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Effect of CO_2 Concentration, Temperature and Light on Macro Biomolecules Accumulation in *Chlorella protothecoides*

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A B S T R A C T

The content of chlorophyll, carbohydrate, proteins and lipids in *Chlorella protothecoides* was observed in treated as well as untreated cells of *C. protothecoides* using different concentration of carbon dioxide with time period *i.e.* 30min, 12hr and 30min and 12hr at culture room conditions. The content of chlorophyll, carbohydrate, protein and lipids varied with the temperature and time period.

Introduction

The environment is going to rapid change due to human activities like industrialization, deforestation *etc*. and undergoes global warming due to increasing CO_2 concentration in atmosphere.

The temperature on our planet increased due to increase in green house gases *i.e.* CO_2, Methane, nitrous oxide *etc*.

To remove effect of carbon and storage of carbon from atmosphere the carbon sequestration through bio-logical methods play most important role in green growth *i.e.* algae which fix carbon present in water and through photosynthesis. *Chlorella species* play an important role as it contains chlorophyll pigment.

In fresh cultures protein content is high but as the culture grows older fat and carbohydrates content increases and become a rich source of food for mankind.

In *Chlorella*, carbohydrates source *i.e.* starch and sucrose is present and multiply rapidly in the presence of CO_2, sunlight and nutrients. It can be used as food supplement and in spacecrafts. *Chlorella* is also used as drug *i.e.* antibiotic chlorellin against bacterial disease. It can also be used in sewage disposal plant to fix CO_2.

Materials and Methods

Isolation of *Chlorella* sp.

The algal field sampling was done. Further screening of predators, debris removal, filtration, Centrifugation, pellet suspended in sterilized media, agar streaks, micropipette isolation and serial dilution were performed to get the single axenic culture of the algae.

Preparation of Bold Basal (BBM) Medium

About 10 ml (Each Macronutrients) +1ml (EDTA sol., Iron sol., Boron sol. and macronutrients) were taken and total volume was made upto one litre by using sterilized distilled water. The pH of medium was adjusted to 6.5 for the ideal development of culture. Autoclaved was done at 121.5 ^{0}C for 15 min at 15lbs.

Streaking cells across agar plates

Specimen was spread with the loop over the BBM medium containing 1.4% agar (Heaney and Jaworski, 1977) and process was repeated to get the pure culture (Richmond, 2004).

Raising of mass culture of algae

The pure cultures were exchanged to fluid and solid medium to grow at small scale in laboratory conditions. Culture attained slack stage and became light green which showed the exponential development stage. After 24 days dull green shading was observed.

Biochemical analysis

Chlorophyll Content was detected using the standard available method (Mackinney, 1941; Jaffery and Humphrey, 1975); Carbohydrate content by using Dubois *et al.*, (1951 &1956); Protein content by Lowry *et al.,* (1951); Lipid extraction and determination of total lipid content was detected by using Takagi *et al.*, (2006) and Bligh and Dyer (1959).

Results and Discussion

Total chlorophyll content

The content of chlorophyll in *Chlorella protothecoides* varied with temperature, CO_2, treatment time, hatching temperature, photoperiod and light force. The chlorophyll content of *C. protothecoides* with 30 min CO_2treatment was most elevated in 12% CO_2 at 30 ^{0}C (7.84±.35mgL^{-1}) (Table 1 and Fig. 1). On the other side, During 12hr CO_2 treatment, the chlorophyll content was observed as 18.2±0.08mgL^{-1} (Table 2 and Fig. 2).

CO_2 treatment for 30min and 12hr at culture room condition

In *C. protothecoides,* the aggregate chlorophyll substance was most elevated in case of 550 ppm (9.15±0.15mgL^{-1}) and 4% (13.97±0.09mgL^{-1}) in 30 min and 12h CO_2 treatment, respectively (Fig. 3).

Total Carbohydrate Content

Sugar content in CO_2 treated cell was higher than the untreated cell. Further it was observed that sugar content also depended on light intensity and CO_2 concentration.

