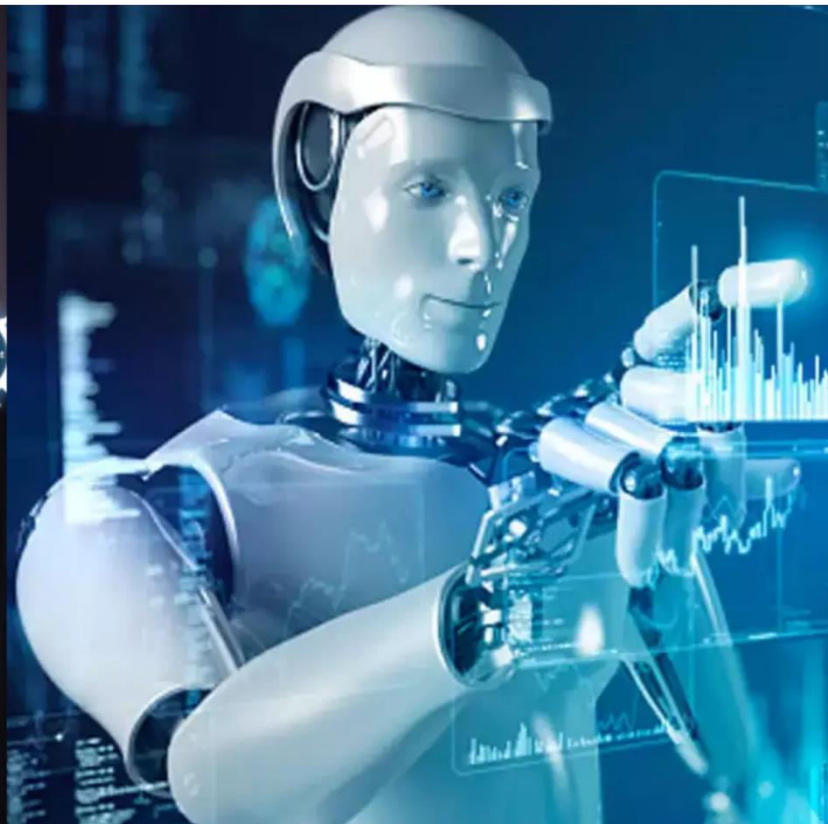




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Impact of CO₂ Concentration on its Growth and Pigment Formation of Anabaena Ambigua

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ABSTRACT: Due to fast growing concern about global warming, which is attributed primarily to the elevated CO₂ level in atmosphere, photosynthetic CO₂ mitigation has attracted much attention. The present study focuses on growth of *Anabaena ambigua* under different CO₂ concentrations. *Anabaena ambigua* was grown in BG-11 media at a light intensity of 7000 lux in eight different bottles, arranged in columns. Each pair is named as control(0min), 2min, 5min, 8min respectively. Control indicates CO₂ is not passed in first case, CO₂ is passed for 2min twice daily with a time interval of 8 hrs at a flow rate of 3 Lpm in second case, CO₂ is passed for 5min twice daily with a time interval of 8 hrs at a flow rate of 3 Lpm in third case, CO₂ is passed for 8min twice daily with a time interval of 8hrs at a flow rate of 3 Lpm in fourth case. Pigment analysis was performed. Phycobilin pigments are more in *Anabaena ambigua* grown in CO₂ concentration of 2min, 5min, then control and lastly 8min. No much difference was observed in total chlorophyll content in the algal species grown at different CO₂ concentrations. Carotenoids are more in algal cultures grown in CO₂ concentrations of 2min, 5min than in control and finally in 8min

