institute of Science and Technology, Gauhati University. Guwahati, Assam, India.

Accepted 5 September, 2011

Growth studies were conducted on green algae *Chlorella*, *Scenedesmus* and *Haematococcus* strains in batch mode cultures. In this study, the effect of sodium bicarbonate salt ( $\text{NaHCO}_3$ ) and carbon dioxide ( $\text{CO}_2$ ) gas as carbon source on microalgal cultures were investigated. For this purpose, growth response of the aforementioned three strains under varying concentrations of  $\text{NaHCO}_3$  (15, 30, 45, 60 and 75 mg/L,) and  $\text{CO}_2$  gas (7929, 4758 and 4400 mg/L,) were investigated. The best growth response showed by *Chlorella* strain was observed at 75 mg/L (ppm) bicarbonate (% increase in biomass=82.6mg/L/day for 12 days) and 4758 mg/L  $\text{CO}_2$  gas concentration (189.1 mg/L/day for 7 days). While *Haematococcus* strain showed its best growth in 30 ppm bicarbonate (72.9 mg/L/day for 17 days) and 4758 mg/L  $\text{CO}_2$  gas (134.1 mg/L for 7 days), the *Scenedesmus* strain showed its best growth in 45 ppm bicarbonate (30.9 mg/L/day for 17 days) and 4758 mg/L  $\text{CO}_2$  gas (103.8 mg/L for 7 days). All the strains showed good growth when  $\text{CO}_2$  gas was supplied in terms of increase in cell number, biomass and lipid content compared to bicarbonate utilization as carbon source, except *Haematococcus* strain which fail to grow when high concentration of  $\text{CO}_2$  gas (7929 ppm) was supplied. Out of the three strains, it was *Chlorella* sp. which showed highest growth rate and lipid content when  $\text{CO}_2$  gas was supplied, (specific growth=0.704; 189.1% increase in biomass, g/L/day and 1.015 doubling/day, 31% lipid content in terms of dry cell weight).

**Key words:** Microalgae, bicarbonate, biomass, lipid.

## INTRODUCTION

Biological carbon IV oxide ( $\text{CO}_2$ ) mitigation has attracted much attention in terms of  $\text{CO}_2$  fixation through photosynthesis as it leads to production of biomass energy (Kondili and Kaldellis, 2007), (Ragauskas et al., 2006), (de Morais and Costa, 2007). However, the potential for increased  $\text{CO}_2$  capture in agriculture by plants has been estimated to contribute only 3-6% of fossil fuel emissions (Skjanes et al., 2007), largely due to

the slow growth rates of conventional terrestrial plants. On the other hand, microalgae a group of fast growing unicellular or simple multicellular micro organism has the ability to fix  $\text{CO}_2$  while capturing solar energy with an efficiency of 10 to 50 times greater than that of terrestrial plants and higher biomass production compared to energy crops (Wang et al., 2008).

The main environmental factors influencing microalgal growth and chemical composition are light, nutrients, temperature and pH (Rousch et al., 2003). Carbon source is an essential factor for microalgal growth (Wen and Chen, 2003). Generally, the carbon source for microalgae in culture condition is  $\text{CO}_2$  from atmosphere which is naturally present at approximately 300 ppm. Microalgae has higher growth rate and  $\text{CO}_2$  fixing ability

\*Corresponding author. E-mail: [rajivgoswami23@gmail.com](mailto:rajivgoswami23@gmail.com).  
Tel: 91-361-2268268(0) /09864108070. Fax: 91-361-2700311.

**Abbreviations:** CA, Carbonic anhydrase; DIC, dissolved inorganic carbon.

**Table 1.** CO<sub>2</sub> Tolerance of various species

Species	Known maximum CO <sub>2</sub> concentration (%)	Reference
<i>Cyanidium caldarium</i>	100	Seckbach et al., 1971
<i>Scenedesmus</i> sp.	80	Hanagta et al., 1992
<i>Chlorococcum littorale</i>	60	Kodama et al., 1993
<i>Synechococcus elongatus</i>	60	Miyairi, 1997
<i>Euglena gracilis</i>	45	Nakano et al., 1996
<i>Chlorella</i> sp.	40	Hanagta et al., 1992
<i>Eudorina</i> sp.	20	Hanagta et al., 1992
<i>Dunaliella tertiolecta</i>	15	Nagase et al., 1998
<i>Nannochloris</i> sp.	15	Yoshihara et al., 1996
<i>Chlamydomonas</i> sp.	15	Miura et al., 1993
<i>Tetraselmis</i> sp.	14	Matsumoto et al., 1995

Source, Mark E. Huntley (University of Hawaii) and Donald G. Redalje (University of Southern Mississippi).

and it could completely recycle CO<sub>2</sub> as again CO<sub>2</sub> is converted to chemical energy via photosynthesis, which can be converted to fuels using existing technologies.

Microalgae can fix CO<sub>2</sub> from different sources which can be categorized as (i) CO<sub>2</sub> from atmosphere, (ii) CO<sub>2</sub> from industrial flue gases, and (iii) fixed CO<sub>2</sub> in the form of soluble carbonates (NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub>). CO<sub>2</sub> could be directly fed into microalgae culture for biofixation but sometimes it is difficult to obtain a stable and consistent supply of CO<sub>2</sub> unless the location of microalgae cultivation system is very close to a factory or power plant. So one of the alternative way to prepare a large amount of inorganic carbon source for microalgal growth is to capture the CO<sub>2</sub> emitted from industries by alkali absorption and stored it in the liquid as HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup> ions (Wang et al., 2008). In this way, it would be easier and more convenient to supply carbon source for phototropic growth of microalgae. Also, since bicarbonate and carbonate are much more soluble than CO<sub>2</sub> the problem with the low solubility and low retention time of CO<sub>2</sub> in the medium could be avoided. However the metabolic efficiency and resulting microalgae composition of using CO<sub>2</sub> and bicarbonate /carbonate as carbon source could be different from species to species (de Morais and Costa, 2007). It was found that the solubility of CO<sub>2</sub> gas is 1.45 g/L at 25°C, 100 K Pa. When CO<sub>2</sub> dissolve in water, three inorganic carbon species are produced, namely CO<sub>2</sub> (aq), bicarbonate and carbonate ions. The equilibrium concentrations of the various carbonate species in aqueous solution are controlled by the pH of the solution. More specifically, at a pH below about 4.5, the carbonate species will consist entirely of carbonic acid (H<sub>2</sub>CO<sub>3</sub>). As the pH is increased to a value of about 8.5, the carbonate species will consist entirely of bicarbonate (HCO<sub>3</sub><sup>-</sup>) and as the pH is raised above 8.5, the predominant carbonate species will be carbonate (CO<sub>3</sub><sup>2-</sup>) (Huber et al., 1999).

