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Biofixation potential of Carbon dioxide by Fresh water species of *Chlorella* and *Closteriopsis*

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Abstract

Microalgae based seizure of CO_2 can be an effective means of carbon sequestration, owing to their higher photosynthetic efficiency, faster growth rate and higher biomass production compared to plants. Several members including Chlorella have been brought in this direction and the present study is an attempt to introduce new algal members in carbon assimilation. Pure cultures of Chlorella and Closteriopsis were maintained in Bolds Basal medium. For experimentation, 1 litre each of the respective algal cultures was added to 4 litres each of the culture medium kept in 3 glass tanks of size 18x18x24 cm. First tank was maintained as such and treated as control. The second and third tanks were supplied with air and CO_2 gas respectively at constant flow rate. pH, Dissolved Oxygen and free carbon dioxide content of all the treatment sets were monitored at an interval of 3 hours and cell count, cell size and biomass were worked out at an interval of 6 hours. All the 3 sets were kept under observation for a period of 48 hours and the results are reported. The results of the present study showed that in spite of slightly acidic pH and higher free CO_2 content, the Chlorella sp. exhibited higher rate of DO production, cell count, cell size and biomass content in treatment sets containing CO_2 supply than Closteriopsis sp. The bending of the tips of the cells of Closteriopsis sp. in CO_2 treated set is indicative of the stressful condition developed within the system.

Keywords: CO_2 sequestration, *Chlorella sp.*, *Closteriopsis sp.*

Introduction

Carbon sequestration is a natural or deliberate process by which CO_2 removed from the atmosphere or diverted from point sources are stored in terrestrial environments (vegetation, soils and sediments), oceans and geologic formations[1]. All these processes can fall in physical, chemical or biological methods. Physical as well as chemical means of CO_2 sequestration mostly require facilities for capturing, storing and transporting CO_2 and hence capital intensive. Therefore it is necessary to develop more cost-effective and sustainable alternatives to curb the soaring emission rate[2].

Biosequestration is the capture and storage of the atmospheric carbon dioxide through biological processes, via increased photosynthesis, carbon trapping in agriculture soil or by means of algal biomass. Many researchers report that the natural process of photosynthesis is the most excellent solution to the problem of riotous carbon emissions. Biological sequestration of CO_2 can be achieved through both terrestrial and aquatic processes. Amongst various CO_2 sequestration strategies, the biological methods particularly the ones using microalgae have several merits[3], which include direct CO_2 capture and fixation from sources by suitable microalgal strains and subsequent biomass conversion. Algae, confining to fresh water and marine systems can be candidates for sequestration as they are responsible for approximately 50% of total photosynthetic primary production[4]. Photosynthetic mitigation of CO_2 using microalgae is promising from many aspects as they offer rapid growth rates due to higher efficiency solar conversion (up to 10 times that of higher plants) and with a doubling time of about three to five days[5]. They are better able to handle extreme environments and hence can be more readily incorporated into engineered systems[6]. Also the biomass produced by them, through various process technologies, can lead to the production of a wide variety of value added products. In this context, the present study has been carried out with the objective of screening newer microalgal members and to compare their carbon dioxide assimilation efficiencies with the standard ones.

Materials and Methods

The micro algal samples were collected using plankton nets from various fresh water environments, including Bharathapuzha at Shornur, Chaliyar at Chaliyam and a pond at Calicut University Botanical Garden (CUBG). Identification of the collected algal members was done with the help of experts. From these collections, pure cultures of the test organisms were prepared using standard procedures using Bolds Basal medium[7].

For experimentation, 12 litres of Bolds Basal medium was prepared each time and to these, 3 litres of respective culture medium containing pure cultures of the test organism was added and kept for incubation. Five litres each of the incubated micro

algal culture was transferred to three glass tanks. The first glass tank containing the culture was maintained as such and was treated as control. To the culture (5 litres) contained in second tank, air has been bubbled and was treated as aerated set. To the third tank, carbon dioxide from a cylinder has been bubbled and was treated as CO_2 treated set. The control was kept idle and through other sets, air and CO_2 has been bubbled at a constant flow rate (6 – 9 bubbles per minute) from 6 am to 6 pm for 3days. All the three sets were kept at illumination during the day time. Sampling and monitoring of cultures were carried out at regular intervals for a period of 48 hours. pH, dissolved oxygen and free carbon dioxide content of the culture was monitored at an interval of 3 hours, whereas, cell count, cell size (micrometry) and biomass were worked out at regular intervals of 6 hours.