I. INTRODUCTION

Fossil fuels include oil, coal, natural gas. Dead plants, animals trapped beneath the earth's surface over a period of hundred's of millions of years produced fossil fuels(Ragauskas et al., 2006). Fuels consists of hydrocarbons which on burning releases energy. Main products obtained on burning the fossil fuels are carbon dioxides, nitrous oxides, and hydrocarbons (Veziroglu et al., 2008). These gases are referred as green house gases. These green house gases are heating the earth's atmosphere by trapping the infrared radiation which is otherwise reflected back towards space (Ramanathan, 1988, Schneider, 1989, and Green, et al., 2000). Among the gases emitted by fossil fuels carbon dioxide is one of the most significant. The earth's ecosystem provides a natural carbon cycle but humans are producing more amount of carbon dioxide from industries, automobiles which are the major sources for it (Benemann, 2001, and Govindjee et al., 1999). With risk of climate changes and inevitable fuel shortages scientists are focussing more on alternative energy sources as well as carbon dioxide sequestration(Ayhan Demirbas, 2008). Plants are the best source to remove the carbon dioxide from atmosphere. Many researchers are working on biodiesel, ethanol as the sources of fuel obtained from plants as burning of these fuels releases less pollutants. Different sources are identified like corn, soya bean, jatropha, microalgae etc(Pahl. Greg, 2008 and Knothe et al., 2005). The most important microalgae are diatoms, green algae, blue-green algae etc. Microalgae are photosynthetic microorganisms that convert sunlight, water and carbon dioxide to sugars, from which biological macromolecules, as lipids, can be obtained (Khan et al., 2009, GuanHua Huang et al., 2010, and Mandal et al., 2009). Photosynthetic carbon dioxide fixation by microalgae is a feasible technology with energy saving and environment friendly. In the applications of microalgae for carbon dioxide removal (CO₂ biofixation), and energy production, the tolerance to CO₂, biomass, fatty acid content of microalgae are important(Teresa et al., 2010). Blue-green algae has wide applications in pharmaceutical industry, food industry, biofertilizers etc(Borowitzka, 1995, Spolaore et al., 2006,). It is also source of valuable products like pigments, polysaccharides etc(Rodgers et al., 1979).

The present work focusses on growth rates, pigments accumulation in *Anabaena ambigua* (blue-green algae) grown at different levels of carbon dioxide concentration, at light intensity of 7000 lux and in BG-11 media.

II. MATERIALS AND METHODS

The culture *Anabaena ambigua* (blue-green algae) used in this experiments was obtained from NCIM, Pune with Accession number 2785. The culture grown on solid agar media was subcultured in 150ml Erlenmeyer flasks. This culture was grown photoautotrophically in the BG-11 media at 28°C under light and dark illumination (18:6) which was kept in the orbital shaker at 110 rpm for 12 days. Now sub-culture is available to perform further set of

experiments. All the chemicals used in preparing the BG-11 media are of analytical grade purchased from Hi-Media. Distilled water is used in making BG-11 media

III. EXPERIMENTAL DESIGN

The subcultured *Anabaena ambigua* was inoculated into 8 fresh bottles containing 700 ml of BG-11 media in the laminar air flow chamber. The caps of the bottles were fitted with pipes to provide aeration for culture growth and were sealed with leak proof agent to prevent leakage of the media. The bottles were placed in an inverted position in a specific arrangement fitted with fluorescent bulbs with a light intensity of 7000 lux. The light intensity was measured using the lux meter. The bottles were placed in an upside down position within the arrangement and were fitted with aerators through the pipes for aeration to the cultures. Holes were made on the bottom of the bottles for the gases to escape. The culture in the media was allowed to grow for 10 days.

Now CO₂ was supplied to the culture, growth was observed with change in colour in each bottle. CO₂ was supplied from the cylinder to the arrangement as shown in the Fig1.