A number of microalgal species have been shown to be able to utilize carbonate such as NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>

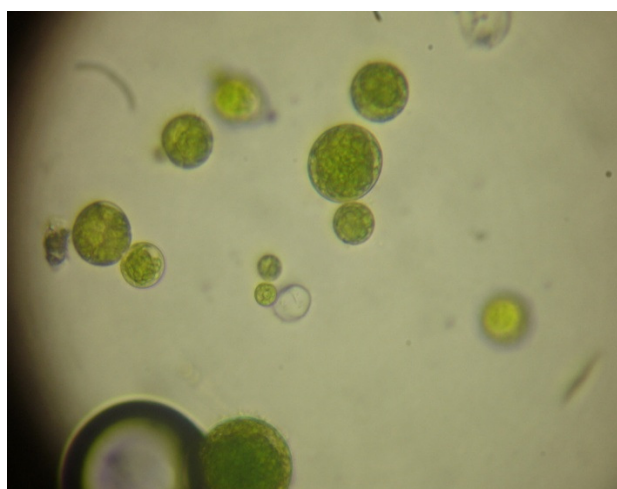
for cell growth (Huertas et al., 2000), (Ginzburg, 1993), (Merrette et al., 1996). Some microalgal species have high extracellular carbonic anhydrase (CA) activities (Huertas et al., 2000), which is responsible for the conversion of carbonate to free CO<sub>2</sub> to facilitate CO<sub>2</sub> assimilation. In addition, the direct uptake of bicarbonate by an active transport system has been found in several species (Colman and Rotatore, 1995). It was also observed previously that NaHCO<sub>3</sub> is a better carbon source for most of the microalgal strains than using Na<sub>2</sub>CO<sub>3</sub>. Adoption of carbonate utilizing strains for CO<sub>2</sub> fixation could be advantageous in many aspects: (i) CO<sub>2</sub> released in night time from industrial facilities could be converted to carbonate salts and stored for conversion in day time, (ii) as only a limited number of microalgal species thrive in media containing high concentration of carbonate salts, so species control become simple (that is, preventing contamination), and (iii) most of microalgal species have high pH optima (in the range of 9.0-11) further simplifying species control (Table 1).

Microalgae have CA on their cell surface and can utilize bicarbonate as well as CO<sub>2</sub>. Kinetic study revealed that most of the bicarbonate is utilized after this ion is converted to CO<sub>2</sub> via CA located on the cell surface. Therefore, the actual molecular species which crosses the plasma lemma is mostly free CO<sub>2</sub>. The apparent Km (CO<sub>2</sub>) values for photosynthesis in most microalgae grown in ordinary air (low CO<sub>2</sub> cells) are as low as in terrestrial C<sub>4</sub> plants, although the algal cells fix CO<sub>2</sub> via C<sub>3</sub> pathway. In contrast the apparent Km (CO<sub>2</sub>) values in cells grown on CO<sub>2</sub> enriched air (high CO<sub>2</sub> cells) are as high as those in the terrestrial C<sub>3</sub> plants. This indicates that efficiency of dissolved inorganic carbon (DIC) utilization for photosynthesis in low CO<sub>2</sub> cells is very high. The activity of CA in low CO<sub>2</sub> cells is higher than that in high CO<sub>2</sub> cells (Aizawa and Miyachi, 1986).

This study was aimed at determining the effect of elevated NaHCO<sub>3</sub> and CO<sub>2</sub> gas concentration under different pH conditions on green algae *Chlorella*,

**Table 2.** The composition of BG11 media

Major element	Composition
NaNO <sub>3</sub>	1.5 g
K <sub>2</sub> HPO <sub>4</sub>	0.04 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.075 g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
NaCO <sub>3</sub>	0.02 g
Trace metal mix A5	1.0 ml

**Photograph 1.** *Haematococcus* strain under 40X magnification.

*Haematococcus* and *Scenedesmus*. The response was monitored in terms of changes in biomass, cell count, cell densities and lipid content.

## MATERIALS AND METHODS

In our study, three microalgae strains namely *Chlorella*, *Scenedesmus* and *Haematococcus* were considered, all the aforementioned strains were isolated from north eastern region of India, Assam, out of which *Chlorella* is the fastest growing microalgae. The aforementioned strains were explored in terms of growth study in air, different concentration of NaHCO<sub>3</sub> salt, and different concentration of CO<sub>2</sub> gas.

CO<sub>2</sub> is one of the critical factors for photosynthesis in microalgae along with light water and nutrients. Attempts were made to blow CO<sub>2</sub> in the media but there is loss of CO<sub>2</sub> in the air because the solubility of CO<sub>2</sub> in media is very low (Wieler et al., 1940). For that CO<sub>2</sub> gas is supplied periodically to the cultures by maintaining the pH of the cultures (a particular CO<sub>2</sub> concentration has a particular pH value, so a standard curve was plotted using titration method; data not shown). Carbonate salts have higher solubility than CO<sub>2</sub>, example: Na<sub>2</sub>CO<sub>3</sub> has a solubility of 29.4/100 g of water at 25°C

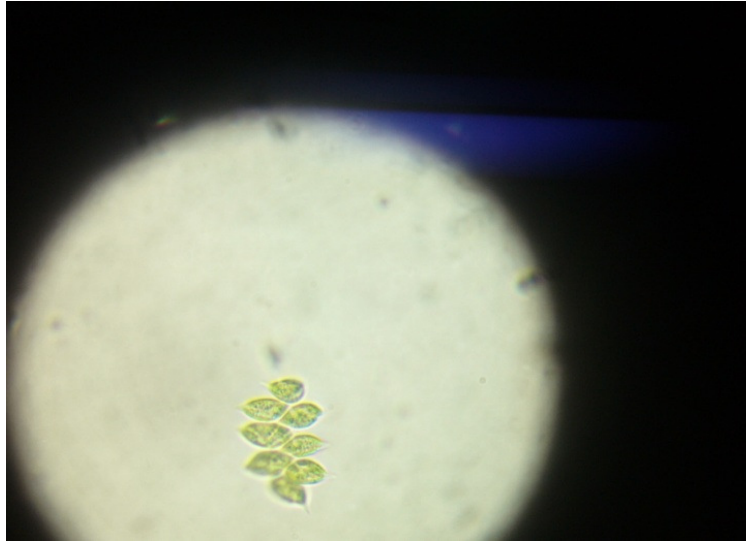
(Kobe and Sheety, 1948).

In our study, NaHCO<sub>3</sub> and CO<sub>2</sub> gas is used as a source of inorganic carbon for photosynthesis of three strains. Adding bicarbonate does not require power consumption for bubbling CO<sub>2</sub> gas in the aqueous phase and can minimize the carbon loss to the atmosphere by saturating the bicarbonate concentration in an appropriate pH range for algae culture as well as can be used for photosynthesis in algae (Aizawa and Miyachi, 1986).