Results and Discussion

Results of cell size and number of cells of *Chlorella sp. and Closteriopsis sp.* in response to changes in pH, dissolved oxygen and free CO_2 content are depicted in table 1a and 2a and results of biomass are depicted in table 1b and 2b respectively. Similarly trends in pH, dissolved oxygen, free CO_2, cell size, cell count and biomass content are depicted in Figures 1-6.

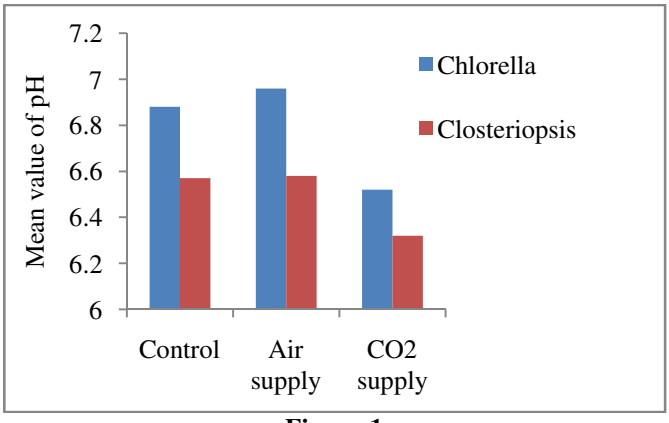

Figure-1
Variation in the pH of microalgal members studied

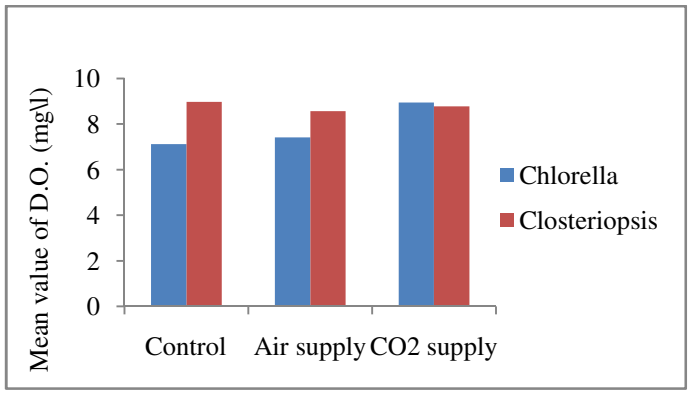

Figure-2
Variation in the DO of microalgal members studied

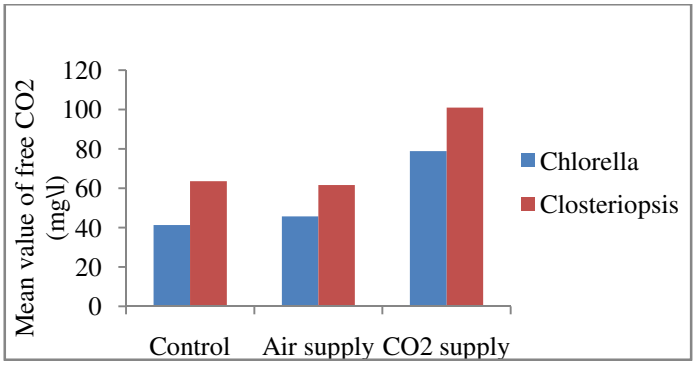

Figure-3
Variation in the Free CO_2 of microalgal members studied

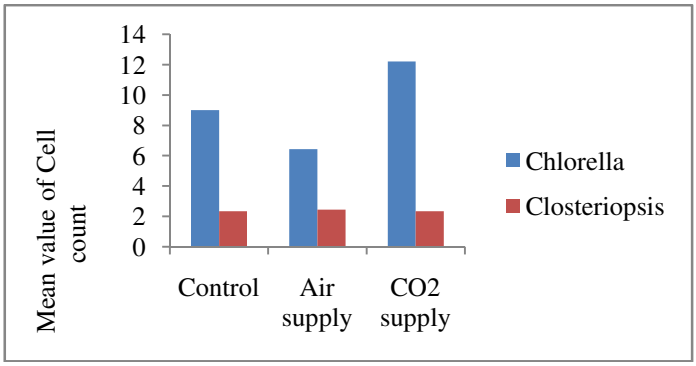

Figure-4
Variation in the Cell count of microalgal members studied.