CO_2 treatment up to 30 min

The sugar content of *C. protothecoides* expanded with increasing temperature with CO_2 concentrations. The starch content in control was 4.82±0.74mg/L but it increased with CO_2 concentration 7.59±0.35mg/L at 4% CO_2 and 6.48±0.54mg/L, respectively.

In case of 4% and 12% CO_2 at 30^{0}C the starch content was observed as maximum (Table 3 and Fig. 4).

Effect of CO_2 treatment upto 12 hr

The starch content increased in *Chorella protothecioides* when the cell treated with 15% CO_2 at 25^0C *i.e.* 14.746 ± 0.53mg/L as compared to untreated cell (4.087±0.6mg/L).

In case of 4% CO_2 the starch content increased with temperature from 6.762±0.2mg/L at 25^0C to 12.496±0.27mg/L at 35^0C (Fig. 5).

CO_2 treatment for 30 min 12 hour in culture room conditions

The aggregate sugar substance of *C.protothecioides* were most noteworthy in 1% CO_2 (5.756±0.89mg/L), 12% CO_2 (6.19±0.89mg/L) and 15% (5.98±0.72mg/L) CO_2 for 30min in comparison to control (2.109±0.5 mg/L) (Table 4).

Total protein content in the cell

The total protein content varied with the temperature, CO_2 treatment time, photoperiod and light intensity.

The content was reported lower after treating the cells with CO_2 but also depended on the temperature and concentration of CO_2.

Treatment up to 30 min

Maximum protein was observed in case of 15% CO_2 at 30°C (15.56±0.32mg/L) in comparison to control condition (15±1.5mg/L) and lower at 25oC and 30^0C at 1%, 4% and 12% (Table 5 and Fig. 6).

At 25 ^{0}C the protein content was reported higher at 15% CO_2 (16.1±0.2mg/L as compared to controlled condition (14.5±1.8mg/L).

The cells treated with 12% CO_2 showed lower concentration of protein at 30 ^{0}C and at 35 ^{0}C in case of 1%, 4%, 12% and 15% CO_2 as compared to untreated cell (Fig. 7).

CO_2 treatment for 30min and 12hr in culture room condition

When *C. protothecodes* treated with different CO_2 conc. the protein content was higher in 1% CO_2 (15.1±0.26mg/L) and in 12% CO_2 (15.12±0.56mg/L) at 25ºC in 30 min. expanded in protein content than the control (13.2±0.2mg/L) when treated for 12 hr. in other concentration of CO_2 concentration protein content decreases in 30 min and 12hr treatment (Table 6).

Total lipid content in the cells of *C.protothecoides*

Presence of lipid in microalgae was tested by Nile red stain which is lipophilic in nature. In *C. protothecoides* the lipid content varied with temperature and CO_2 concentration.

During 30min treatment time, *Chlorella protothecoides* showed more lipid content in all the CO_2concentrations *i.e.* 550ppm, 1%, 4%, 12% and 15% at all temperature as compared with control room condition.

At 25°C, indicate lipid substance were 41.7±1.7%, 38.25±1.33%, 37.23±1.67%, 41.36±0.9% and 40.24±1.15% in 550ppm, 1%, 4%, 12% and 15% CO_2 treatment when diverged from control (33.28±1.3%) while at 30°C, mean lipid substance were most prominent in 550ppm (34.08±0.9%), 4% (39.87±1.1%) and 12% (43.49±1.74%) trailed by 15% (37.83±0.18%) and 1% (38.26±0.93%) CO_2 treatment appeared differently in relation to control (34.08±0.37%). Strikingly at 35°C the lipid substance was higher in 1% (42.22±1.51%), 4% (43.26±1.1%), 12% (44.38±1.4%) and 15% (46.33±1.34%) with 38.42±0.7% lipid in control (Table 7).