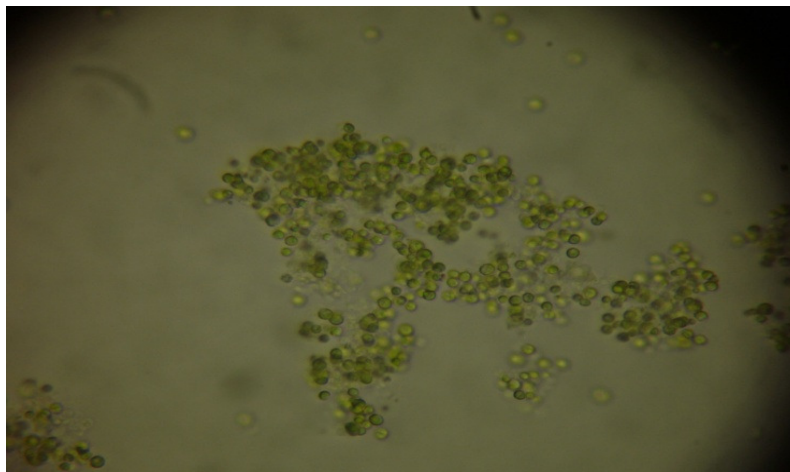
## Preparation of medium

BG11 culture media was selected and prepared for the growth of microalgae strains (without adding carbon source). The composition of BG11 media is shown in Table 2, aforementioned strains were isolated from north eastern region of Assam, out of which *Chlorella* is the fastest growing microalgae. In our study, three microalgae strains namely *Chlorella*, *Scenedesmus* and *Haematococcus* were considered. Control media are prepared having normal BG11 for each of the three strains. Again five + three culture flasks each having different concentration of bicarbonate salts and gaseous CO<sub>2</sub> were prepared separately for each of the three strains. For this purpose, NaHCO<sub>3</sub> salt in 15, 30, 45, 60 and 75 mg/L (1 mg/L=1 ppm) were freshly weighed and added to each of the flasks. For bicarbonate study, before inoculation the pH of the each flasks were adjusted to 7.5 with 0.1N HCl and 0.1 N NaOH with the help of L1 120 pH meter, Elico India. About 150 ml of media were distributed to each of the flasks including the blank and all are inoculated with 20 ml of inoculums (cell densities of inoculums; *Chlorella*=188.48X10<sup>4</sup>, *Haematococcus*=30.4X10<sup>4</sup>, *Scenedesmus*=27.2X10<sup>4</sup>) (Photographs 1, 2 and 3). The optical density (O.D) of each the flasks were measured at 680 nm of wavelength at regular interval of time (24 h) with the help of systronics spectrophotometer-104. The strains were checked for 17 days growth period in varying bicarbonate concentration except 12 days for *Chlorella* strain (12 days for *Chlorella* as it has a faster growth rate than the other two).

Gaseous CO<sub>2</sub> is also supplied to cultures in variable amounts (7929, 4758 and 4400 mg/L). Firstly, the pH of the cultures were elevated to 10.5, then CO<sub>2</sub> gas is supplied till the pH falls to 5.5, 7 and 8 so that mentioned amount of CO<sub>2</sub> concentration can be reached. It was calculated previously, when we lowered the pH from 10.5 to 5.5 by adding CO<sub>2</sub> gas, the amount of CO<sub>2</sub> gas dissolved is 7929 mg/L, hence a standard curve was plotted using pH and CO<sub>2</sub> concentration using titration method. As CO<sub>2</sub> gas has a poor solubility so there is addition of CO<sub>2</sub> gas periodically by checking the pH of the cultures. Percentage biomass increase/day, cell count and O.D. readings were taken daily. All the strains were checked for 7 days growth period in varying amount of CO<sub>2</sub> gas.



**Photograph 2.** Scenedesmus strain under 40X magnification.



**Photograph 3.** Chlorella strain under 40X magnification.

Source, All appendixes were taken at Environmental Biotech Lab. Department of Biotechnology, Gauhati University, Guwahati Assam.

### Light condition

Light intensity is a very important factor for microalgae cultures as requirements of microalgae are relatively low in comparison to higher plants. For our experiment, fluorescent lamps were used as repeated for growth of all the cultures. The temperature was adjusted to 25 °C for all the flasks.

### Analytical method

Direct microscopic cell count by Neubour haemocytometer was performed using microscope (Labomed). O.Ds of microalgae cultures were measured at regular interval of time (24 h) by absorbance at 680 nm with the help of spectrophotometer (Systronics). The spectrophotometer was blanked every time with each medium, respectively. At the end of the experiment, all the

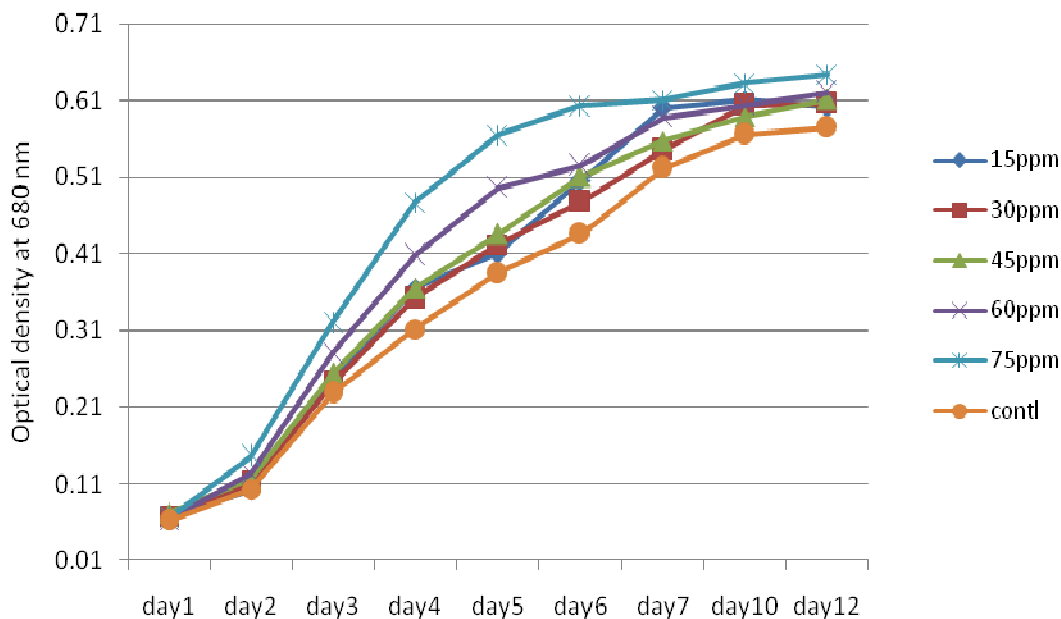
culture flasks were centrifuged and filtered and dry weights of pellets were measured (80 °C for 3 h) to study the increase in biomass, cell count and lipid content.

### Determination of specific growth rate

Specific growth rate is a measure of number of generations (the number of doublings) that occur per unit of time in an exponentially growing culture. The exponential (straight line) phase of growth was carefully determined and specific growth rate was obtained using following equation (Guillard and Ryther, 1962).

$$\mu = \ln(N_t/N_0) / T_t - T_0$$

Where,  $N_t$  is the no of cells at the end of log phase;  $N_0$  is the no of cells at the start of log phase;  $T_t$  is the final day of log phase and  $T_0$



**Figure 1.** Growth response of *Chlorella* sp. (at 680 nm) under different levels of sodium bicarbonate (NaHCO<sub>3</sub>) salt concentration.

is the starting day of log phase.