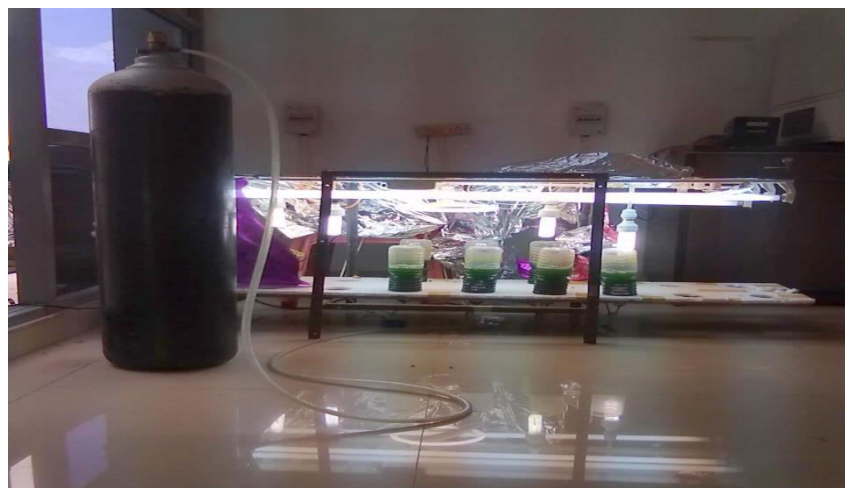


Fig 1: Growth of *Anabaena ambigua* at a light intensity of 7000 lux and different CO₂ Concentrations

As shown in the above Fig1 the bottles were arranged in four columns with two bottles in each column. The two bottles in each column represent the same conditions (i.e duplicates). Now CO₂ was passed to the bottles twice a day with a time interval of 8hrs with a flow rate of 3 Lpm. CO₂ was supplied till harvesting of biomass was done. Different concentrations of CO₂ is supplied to the bottles as shown in the Table 1

Table 1 Supply of CO₂ at different concentrations

Serial No(From left in Fig 1)	Carbon dioxide Concentrations supplied
First column of bottles	No carbon dioxide was passed. It is referred as control
Second column of bottles	CO ₂ was passed for 2min twice a day with a time interval of 8hrs at a flow rate of 3 Lpm. It is referred as 2 min.
Third column of bottles	CO ₂ was passed for 5min twice a day with a time interval of 8hrs at a flow rate of 3 Lpm. It is referred as 5min
Fourth column of bottles	CO ₂ was passed for 8min twice a day with a time interval of 8hrs at a flow rate of 3 Lpm. It is referred as 8min

The above experiments were performed in duplicates from third day of inoculation the average absorbance values are recorded after passing the carbon dioxide gas, using colorimeter at 540nm. The recorded optical density values are shown in table 2.

Biomass extraction: After 10 days of growth the absorbance values found to be constant and then slowly decreased. CO₂ supply was stopped and the culture media was transferred into reagent bottles and was allowed to settle for 24 hours. When the particulate matter was found to be settled, the upper layers were removed by pipetting. To obtain the biomass, the matter was centrifuged at 6000 rpm for 6 minutes, pellets were obtained and the supernatant was discarded. The pellet was transferred into a petriplate and was allowed to dry in hot air oven at 60 degrees centigrade for 6 hours which gives the dry weight. The dry weight of the culture was noted.