Table.1 Comparison of total chlorophyll content accumulation up to 30 min in *C. protothecoides*

S.No.	Temp.°C	Control	550ppm	1% CO_2	4% CO_2	12% CO_2	15% CO_2
1	25 °C	4.087	4.765	3.998	6.762	7.242	6.813
2	30 °C	5.513	3.948	6.242	5.934	7.848	3.838
3	35 °C	5.231	4.388	6.192	4.291	7.675	5.485

Table.2 Total chlorophyll content accumulation up to 12hr in *C. protothecoides*

S.NO.	Temp.°C	Control	550ppm	1% CO_2	4% CO_2	12% CO_2	15% CO_2
1	25 °C	13.929	17.637	13.288	17.271	18.023	16.019
2	30 °C	14.011	15.382	12.299	17.593	17.627	16.291
3	35 °C	14.118	12.12	13.928	18.11	18.229	15.273

Table.3 Carbohydrate content accumulation up to 30 min in *C. protothecoides*

S.NO.	Temp.°C	Control	550ppm	1% CO_2	4% CO_2	12% CO_2	15% CO_2
1	25 °C	4.82	5.66	5.24	7.59	5.38	6.485
2	30 °C	13.7	15.8	14.751	17.6	17.5	15.7
3	35 °C	14.2	12.4	13.301	12.851	13.756	13.978

Table.4 Carbohydrate content accumulation up to 30 min &12 hr in *C. protothecoides*

S.No.	Temp.°c	Control	550ppm	1% CO_2	4% CO_2	12% CO_2	15% CO_2
1	25 °C	2.193	9.193	5.756	9.092	6.192	5.988
2	30 °C	2.109	11.192	10.234	14.832	13.874	12.038

Table.5 Protein content accumulation up to 30 min

S.NO.	Temp.°C	Control	550ppm	1% CO_2	4% CO_2	12% CO_2	15% CO_2
1	25 °C	13.49	14.42	13.34	8.32	9.9	10.55
2	30 °C	15	5.95	10.95	11.76	14.6	15.36
3	35 °C	14.24	10.18	12.145	10.04	12.25	12.955

Table.6 Pattern of protein content accumulation up to 30min &12 hr in*C. protothecoides*

S.NO.	Temp.°C	Control	550ppm	1% CO_2	4% CO_2	12% CO_2	15% CO_2
1	25 °C	13.2	14.15	15.1	11.172	15.12	14.41
2	30 °C	13.675	12.41	13.36	11.015	9.98	8.83

Table.7 Total lipid content accumulation up to 30 min in *C. protothecoides*

S.NO.	Temp. °C	Control	550ppm	1% CO_2	4% CO_2	12% CO_2	15% CO_2
1.	25^0C	33.28	41.7	38.25	37.23	41.36	40.24
2.	30^0C	34.08	44.26	38.26	39.87	43.39	37.83
3.	35^0C	38.42	39.87	42.22	43.26	44.38	46.36

Fig.1 Pattern of chlorophyll content accumulation up to 30 min in *C. protothecoides*