If T expressed in days from the growth rate ( $\mu$ ) can be converted to division or doublings per day (k) by dividing ( $\mu$ ) by the natural log of 2(0.6931).

$$K = \mu / 0.6931$$

The time required to achieve a doubling of the number of viable cells is termed as doubling time ( $T_d$ ) which is calculated by the following formula.

$$T_d = 0.6931 / \mu$$

#### Determination of total lipid

Microalgal lipid extraction was done by Bligh and Dyer method. For that microalgal biomass were collected by centrifuging the cells at 4000 Xg for 10 min. The cells were washed with distilled water, lyophilized and weighed. The known amount of biomass (100 mg) was then homogenized with chloroform: methanol 1:2 at 35°C. Extract was centrifuged for 7 min at 10000 Xg and supernatant was collected in a separating funnel. The residue was further homogenized with chloroform and again centrifuged (10000 Xg) to collect the supernatant. Now 0.9% sodium chloride (NaCl) solution was added to the filtrate and washed, lower layer of chloroform was separated and treated with anhydrous Na<sub>2</sub>SO<sub>4</sub> to remove the traces of water. The lipid content was determined gravimetrically and expressed as dry weight % after evaporating the chloroform (Bligh and Dyer, 1959).

## RESULTS AND DISCUSSION

This study confirms that all the three strains namely

*Chlorella*, *Scenedesmus*, and *Haematococcus* could grow well in modified media composition that is, in higher bicarbonate concentration and CO<sub>2</sub> gas. Maximum growth recorded in case of *Chlorella* strain is in 75 ppm of bicarbonate, which is equivalent to 1191 ppm of CO<sub>2</sub> (Figure 1; Tables 3 and 4). It was found earlier that 15.3 ppm of bicarbonate is equivalent to 243 ppm of CO<sub>2</sub> (Jeong et al., 2003), whereas in case of *Haematococcus* and *Scenedesmus* strains, showed their best growth in 30 and 45 ppm of bicarbonate concentration, respectively (equivalent to 476 and 714 ppm of CO<sub>2</sub>, respectively) (Tables 3 and 4). Although, it was observed that *Haematococcus* strain showed cell coagulation when high CO<sub>2</sub> gas (7929 ppm) was supplied (Figure 2). *Scenedesmus* strain also tends to coagulate a little bit at high CO<sub>2</sub> concentration (7929 ppm) but it continues to grow slowly (Figure 3). Out of the three strains, it was *Chlorella* which showed its best growth in high bicarbonate concentration (75 ppm bicarbonate ~ 1191 ppm CO<sub>2</sub>) and all the three strains showed maximum growth in 4758 ppm of CO<sub>2</sub> gas (Figures 4, 5 and 6).

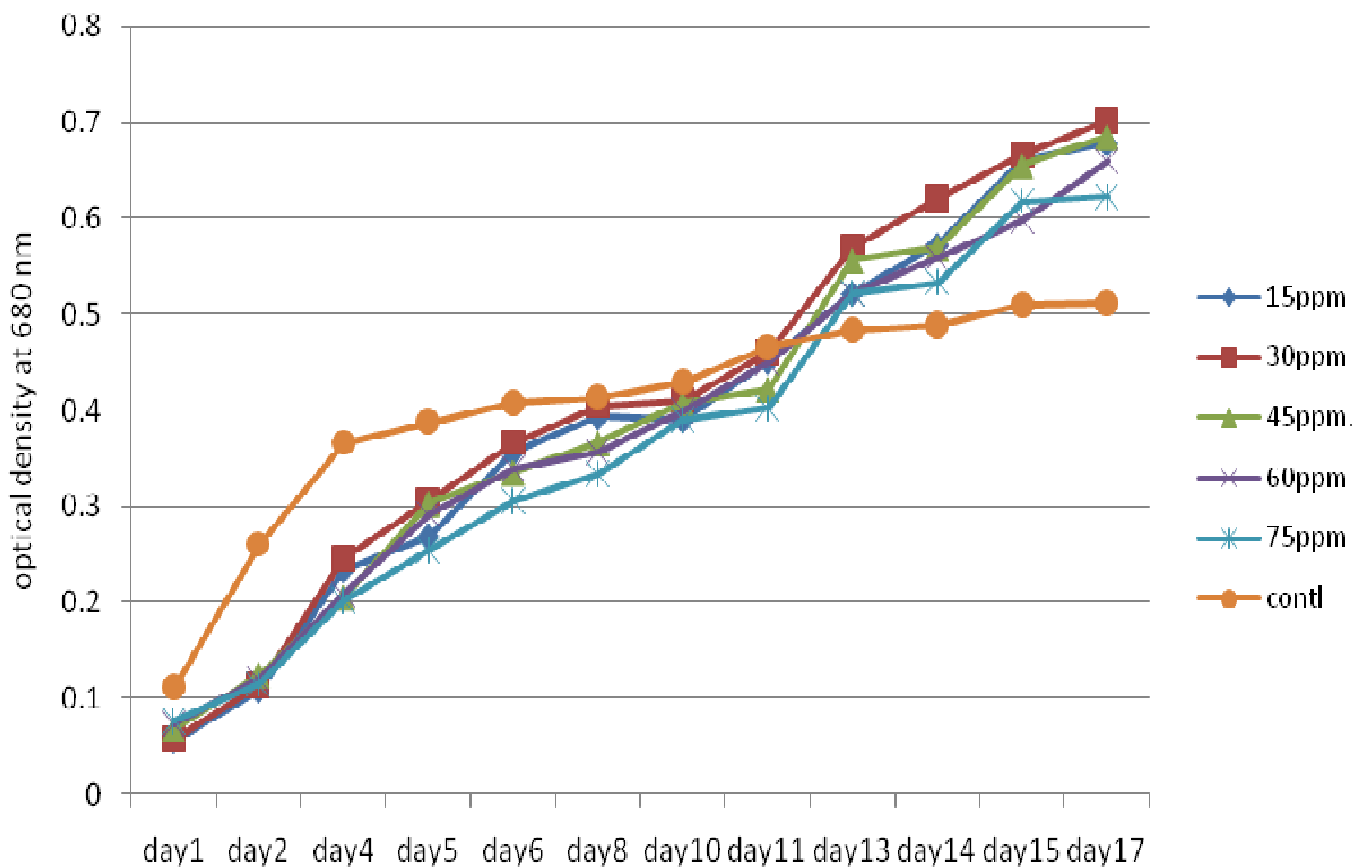
From the above study, it was found that the lipid content of all the strains increased when they were grown in media supplemented with bicarbonate salt and carbon dioxide gas. Addition of bicarbonate at 75 ppm in case of *Chlorella*, 30 ppm in case of *Haematococcus* and 45 ppm in case of *Scenedesmus* strain showed highest accumulation of lipid (18, 15 and 14% of dry cell weight), along with highest growth response (Figures 7 and 8). Also, addition of gaseous CO<sub>2</sub> at 4758 ppm to the cultures of *Chlorella*, *Haematococcus* and *Scenedesmus* strains

**Table 3.** Specific growth rate and doubling/day calculated for *Chlorella*, *Haematococcus* and *Scenedesmus* sp. under different levels of sodium bicarbonate salt (NaHCO<sub>3</sub>) concentration-