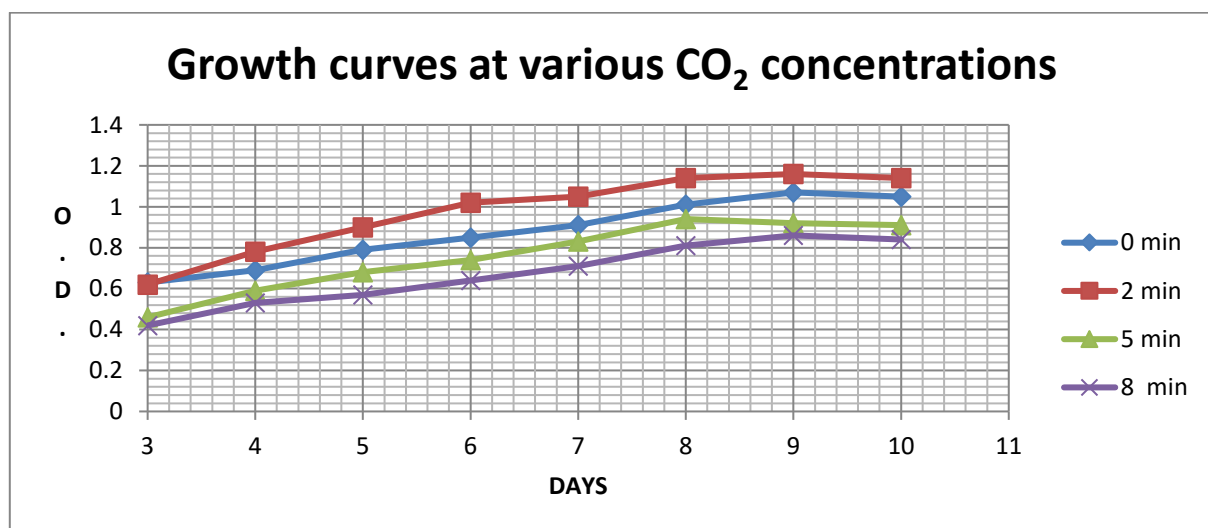
IV. RESULTS AND DISCUSSIONS

The average absorbance values were recorded from 3rd day to 10th day and are given in table 2 as follows:

Table 2 (Absorbance values at different days)

S.No	Absorbance values at 540nm			
	Control(0 min)	2 min	5 min	8 min
Day 3	0.63	0.62	0.46	0.42
Day 4	0.69	0.78	0.59	0.53
Day 5	0.79	0.9	0.68	0.57
Day 6	0.85	1.02	0.74	0.64
Day 7	0.91	1.05	0.83	0.71
Day 8	1.01	1.14	0.94	0.81
Day 9	1.07	1.16	0.92	0.86
Day 10	1.05	1.14	0.91	0.84

The growth curves of *Anabaena ambigua* grown at different carbon dioxide concentrations was shown in the graph as follows:



Graph1: Growth curves of *Anabaena ambigua* at different CO₂ concentrations

The above graph indicates that growth was good in *Anabaena ambigua* where CO₂ was supplied for 2min, then comes growth in control(0 min), followed by growth in 5min and 8min.

Estimation of Pigments:

Phycobiliproteins (Bennett and Bogorad, 1973)

Reagent: Phosphate buffer (0.05M. pH 7.5): Equal volumes of 0.1M K₂HPO₄ and 0.1M KH₂PO₄ were mixed to obtain 0.05 M phosphate buffer.

Procedure: A known volume (10 mL) of cyanobacterial cell suspension was taken and centrifuged (4000g, 10 mins.) to obtain the pellet. Phycobilins were extracted completely from pellet with similar volume of 0.05 M phosphate buffer by repeated freezing and thawing. The absorbance was read at 562, 615 and 652 nm against phosphate buffer as blank. The phycobilins were calculated by using the following equations:

$$\text{Phycocyanin (mg/mL)} = \frac{A_{615} - 0.474 \times A_{652}}{5.34}$$

$$\text{Allophycocyanin (mg/mL)} = \frac{A_{652} - 0.208 \times A_{615}}{5.06}$$

$$\text{Phycoerythrin (mg/mL)} = \frac{A_{562} - [2.41 (\text{PC}) - 0.849 (\text{APC})]}{9.62}$$

Table 4: Absorbance values at different CO₂ concentrations

Carbon dioxide concentration	Absorbance values		
	A ₅₆₂	A ₆₁₅	A ₆₅₂
Control	0.24	0.24	0.25
2 min	1.0	1.04	1.1
5 min	0.36	0.325	0.345
8 min	0.14	0.16	0.17

On substituting these absorbance values in the above equations we have summarized results as follows

Table 5: Phycobilins at different concentrations

Carbon dioxide concentration	Phycobilins		
	Phycocyanin(mg/ml)	Allophycocyanin(mg/ml)	Phycoerythrin(mg/ml)
Control	0.0227	0.0949	0.0346
2 min	0.0132	0.0346	0.0227
5 min	0.0949	0.0132	0.0346
8 min	0.0346	0.0227	0.0132

From the above table 5 we can conclude that the Phycobilin pigments are more in Anabaena ambigua grown in CO₂ concentration of 2min, then 5min, then control and lastly 8min.