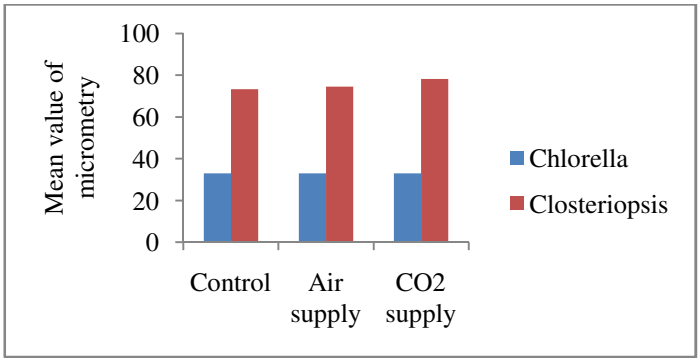

Figure-5
Variation in the Micrometry of microalgal members studied

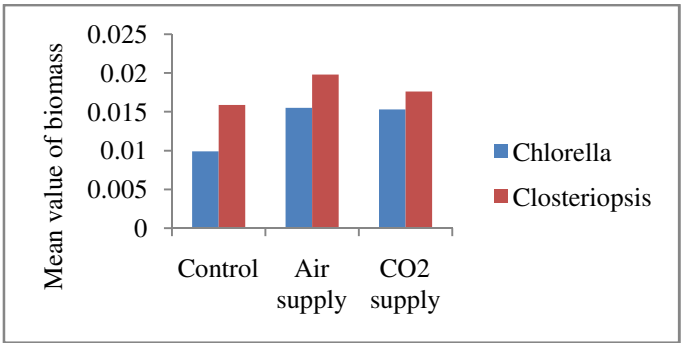

Figure-6
Variation in the Biomass of microalgal members studied

Table-1a
Results on the responses of *Chlorella* sp. to various parameters studied

Parameters analyzed	First day					Second day						Third day		Mean ± SD
	6 am	9 am	12 pm	3 pm	6 pm	12 am	6 am	9 am	12 pm	3 pm	6 pm	12 am	6 am	
pH														
Control	6.89	6.88	6.91	6.97	6.88	6.96	6.87	6.23	6.95	6.98	7.03	6.99	6.91	6.88 ± 0.20
Air supply	6.89	6.87	6.88	6.91	6.96	6.92	6.92	6.91	6.96	7.1	7.04	7.13	6.93	6.96 ±0.08
CO_2 supply	6.89	6.84	6.53	6.48	6.37	6.46	6.51	6.98	6.38	6.36	6.29	6.27	6.46	6.52 ±0.23
Dissolved Oxygen (mg/l)														
Control	6.8	6.8	5.6	7.4	7.2	5.4	6.2	6.2	16.8	7.6	6.4	7.2	3	7.12±3.14
Air supply	6.8	4.4	5.6	7.2	7.8	6	5.8	6	12.2	7.6	6.8	7.6	12.6	7.42±2.41
CO_2 supply	6.8	6.4	7.8	8.4	8.8	6	6.2	7	17.8	9.6	10.2	11	10.4	8.95±3.16
Free CO_2 (mg/l)														
Control	48.4	44	44	44	35.2	26.4	44	39.6	35.2	48.4	44	39.6	44	41.3±6.10
Air supply	48.4	48.4	35.2	39.6	44	52.8	44	44	39.6	61.6	48.4	39.6	48.4	45.7±6.85
CO_2 supply	48.4	52.8	61.6	70.4	79.2	158.4	61.6	79.2	83.6	101.2	83.6	70.4	74.8	78.9±27.74
Cell count (x10^4cells/ ml)														
Control	6		6		16	14	5		11		9	8	6	9.00±3.9
Air supply	6		5		12	8	4		8		6	5	4	6.44±2.56
CO_2 supply	6		11		21	18	7		13		14	11	9	12.2±4.92
Micrometry (µm)														
Control	33		33		33	33	33		33		33	33	33	33± 0
Air supply	33		33		33	33	33		33		33	33	33	33± 0
CO_2 supply	3 3		33		33	33	33		33		33	33	33	33± 0