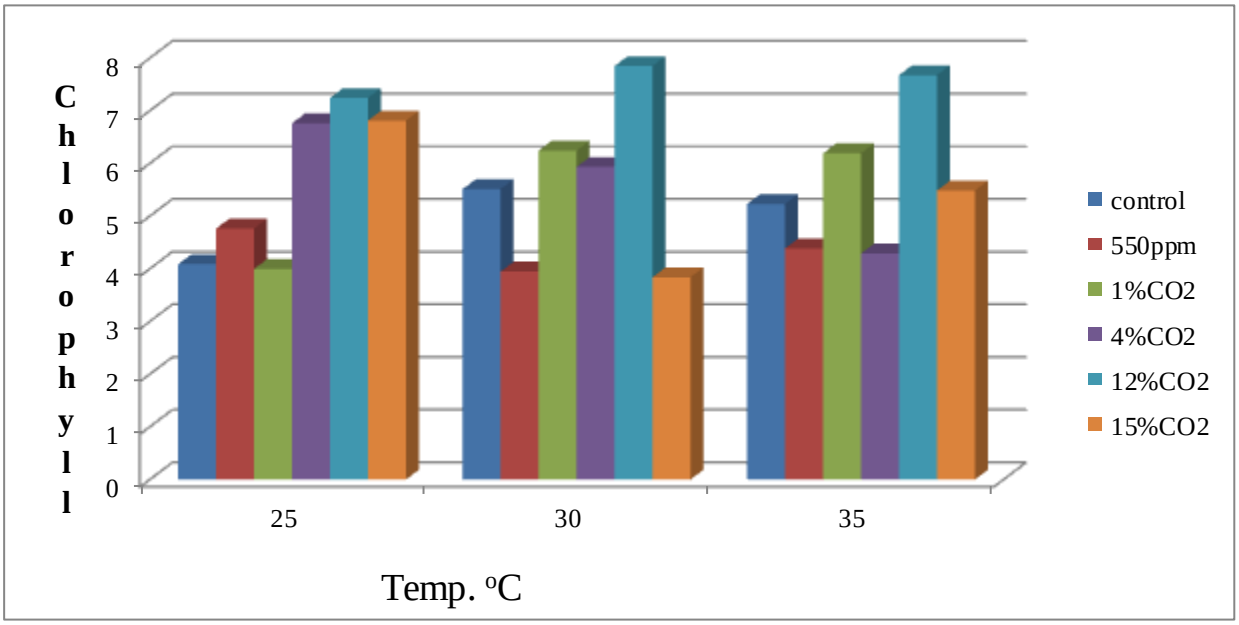


Fig.2 Total chlorophyll content accumulation up to 30 min and 12 hr

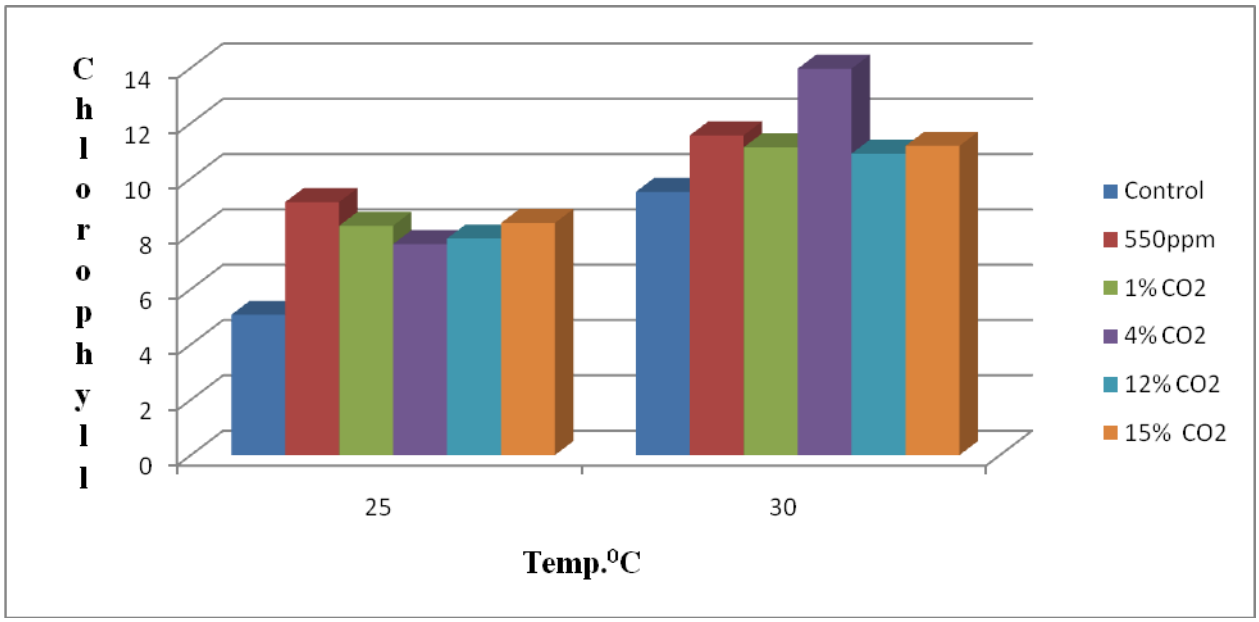


Fig.3 Comparison of total carbohydrate content accumulation upto 30 min

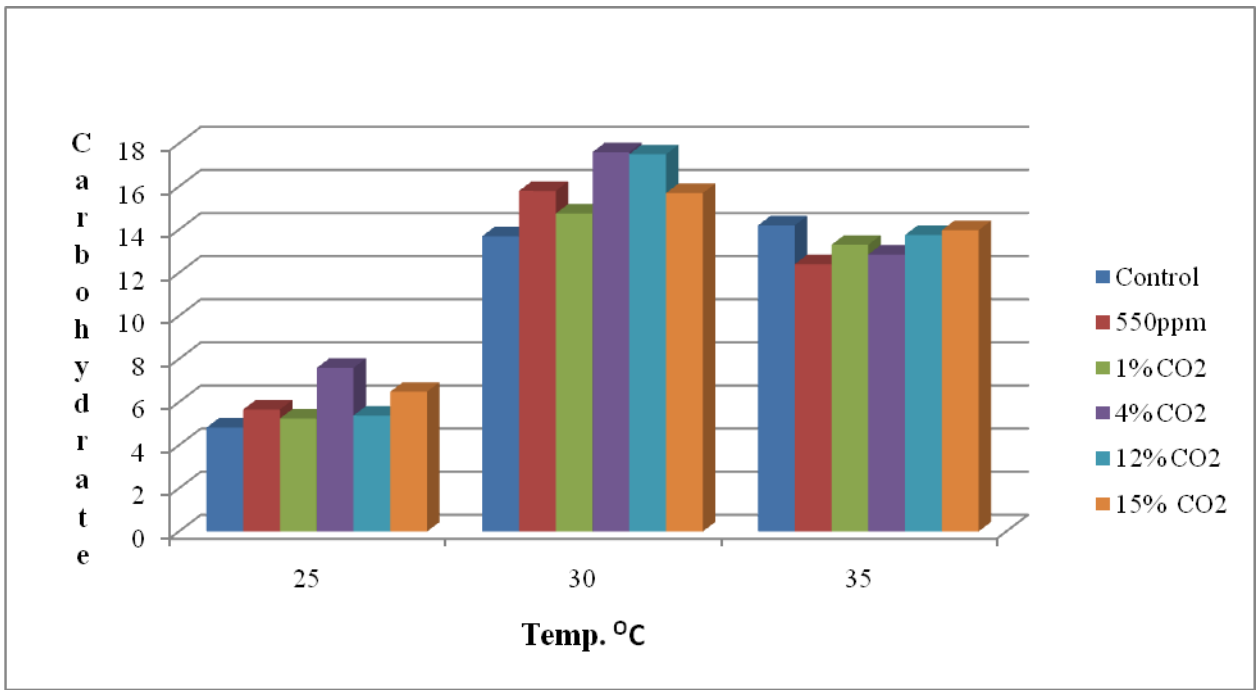


Fig.4 Comparison of total carbohydrate content accumulation up to 12 hr