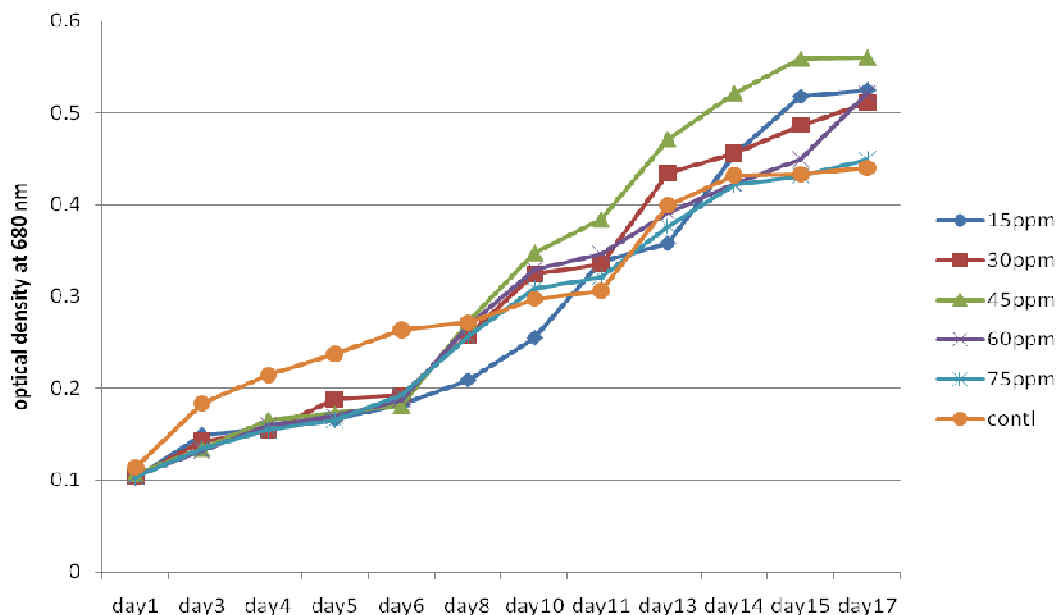
HCO <sub>3</sub> concentration	<i>Chlorella</i> sp.		<i>Haematococcus</i> sp.		<i>Scenedesmus</i> sp.	
	Specific growth	Doubling/day	Specific growth	Doubling/day	Specific growth	Doubling/day
15 ppm	0.394	0.568	0.381	0.511	0.09946	0.143
30 ppm	0.378	0.545	0.384	0.554	0.1138	0.164
45 ppm	0.362	0.522	0.247	0.357	0.1217	0.175
60 ppm	0.372	0.546	0.27	0.391	0.1025	0.147
75 ppm	0.396	0.571	0.283	0.409	0.09246	0.133
cntrl	0.309	0.445	0.17	0.246	0.0458	0.066

**Table 4.** Specific growth rate and doubling/day calculated for *Chlorella*, *Haematococcus* and *Scenedesmus* sp. under different gaseous CO<sub>2</sub> level.

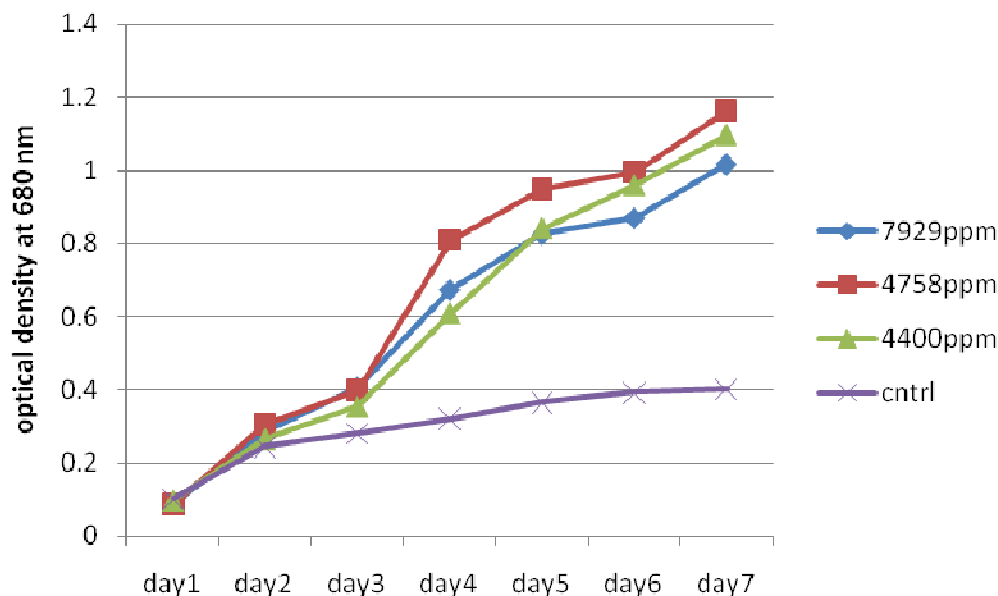
CO <sub>2</sub> concentration	<i>Chlorella</i> sp.		<i>Haematococcus</i> sp.		<i>Scenedesmus</i> sp.	
	Specific growth	Doubling/day	Specific growth	Doubling/day	Specific growth	Doubling/day
7929ppm	0.502	0.724	0.602	0.869	0.642	0.926
4758ppm	0.704	1.01	0.664	0.959	0.673	0.971
4400ppm	0.545	0.545	0.662	0.956	0.551	0.794
cntrl	0.133	0.133	0.483	0.693	0.337	0.486



**Figure 2.** Growth response of *Haematococcus* sp. (at 680 nm) under different levels of sodium bicarbonate (NaHCO<sub>3</sub>) salt concentration.



**Figure 3.** Growth response of *Scenedesmus* sp. (at 680 nm). under different levels of sodium bicarbonate ( $\text{NaHCO}_3$ ) salt concentration.



**Figure 4.** Growth response of the *Chlorella* sp. under different levels of  $\text{CO}_2$  gas for 7 days.

enhance total lipid content (22, 17 and 15% of dry cell weight, respectively) (Figures 9 and 10)

Microalgae have considerable biotechnological potential including producing valuable substances for the food additive, cosmetic, biofuel and pharmaceutical industries. A feasible microalgal  $\text{CO}_2$ -mitigation model can effectively fix  $\text{CO}_2$  and also convert biomass to different valuable byproducts (Ono and Cuello, 2006). Recent

studies showed that *Scenedesmus* sp. not only, is the promising  $\text{CO}_2$ -fixating microalga (de Moraes and Costa, 2007), (Ho et al., 2010), but also a good microalgal lipid producer. It was found that *Scenedesmus* sp. could convert approximately 15-25% atmospheric  $\text{CO}_2$  into biodiesel for transportation fuel (Ho et al., 2010), (Mandal and Mallick, 2009) and its biomass could accumulate lutein and other pigments for health food applications