Table-1b
Results on the responses of *Chlorella* sp. to biomass

Parameters analyzed	First day			Second day				Third day		Mean±SD
	6am	12pm	6pm	12am	6am	12pm	6pm	12am	6am	
Biomass (gm)										
Control	0.0072	0.0113	0.0127	0.0085	0.0012	0.0024	0.0178	0.0176	0.0108	0.0099± 0.0058
Air supply	0.0072	0.007	0.0099	0.0196	0.0135	0.0329	0.0117	0.029	0.0086	0.0155 ± 0.0096
CO_2 supply	0.0072	0.0123	0.0136	0.0014	0.0047	0.0294	0.0194	0.0153	0.0348	0.0153 ± 0.011

Table-2a
Results on the responses of *Closteriopsis* sp. to various parameters studied

Parameters analyzed	First day					Second day						Third day		Mean ±SD
	6 am	9 am	12 pm	3 pm	6 pm	12 am	6 am	9 am	12 pm	3 pm	6 pm	12 am	6 am	
pH														
Control	6.54	6.56	6.59	6.60	6.65	6.53	6.49	6.55	6.64	6.56	6.65	6.55	6.48	6.57±0.05
Air supply	6.54	6.58	6.56	6.61	6.68	6.56	6.50	6.56	6.63	6.59	6.64	6.57	6.47	6.58±0.05
CO_2 supply	6.54	6.32	6.23	6.22	6.21	6.24	6.27	6.38	6.37	6.36	6.38	6.32	6.29	6.32±0.09
Dissolved Oxygen (mg/l)														
Control	10	10.4	10.4	9.4	9.6	7.8	8.8	10.2	5.8	9.6	9.8	9.4	5.6	8.98±1.62
Air supply	10	11	11	9.8	9.8	6	8.2	9.2	7	8.8	7.2	8.4	5	8.57±1.85
CO_2 supply	10	12.6	12.8	10.6	10	8.6	9.2	8.2	7	5.2	8	7.2	4.8	8.78±2.46
Free CO_2 (mg/l)														
Control	61.6	57.2	74.8	61.6	61.6	61.6	79.2	61.6	66	61.6	61.6	57.2	61.6	63.6±6.38
Air supply	61.6	57.2	88	61.6	61.6	57.2	61.6	52.8	57.2	61.6	57.2	61.6	61.6	61.6±8.42
CO_2 supply	61.6	88	277.2	105.6	96.8	83.6	83.6	88	88	88	83.6	83.6	83.6	101.0±53.9
Cell count (x10^4cells/5 litre)														
Control	2		2		2	2	2		2		3	3	3	2.33±0.5
Air supply	2		2		2	3	3		3		3	2	2	2.44±0.52
CO_2 supply	2		3		3	3	3		3		2	1	1	2.33±0.86
Micrometry (mm)														
Control	66		66		66	77	77		77		77	77	77	73.3±5.5
Air supply	66		66		66	77	88		77		77	77	77	74.6±7.33
CO_2 supply	66		77		77	77	88		77		88	88	66	78.2±8.59

Table-2b
Results on the responses of *Closteriopsis* sp. to biomass

Parameters analyzed	First day			Second day				Third day		Mean ±SD
	6am	12pm	6pm	12am	6am	12pm	6pm	12am	6am	
Biomass (gm)										
Control	0.0103	0.0142	0.0076	0.0237	0.0289	0.0049	0.0315	O.O151	0.0222	0.0159±0.01
Air supply	0.0103	0.015	0.0148	0.0317	0.0272	0.0087	0.0081	0.0416	0.0207	0.0198±0.01
CO_2 supply	0.0103	0.0053	0.0184	0.0181	0.0301	0.009	0.0316	0.0158	0.0202	0.0176±0.00

Upon comparison of the results of various parameters, it has been noticed that all treatment sets with CO_2 supply exhibited pH in acidic range. This can happen as a result of residual CO_2 in the culture media, bringing the pH to acidic conditions. It is also noted that all the algal members survived in acidic range of pH. There are reports on the capability of *Chlorella* sp. to survive at pH ranging from 4.0–6.0[7] and 4.0 to 7.0[9]. However such reports with respect to *Closteriopsis* species is scanty. The better growth performances of both the micro algal members at acidic pHs and the maintenance of culture pH by the algal members within a range are indicative of their adaptability and CO_2assimilation capabilities.

