Original Research Article

Effect of CO₂ Concentration, Temperature and Light on Macro Biomolecules Accumulation in *Chlorella protothecoides*

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**Abstract**

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 CO₂ concentration in atmosphere.

The temperature on our planet increased due to increase in green house gases i.e. CO₂, 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 CO₂, 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 CO₂.

**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 °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, CO₂, treatment time, hatching temperature, photoperiod and light force. The chlorophyll content of *C. protothecoides* with 30 min CO₂ treatment was most elevated in 12% CO₂ at 30 °C (7.84±.35mgL⁻¹) (Table 1 and Fig. 1).

On the other side, During 12hr CO₂ treatment, the chlorophyll content was observed as 18.2±0.08mgL⁻¹ (Table 2 and Fig. 2).

**CO₂ 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 CO₂ treatment, respectively (Fig. 3).

**Total Carbohydrate Content**

Sugar content in CO₂ treated cell was higher than the untreated cell. Further it was observed that sugar content also depended on light intensity and CO₂ concentration.

**CO₂ treatment up to 30 min**

The sugar content of *C. protothecoides* expanded with increasing temperature with CO₂ concentrations. The starch content in control was 4.82±0.74mg/L but it increased with CO₂ concentration 7.59±0.35mg/L at 4% CO₂ and 6.48±0.54mg/L, respectively.

In case of 4% and 12% CO₂ at 30°C the starch content was observed as maximum (Table 3 and Fig. 4).

**Effect of CO₂ treatment upto 12 hr**
The starch content increased in *Chlorella protothecoides* when the cell treated with 15% CO$_2$ at 25°C 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°C to 12.496±0.27mg/L at 35°C (Fig. 5).

**CO$_2$ treatment for 30 min 12 hour in culture room conditions**

The aggregate sugar substance of *C.protothecoides* 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 25°C and 30°C at 1%, 4% and 12% (Table 5 and Fig. 6).

At 25°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°C and at 35°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. protothecoides* 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 25°C 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$_2$ concentrations 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% CO2 | 4% CO2 | 12% CO2 | 15% CO2 |
|-------|----------|----------------|--------|--------|---------|---------|
| 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% CO2 | 4% CO2 | 12% CO2 | 15% CO2 |
|-------|----------|----------------|--------|--------|---------|---------|
| 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% CO2 | 4% CO2 | 12% CO2 | 15% CO2 |
|-------|----------|----------------|--------|--------|---------|---------|
| 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% CO2 | 4% CO2 | 12% CO2 | 15% CO2 |
|-------|----------|----------------|--------|--------|---------|---------|
| 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% CO2 | 4% CO2 | 12% CO2 | 15% CO2 |
|-------|----------|----------------|--------|--------|---------|---------|
| 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% CO2 | 4% CO2 | 12% CO2 | 15% CO2 |
|-------|----------|----------------|--------|--------|---------|---------|
| 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    |

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

| S.NO. | Temp. °C | Control | 550ppm | 1% CO₂ | 4% CO₂ | 12% CO₂ | 15% CO₂ |
|-------|----------|---------|--------|--------|--------|---------|---------|
| 1.    | 25°C     | 33.28   | 41.7   | 38.25  | 37.23  | 41.36   | 40.24   |
| 2.    | 30°C     | 34.08   | 44.26  | 38.26  | 39.87  | 43.39   | 37.83   |
| 3.    | 35°C     | 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 up to 30 min

![Comparison of total carbohydrate content accumulation up to 30 min](image)

**Temp. °C**

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

![Comparison of total carbohydrate content accumulation up to 12 hr](image)

**Temp. °C**
Fig. 5 Comparison of total protein content accumulation up to 30 min

![Bar graph showing comparison of total protein content accumulation up to 30 min.](image)

Temp. °C

Protein

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

![Bar graph showing comparison of total protein content accumulation up to 12 hr.](image)

Temp. °C

Protein
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. protothecioides* 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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