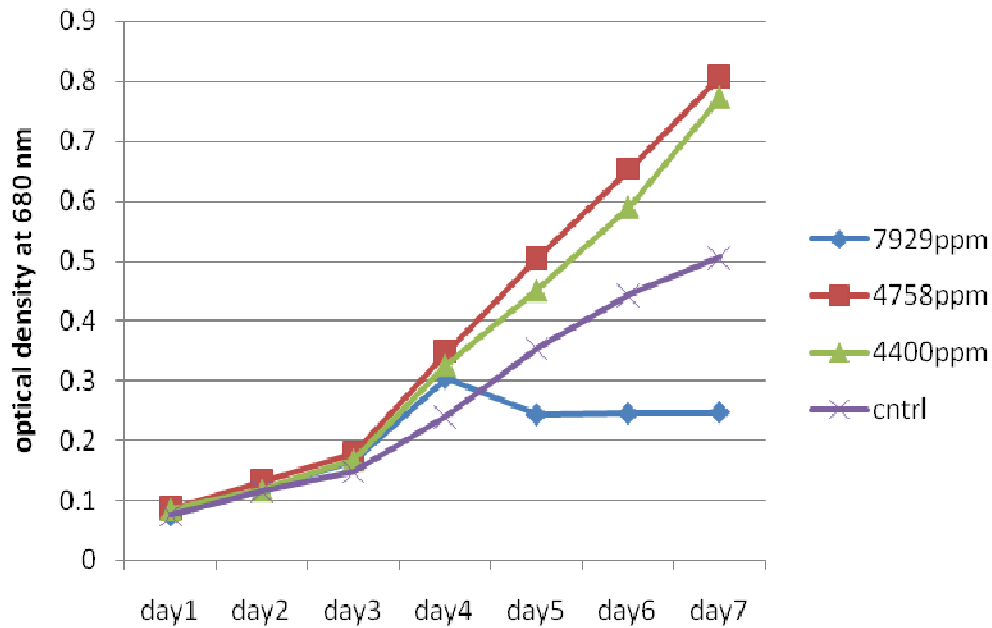


Figure 5. Growth response of the *Haematococcus* sp. under different level of CO<sub>2</sub> gas for 7 days.

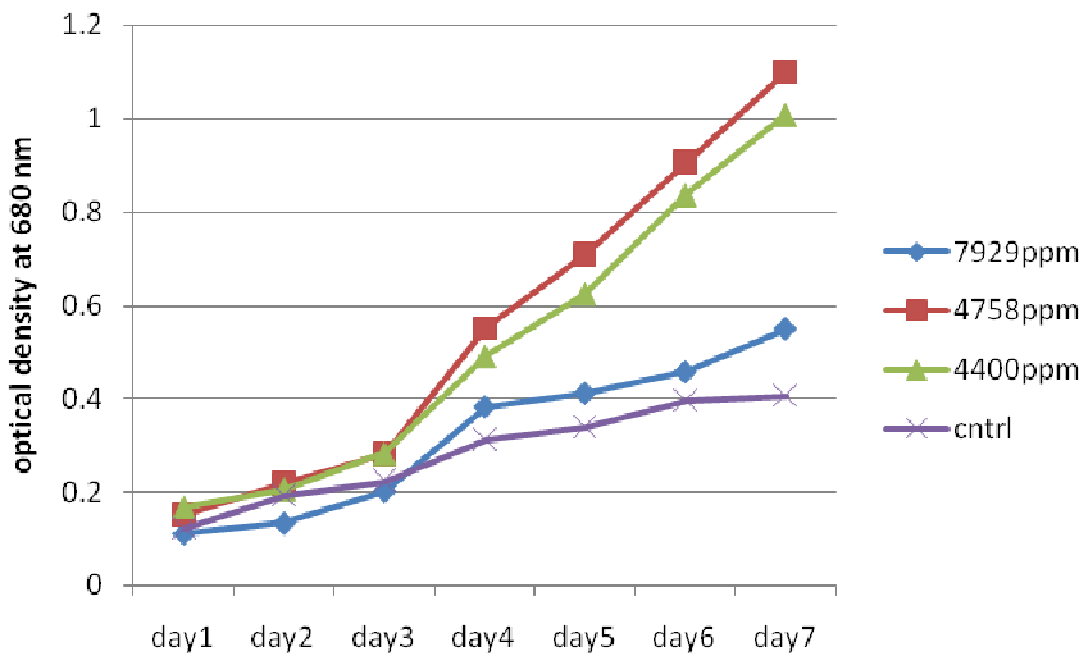
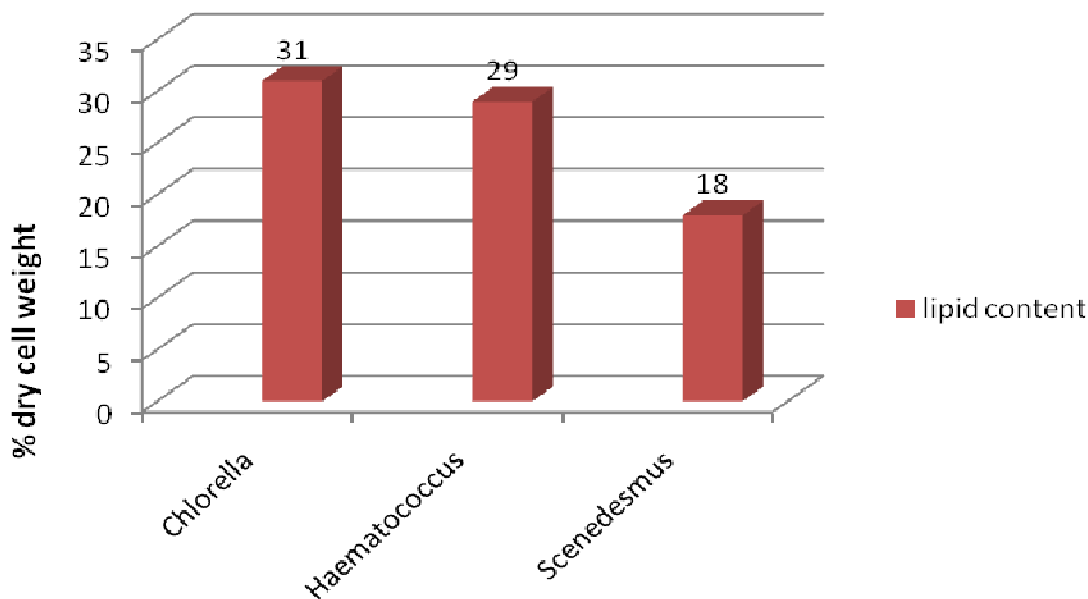


Figure 6. Growth response of the *Scenedesmus* sp. under different level of CO<sub>2</sub> gas for 7 days.