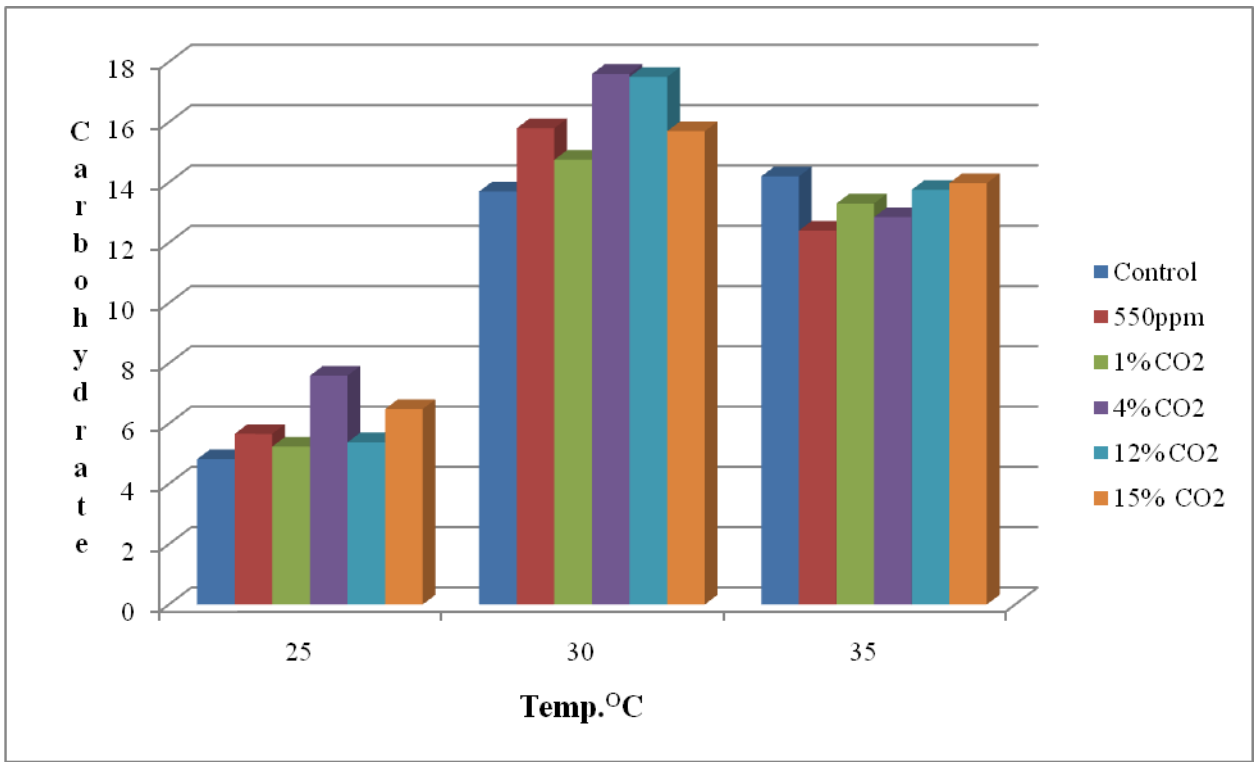


Fig.5 Comparison of total protein content accumulation up to 30min

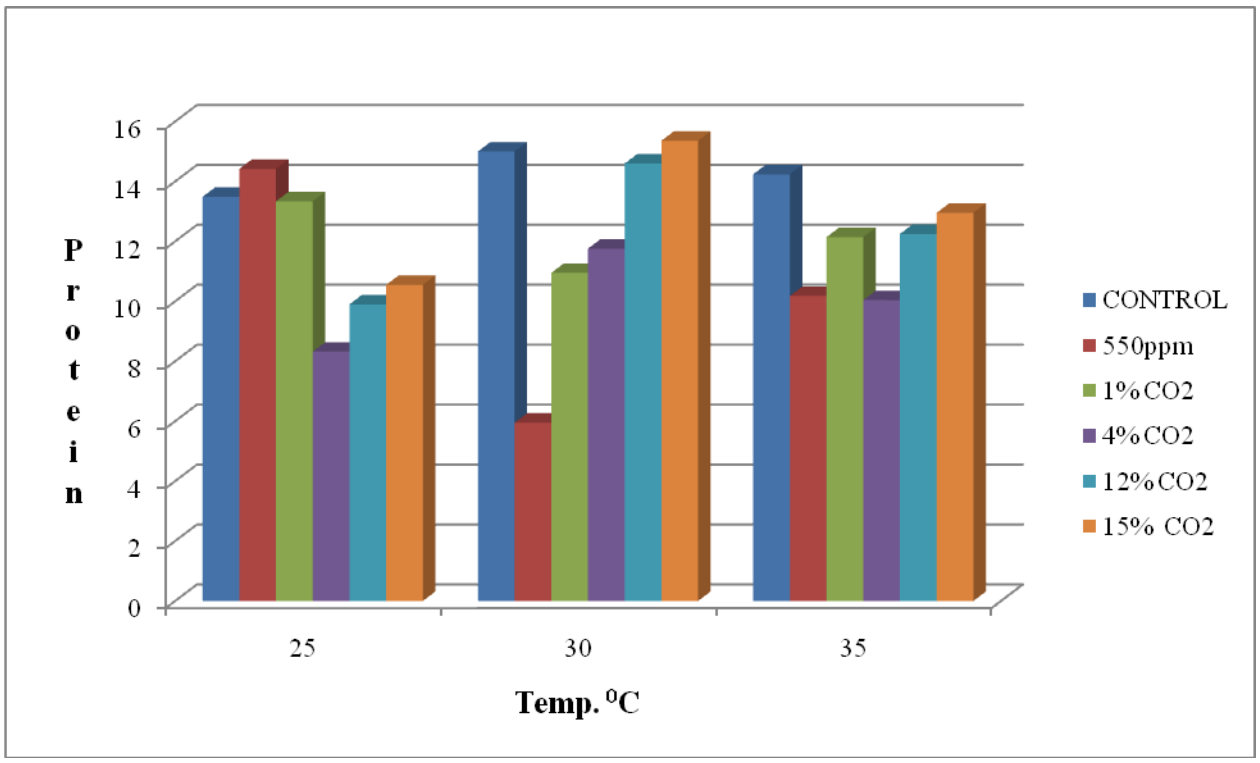


Fig.6 Comparison of total protein content accumulation up to 12 hr

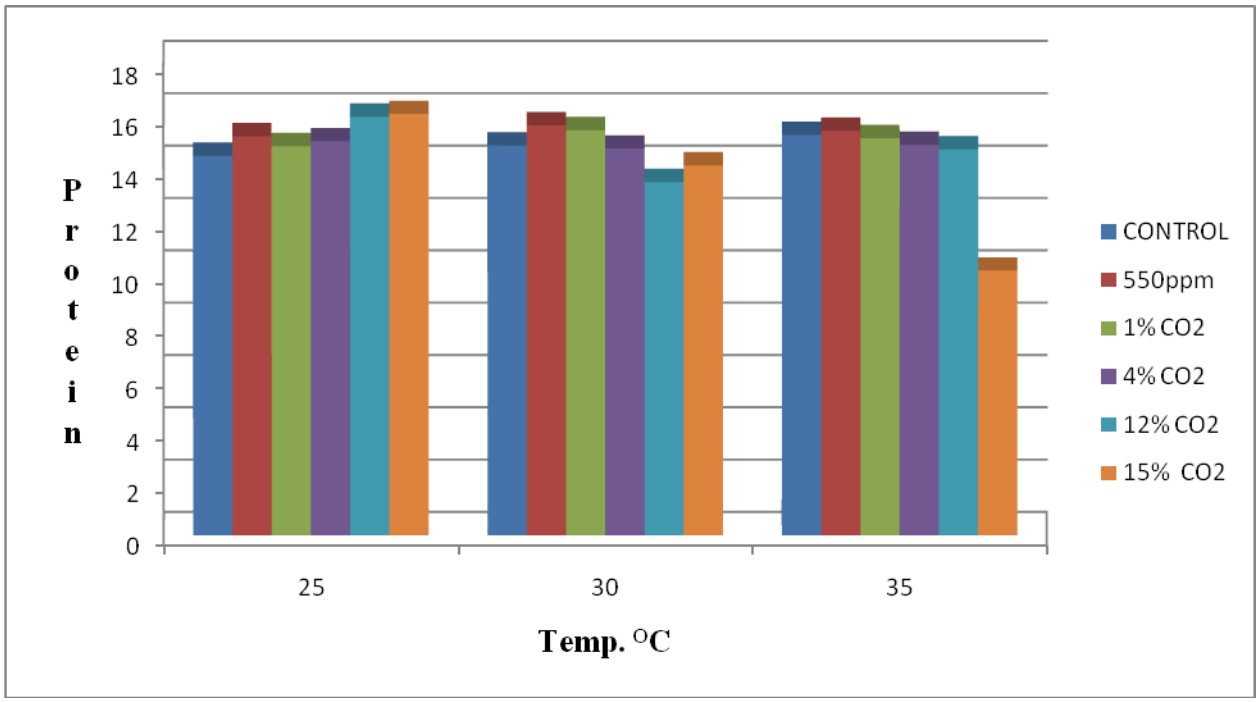
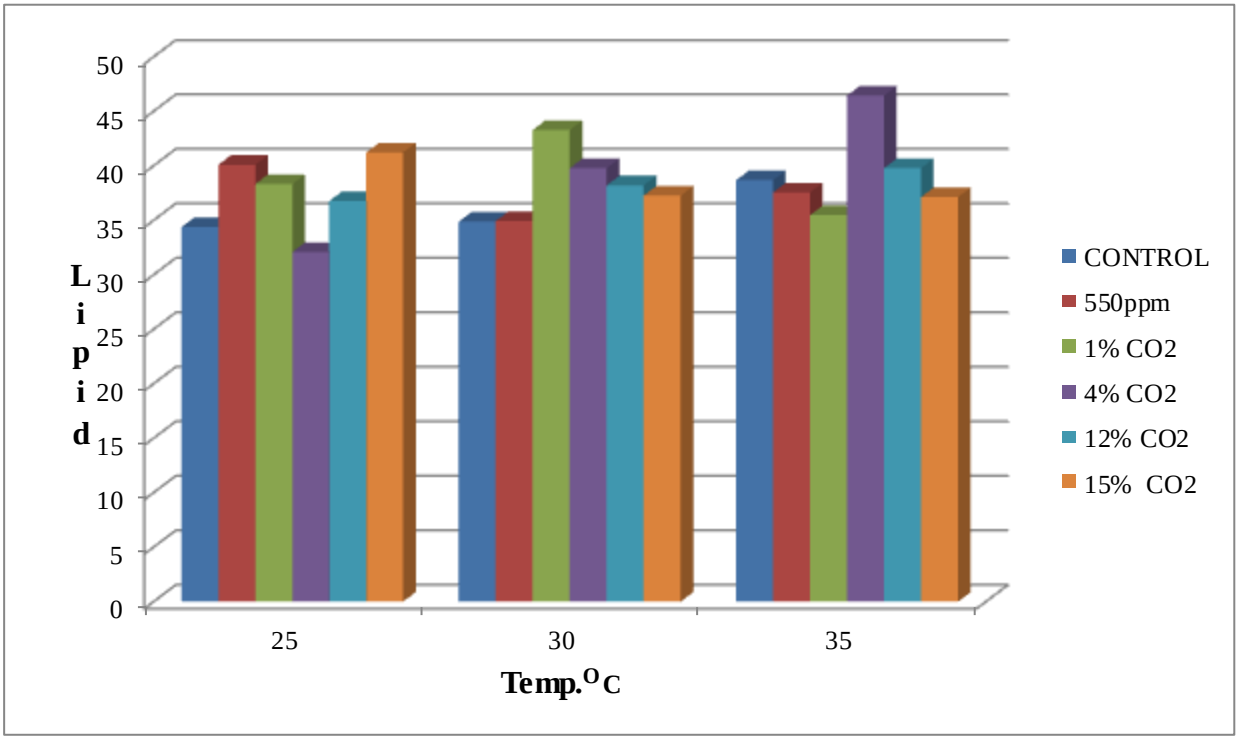


Fig.7 Comparison of total lipid content accumulation up to12hr



The lipid substance was higher than the control (34.36±1.3%) for *Chlorella protothecoides* in 550ppm (40±0.84%), 1% (38.32±0.8%), 4% (32.09±1.06%), 12% (36.7±1.08%) and 15% (41.2±1.1%) CO_2 treatment at 25°C At 30°C, it was watched that *C. protothecoides* treated cells with 4%, 12% and 15% CO_2 extended in lipid content and at 35°C only 4% (46.6±1.21%) CO_2 showed an extension in lipid content diverged from control while other CO_2 centers demonstrated cut down lipid content (Fig. 8).

During present investigations, it was observed that *C. prototheciodes* fix the CO_2 and the content of chlorophyll, carbohydrate, protein and lipid which vary according to CO_2, temperature and environmental conditions. The micro algae hsg theotential to control the increasing levels of CO_2 and can ultimately decrease the global warming which is the demand of the hour.

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