Screening of Thermotolerant Microalgal Species Isolated from Western Ghats of Maharashtra, India for CO_2 Sequestration

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Abstract: Extensive work has been carried out to find suitable strain of algae which are tolerant to high concentration of CO_2. In the previous studies *Chlorella vulgaris* (SAG 211.12), a mesophilic strain, showed CO_2 sequestration upto 23% CO_2 concentration. The main constraint using such strains is that they don't tolerate temperatures beyond 35-40ºC. Hence attempts were undertaken to isolate algal strains from hot springs located across the western ghats of Maharashtra. *Limnothrix redekei*, *Planktolyngbya crassa* and *Geitlerinema sulphureum* could easily sequester about 28% of CO_2 without hampering biomass production. High temperature adaptability (42ºC), high CO_2 tolerance (23.08%), reasonably high biomass production and easy harvesting make *G. sulphureum* a promising candidate for the CO_2 sequestration in the tropical climatic conditions. Attempts are being made here to screen the isolated strains for lipid production.

Key words: CO_2 sequestration, microalgae, Cyanophyceae, hot springs.

1. Introduction

Accumulation of anthropogenic carbon dioxide in the atmosphere is a reality and a challenge for science and technology today. At the beginning of industrial era, the concentration of CO_2 in the air was around 280 ppm. Since then, it has raised upto 370 ppm in 2005 and thereby contributed to greenhouse effect and global warming [1]. United Nations promoted the Kyoto protocol (1997) with objective of reducing GHG by 5.2%. More than 170 countries have ratified the protocol. Various methods are employed for CO_2 sequestration including physiological, geological, oceanic, chemical and biological. The first three methods are short term options but do not address the issues of sustainability [2]. The fourth method, chemical reaction based CO_2 sequestration typically consists of three steps; separation, transportation and sequestration. The cost of CO_2 separation and compression is estimated to be $30-50 per ton of CO_2 and transportation and further sequestration cost is estimated to be $1-3 per T. The method is costly and energy consuming the mitigation benefits become marginal [3].

Biological CO_2 mitigation has attracted much attention as an alternative strategy, which involved fixation of atmospheric CO_2 with photosynthesis leading to biomass energy through plants and microalgae. Terrestrial plants contributed to about 3-6% of total CO_2 sequestration [3]. On the other hand microalgae, photosynthetic bacteria are fast growing and reported to have an ability to fix CO_2 10-50 times more efficiently than terrestrial plants [4].

Microalgal CO_2 sequestration has several other merits. Very important one is direct CO_2 utilization from flue gases. This fact is very important as separation of CO_2 from flue gas contributed to 70% of total sequestration cost [5]. CO_2 sequestration can be made still cost effective when wastewater is utilized for algae cultivation [6-8].

Extensive work has been carried out to find suitable strains of algae tolerant to high concentration of CO_2. The effect of pure CO_2 on a wide range of microalgal strains have been examined [9-10]. Wantanabe et al. (1992) isolated fresh water green algae *Chlorella* HA-1 from paddy field [11]. It showed maximum growth at 5-10% CO_2. Growth was remarkably decreased at increased concentration of CO_2 higher than 10%. Kodama et al. (1993) reported a new highly CO_2 tolerant marine algae *Chlorococcum littorate*, it showed optimum growth at 5% and 10% CO_2 [12]. With 20 % CO_2 concentration growth rate was comparable to optimum and they proposed that the strain will be applied to power plants located near the sea shore.

Several studies have been carried out to study effect of simulated or direct flue gases on algal growth. Sung et al. (1998) isolated highly CO_2 tolerant microalgae *Chlorella* KR-1 [13]. Growth of this strain is not inhibited up to 20% CO_2, 100 ppm NOx and 50 ppm SOx. Controlling pH of the media caused increase in tolerance of microalgae to SOx and NOx, which allowed them to grow well up to 250 ppm SOx and 300 ppm NOx. Maeda et al. (1995) found good tolerance of *Chlorella* T1 to flue gas (13% CO_2, 5% O_2, 10 ppm SOx and 30 ppm NOx) direct from boiler [14]. Use of microalgae for CO_2 sequestration from flue gases originated from various sources has not been simply restricted to academic level but it has been going on globally in various academic and commercial organizations. Aquatic species program; 1978-1996, Cyanotech corporation Kona, Hawaii, Seambiotic, Israel successfully cultivated microalgae in open ponds for CO_2 sequestration.