Chlorophyll (MacKinney, 1941)

(i) Reagent: Methanol (95%)

Procedure: A known volume (10 mL) of homogenized cyanobacterial suspension was taken and subjected to centrifugation (4000g, 10 mins.). The chlorophyll was extracted from pellet with similar volume of methanol (95%) in a water bath (60°C, 30mins.). The suspension was centrifuged and the absorbance of the supernatant was read at 650 and 665 nm against 95% methanol as blank.

The concentration of chlorophyll was calculated using the formula:

Total chlorophyll (mg/mL) = $2.55 \times 10^{-2} E_{650} + 0.4 \times 10^{-2} E_{665}$

Where, E_{650} = Absorbance at 650 nm

E_{665} = Absorbance at 665 nm

Table 6 Chlorophyll absorbance at different CO₂ concentrations

Carbondioxide concentration	Absorbance values	
	E_{650}	E_{665}
Control	0.147	0.304
2 min	0.151	0.288
5 min	0.1346	0.264
8 min	0.17	0.304

On substituting the above absorbance values we get the results as follows:

Table 7 Total chlorophyll at different CO₂ concentrations

Carbon dioxide concentration	Total chlorophyll (mg/ml)
Control	0.495×10^{-2}
2 min	0.5×10^{-2}
5 min	0.448×10^{-2}
8 min	0.55×10^{-2}

From the above table 7 it was observed no much difference in total chlorophyll content in algal species grown in different CO₂ concentrations.

Carotenoids (Jensen, 1978)

Reagent: Acetone (85%)

Procedure: A known volume (10 mL) of homogenized cyanobacterial suspension was taken and subjected to centrifugation (4000g, 10 mins.). Discarded the supernatant and washed the pellet 2-3 times with distilled water to remove traces of adhering salts. To the pellet, added 2-3 mL of acetone (85%) and stored at 4°C for a week. Centrifuged and collected the supernatant containing pigment. Repeated the extraction from pellet with acetone till the supernatant became colorless. Pooled all the fractions of supernatants and made up to a final known volume. Took the OD at 450nm using 85% acetone as blank and calculated the total amount of carotenoids as follows:

$$\text{Carotenoids (mg/mL)} = \frac{D \times V \times f \times 10}{2500}$$

Where, D = Absorbance at 450nm

V = Volume of the extract, and

f = Dilution factor

It is assumed that these pigments have an average extinction coefficient of 2500.

Table 8 Carotenoid absorbance at different CO₂ concentrations

Carbon dioxide concentration	D(Absorbance at 450nm)	V(Volume of the extract)	f(Dilution factor)
Control	0.3	10	10
2 min	0.5	10	10
5 min	0.53	10	10

8 min	0.29	10	10
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On substituting the above values in the equation we have the results as follows

Table 9 Carotenoids at different CO₂ concentrations

Carbon dioxide concentration	Carotenoids (mg/ml)
Control	0.12
2 min	0.2
5 min	0.212
8 min	0.116

From the above table 9 it was observed that carotenoids are more in algal cultures grown in CO₂ concentrations of 2min and 5min then in control and finally in 8min.

V. CONCLUSION

From the above experiments it can be concluded that growth was good in *Anabaena ambigua* where CO₂ was supplied for 2min, then comes growth in control(0 min), followed by growth in 5min and 8min. Phycobilin pigments are more in *Anabaena ambigua* grown in CO₂ concentration of 2min, 5min, then control and lastly 8min. Carotenoids are more in algal cultures grown in CO₂ concentrations of 2min, 5min than in control and finally in 8min

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