On a comparative assessment of the mean values of DO, it was noted that even with continuous supply of CO_2, cultures containing *Chlorella* sp. maintained higher levels of DO than their respective controls. This is indicative of the active photosynthetic process associated with the micro algal members in presence of available CO_2 and subsequent release of oxygen. Treatment sets containing *Closteriopsis* sp. also showed better DO than their aerated sets, indicating their efficiency next to *Chlorella* in assimilating CO_2.

Increase in cell count in most cases is considered to be an index of growth[10,11]. In the present study, mean values of the result of cell count of *Chlorella* sp. was found to increase in treatment sets fed with continuous supply of CO_2. In the treatment set containing *Closteriopsis* sp., control and CO_2 treated set showed similar values while aerated set showed higher value. Hence it can be concluded that in the present study, continuous supply of CO_2 have accelerated the growth of *Chlorella* sp. than *Closteriopsis* sp.

Responses of micro algal cells to external stimulus vary[12]. In the case of *Chlorella*, control and CO_2treated set exhibited uniform cell size (33 µm) throughout the period of study. Normally shrinkage of cells is noticed during stressful conditions. In case of stress due to hypertonic conditions, the water molecules leave the cells resulting in its shrinkage[13]. Also cell-bursting during hypoosmotic conditions is also reported[14]. On a detailed microscopic observation of the micro algal members of the present study, it has been noticed that the cells of *Closteriopsis* were found to exhibit signs of stress, evidence by the tip of cells bending inwards. Despite signs of stress, mean values of cell size were higher with CO_2 treated set followed by aerated set and control. Also in the present study, mean values of the biomass of species like *Chlorella* and *Closteriopsis* in their respective CO_2 treated set showed higher values than their respective control values, indicating a trend in the increase in growth.

A consolidation of the results of the present work indicated that in spite of acidic pH and higher free CO_2 content, all the micro algal members under study exhibited higher rate of DO production, higher cell count and higher cell size in treatment sets containing CO_2 supply. This characteristically indicates their efficiencies in mitigating gaseous CO_2. Results with *Chlorella* sp. was highly promising, as evidenced by high DO production, high cell count, high cell size and high biomass in CO_2 treatment sets compared to control. The only stressful condition noticed was with *Closteriopsis* sp., evidence by bending of cell tips in cultures provided with CO_2 supply. Such a situation can be overtaken by supplying sub lethal dosages of CO_2 to the cultures containing species *Closteriopsis*. The present study thus confirms the efficiency of *Chlorella* sp. in sequestering CO_2 and the efficiency of *Closteriopsis* sp. next to it.

Conclusion

Aquatic sequestration of carbon dioxide is one among the effective methods of carbon sequestration. Microalgae are reported to contribute to the reduction in atmospheric carbon dioxide as they utilize CO_2 as source of carbon for biomass production. The present investigation was an approach to assess the potentialities of species of *Chlorella* and *Closteriopsis* in biological fixation of carbondioxide. Pure cultures of these algal members were maintained in BB medium and have been transferred to three treatment sets kept in different conditions. Of the 3 sets, one set was maintained as control and the other two sets as aerated and CO_2 treated sets respectively. To the CO_2 treatment facility, specific dosages of CO_2 were supplied

intermittently from an external source. Changes in the culture medium and trends in the growth pattern of the respective algal members were monitored at regular intervals.

On an overall assessment, it has been noticed that the continuous supply of carbon dioxide accelerated the dissolve oxygen content, cell size, cell count and biomass of both microalgal members. Among the two species, *Chlorella* exhibited high DO production, high cell count, high cell size and high biomass in CO_2 treated set, indicating their higher efficiencies in carbon sequestration higher than *Closteriopsis*.

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