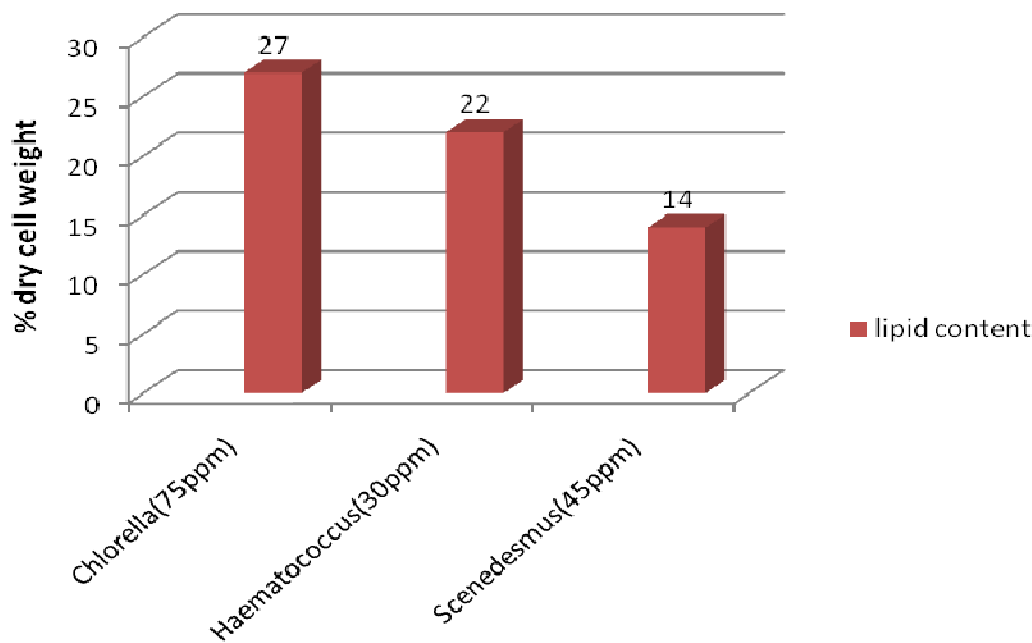
(Ceron et al., 2008). The merit of cultivation of microalgae, CO<sub>2</sub> mitigation and biofuel production which could be combined in an economically sustainable manner, the feasibility of this strategy could be further enhanced by fixing CO<sub>2</sub> from industrial exhaust gases such as flue gases. CO<sub>2</sub> concentration plays an important role in the increase of lipid productivity (Wang et al., 2008). It was

found that at higher CO<sub>2</sub> concentration, growth under normal condition gave higher lipid productivity (Widjaja et al., 2009). All the mentioned three strains namely *Chlorella*, *Scenedesmus*, and *Haematococcus* were tested thoroughly in terms of their growth in high bicarbonate salt and high CO<sub>2</sub> gas concentration. The study reveals that bicarbonate is an effective carbon





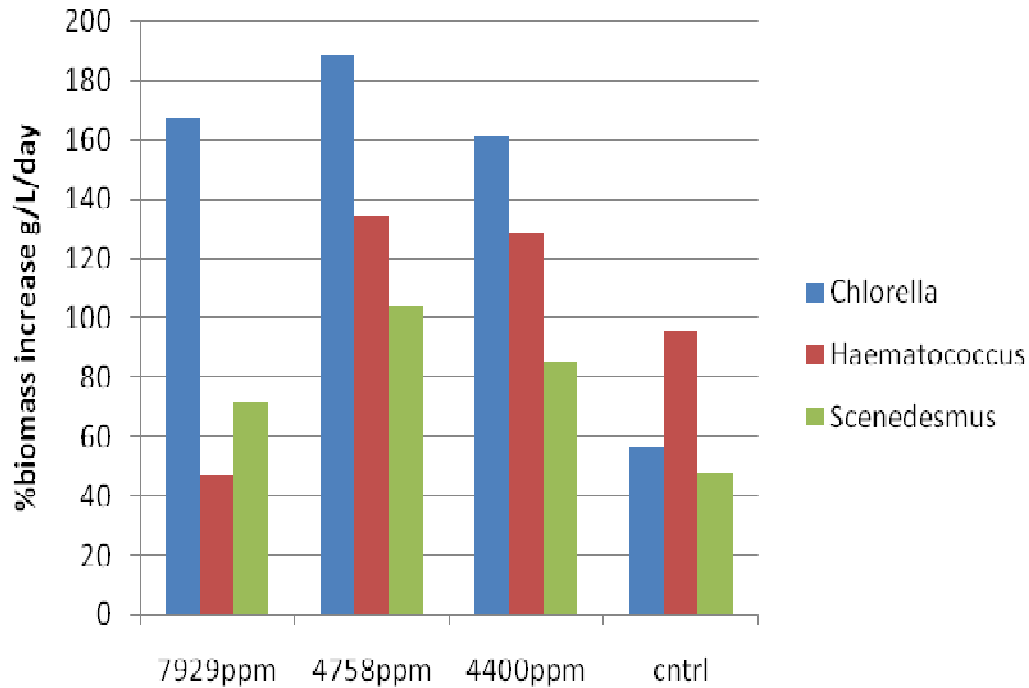
**Figure 7.** Total lipid content in terms of % dry cell weight in *Chlorella*, *Haematococcus*, and *Scenedesmus* sp. under 4758 ppm of CO<sub>2</sub> gas.



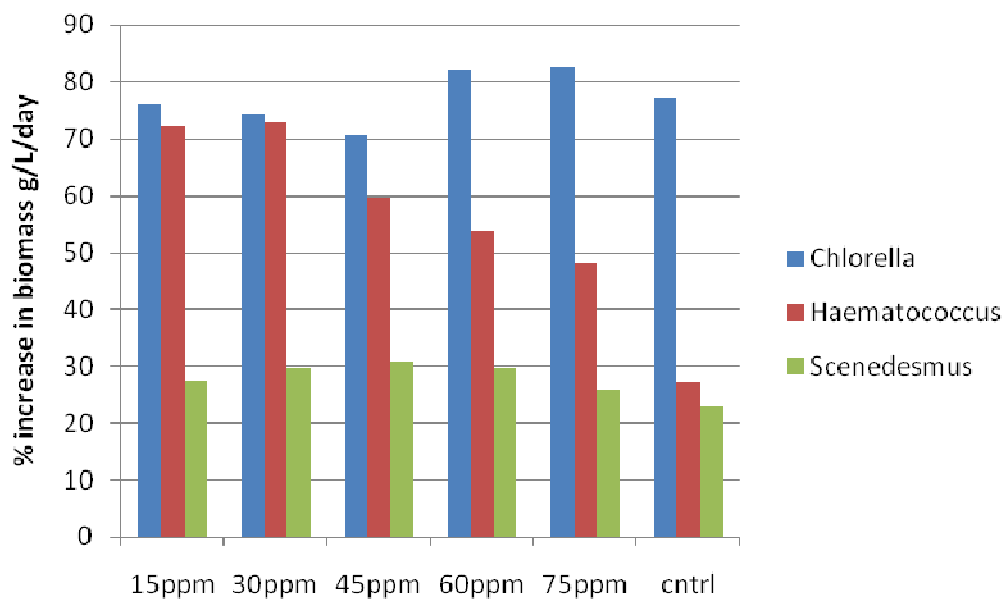
**Figure 8.** Total lipid content in terms of % dry cell weight in *Chlorella*, *Haematococcus*, *Scenedesmus* sp. at 75, 30, 45 ppm of sodium bicarbonate salt (NaHCO<sub>3</sub>), respectively,(where maximum growth rate observed).

source for microalgal growth, as optimum bicarbonate concentration is beneficial for highest biomass production. The findings indicates that CO<sub>2</sub> gas supply can strongly affect the microalgae growth, because CO<sub>2</sub> gas supplied culture flasks showed faster growth rate and

quick accumulation of biomass in all the tested three strains which ultimately leading to high lipid production. It was found that at 15.3 ppm of bicarbonate salt which is equivalent to 243 ppm of gaseous CO<sub>2</sub>, *C. vulgaris* strain exhibited fastest growth rate when tested (Jeong et al.,



**Figure 9.** % Increase in biomass in case of *Chlorella*, *Haematococcus* and *Scenedesmus* sp. when gaseous CO<sub>2</sub> supplied.



**Figure 10.** % Increase in biomass in case of *Chlorella*, *Haematococcus* and *Scenedesmus* strains in different sodium bicarbonate salt (NaHCO<sub>3</sub>) concentration.

2003). In our study, the *Chlorella* sp. performed well in terms of its bicarbonate salt and CO<sub>2</sub> (gas) utilization; it showed its maximum growth at 75 ppm bicarbonate (~1191 ppm CO<sub>2</sub>). So, the study proves a great deal of potential for chlorella strain to use in huge outdoor culture

systems for mitigating flue gases and Industrial waste gases. *Chlorella* sp. is also a potential candidate for the production of biomass which are used in aquaculture for feeding, nutraceutical food additives and animal feed as it is rich in vitamins. So to develop an effective CO<sub>2</sub>

mitigation technology, it is necessary to select an efficient and fast growing microalgae strain which has a good CO<sub>2</sub> fixing efficiency and promising valuable components.

## REFERENCES

- Aizawa K, Miyachi S (1986). Carbonic anhydrase and CO<sub>2</sub> concentrating mechanisms in microalgae and cyanobacteria. *FEMS Microbiol. Rev.* 39: 218-233.
- Bligh EG, Dyer WJ (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37: 911-917.
- Ceron MC, Campos I, Sanchez JF, Acien FG, Molina E, Fernandez-Sevilla JM (2008). Recovery of lutein from microalgae biomass: development of a process for *Scenedesmus almeriensis* biomass. *J. Agric. Food. Chem.* 56: 11761-11766.
- Chelf P, Brown LM, Wyman CE (1993). Aquatic biomass resources and carbon dioxide trapping. *Biomass Bioeng.* 4: 175-183.
- Colman B, Rotatore C (1995). Photosynthetic inorganic carbon uptake and accumulation in two marine diatoms. *Plant Cell Environ.* 18: 919-924.
- de Morais MG, Costa JAV (2007). Biofixation of carbon dioxide by *Spirulina sp.* And *Scenedesmus obliquus* cultivated in a three stage serial tubular photobioreactor. *J. Biotechnol.* 129: 439-445.
- de Morais MG, Costa JAV (2007). Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. *Energy Con. Manage.* 48: 2169-2173.
- Emma Huertas I, Colman B, Espie GS, Lubian LM (2000). Active transport of CO<sub>2</sub> by three species of marine microalgae. *J. Phycol.* 36: 314-320.
- Ginzburg BZ (1993). Liquid fuel (oil) from halophilic algae: a renewable source of non-polluting energy. *Renew. Energy.* 3: 249-252.
- Guillard RRL, Ryther JH (1962). Studies on marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Micro.* 8: 229-239.
- Hanagata N, Takeuchi T, Fukujyu Y, Barnes DJ, Karube I (1992). Tolerance of microalgae to high CO<sub>2</sub> and high temperature. *Phytochemistry.* 31: 3345-3348.
- Ho SH, Chen WM, Chang JS (2010). *Scenedesmus obliquus* CNW-N as a potential candidate for CO<sub>2</sub> mitigation and biodiesel production. *Bioresour. Technol.* 101: 8725-8730.
- Huber, Calvin O, Olson, George P (1999). Method and apparatus for measuring the content of dissolved carbon dioxide in an aqueous medium. United States Patent 5904833.
- Jeong ML, Gillis JM, Hwang JY (2003). Carbon dioxide mitigation by microalgal photosynthesis. *Bull. Kor. Chem. Soc.* 24: 1763-1766.
- Kobe KA, Sheehy TM (1948). Thermochemistry of sodium carbonate and its solutions. *Ind. Eng. Chem.* 40: 99-102.
- Kodama M, Ikemoto H, Miyachi S (1993). A new species of highly CO<sub>2</sub>-tolerant fast-growing marine microalga suitable for high-density culture. *J. Mar. Biotechnol.* 1: 21-25.
- Kondili EM, Kaldelis JK (2007). Biofuel implementation in east Europe: current status and future prospects. *Renew. Sustain. Energy Rev.* 11: 2137-2151.
- Mandal S, Mallick N (2009). Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. *Appl. Microbiol. Biotechnol.* 84: 281-91.
- Matsumoto H, Shioji N, Hamasaki A, Ikuta Y, Fukuda Y, Sato M, Endo N, Tsukamoto T (1995). Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. *Appl. Biochem. Biotechnol.* 51: 681-692.
- Merrett MJ, Nimer NA, Dong LF (1996). The utilization of bicarbonate ions by the marine microalga *Nannochloropsis oculata* (Droop) Hibberd. *Plant Cell Env.* 19: 478-484.
- Miura Y, Yamada W, Hirata K, Miyamoto K, Kiyohara M (1993). Stimulation of hydrogen production in algal cells grown under high CO<sub>2</sub> concentration and low temperature. *Appl. Biochem. Biotechnol.* 39: 753-761
- Miyairi S (1995). CO<sub>2</sub> assimilation in a thermophilic cyanobacterium. *Energy Conversion Manage.* 36: 763-766.
- Nagase H, Eguchi K, Yoshihara K, Hirata K, Miyamoto K (1998). Improvement of microalgal NOx removal in bubble column and airlift reactors. *J. Ferment. Bioeng.* 86: 421-423.
- Nakano Y, Miyatake K, Okuno H, Hamazaki K, Takenaka S, Honami N, Kiyota M, Aiga I, Kondo J (1996). Growth of photosynthetic algae *Euglena* in high CO<sub>2</sub> conditions and its photosynthetic characteristics. *Acta Hort.* 440: 49-54.
- Rousch JM, Bingham SE, Sommaerfeld MR (2003). Change in fatty acid profiles of thermo-intolerant and thermo tolerant marine diatoms during temperature stress. *J. Exp. Mar. Biol. Ecol.* 295: 145-156.
- Ragaukas AJ, Williams CK, Davison BH, Britovsek G, Cairnsey J, Eckert CA, Frederick WJ Jr, Hallett JP, Leak DJ, Liotta CL (2006). The path forward for biofuels and biomaterials. *311(5760):* 484-489.
- Seckbach J, Gross H, Nathan MB (1971). Growth and photosynthesis of *Cyanidium caldarium* cultured under pure CO<sub>2</sub>. *Isr. J. Bot.* 20: 84-90.
- Skjanes K, Lindblad P, Muller J (2007). BioCO<sub>2</sub>-a multidisciplinary, biological approach using solar energy to capture CO<sub>2</sub> while producing H<sub>2</sub> and high value products. *Biomol. Eng.* 24: 405-413.
- Wang B, Li Y, Wu N, Lan CQ (2008). CO<sub>2</sub> bio-mitigation using microalgae. *Appl. Microbiol. Biotechnol.* 79: 707-718.
- Wen ZY, Chen F (2003). Heterotrophic production of eicosapentaenoic acid by microalgae. *Biotechnol. Adv.* 21: 273-294.
- Widjaja A, Chien CC, Ju YH (2009). Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. *J. Tai. Inst. Chem. Eng.* 40:13-20.
- Yoshihara K, Nagase H, Eguchi K, Hirata K, Miyamoto K (1996). Biological elimination of nitric oxide and carbon dioxide from flue gas by marine microalga NOA-113 cultivation in a long tubular photobioreactor. *J. Fer. Bioeng.* 82: 351-354.