Closed photobioreactors prevent direct gas exchange with the atmosphere, as there is less out gassing and loss of the CO_2. They have been reputed for very high productivities. Accumulation of O_2 in closed systems inhibits algal growth but can be overcome by having a degassing area. A2BE carbon captures Boulder, Colorado, US made attempts for CO_2 sequestration in photobioreactor, roller film PBR. Ohio State University has designed pilot scale membrane photobioreactor for enhanced CO_2 sequestration and characterisation of the growth of thermophilic strain of *Chlorogleopsis* (SC2) isolated by Dr. Keith Cooksey of Montana State University from Yellowstone National Park. RWE energy, Germany has successfully developed a system, bubble column reactor connected to series of tubular reactor, for binding CO_2 from power plant in microalgae.

In India, the lead was taken by one of the India's leading cement companies in collaboration with KET's VG Vaze College, Mumbai in 2008 [15]. A primary level project was carried out wherein sequestration of upto 23% CO_2 was carried out in a 2 L air lift photobioreactor using a species of *Chlorella vulgaris* (SAG 211.12). The main constraint in using such strains is that they do not tolerate temperatures beyond 30ºC and are not suited for Indian climatic conditions. In tropical countries ambient temperature is 35-40ºC, which does not support algal growth. For CO_2 sequestration microalgal species to be used should not only able to withstand warm temperatures (since the emitted flue gases would raise the overall temperature of the

medium) but also possess a broad pH optima, ability to withstand accessory gases that accompany flue gases, scalable to industrial size photobioreactors and most importantly be capable of accumulating high value metabolites under stressing and non-stressing conditions [16]. Hence in present study attempts are undertaken to isolate algal strains from hot springs located across the Western Ghats of Maharashtra.

The present communication deals with isolation and cultivation of algal strains from hot springs in tubular airlift photobioreactor for CO_2 sequestration. A lab scale tubular airlift reactor was used to test the feasibility of the gaseous CO_2 reduction, carbon fixation and biomass production. The isolated microalgal strains were screened for their growth at different temperatures, different pH, at increased sulphate concentrations and various CO_2 concentrations to find out suitable strain sustaining at higher temperature (30-35ºC) without requirement for pH adjustment for CO_2 sequestration.

2. Experimental

2.1 Sampling locations

Hot springs, well isolated from populated area located in Western Ghats of Ratnagiri, Raigad and Thane district of Maharashtra of West India were selected for the isolation of the algae. Sampling was done thrice a year from three hot springs located in Aravali, Baragaon Mandir, Golawali and Ganeshpuri. Sampled algal masses were first washed with the distilled water to remove mud if present. Hot springs mainly comprises of blue green algae hence, BG-11 medium [17] with or without NO_3 was used for isolation of the algae.

2.2 Isolation of microalgae from hot springs

Water samples were collected in screw cap bottles and enriched with BG-11 medium. The method for isolation and purification of cyanobacteria was adapted from Ferris and Hirsch (1991) [18]. Algal mats were washed properly to remove the mud and then suspended in liquid medium. Sample was separated teased and then placed on the BG-11 agar plate. The plates were incubated for 15 days and were microscopically examined for the growth of cultures. Individual species were picked aseptically, sub-cultured in 250 ml Erlenmeyer flasks and incubated under continuous illumination (2000 lux) at 22ºC with 16 hr light regime. The BG-11 medium was used for the isolation and maintenance of cultures. Filamentous axenic strains were then conserved and maintained on agar slants. Cultures were maintained at the 32ºC and at 2000 lux light intensity.

On the basis of the preliminary experiments for biomass production out of the ten strains isolated only four strains namely, *Scenedesmus* sp., *Limnothrix redekei* isolated from Aravali, *Planktolyngbya crassa* isolated from Baragaon mandir, *Chroccoccus* sp. isolated from Golavali and *Geitlerinema sulphureum* isolated from Ganeshpuri were short listed for the further studies. Identification of the microalgal cultures was done purely on the basis of the morphological and microscopical observation. The mesophilic algal strain *Scenedesmus* sp. was used as a control for all the experiments.

2.3 Standardization of culture conditions

2.3.1 Growth at different temperatures

The study involved the effect of the various temperatures on the growth of algae the isolated strains were inoculated in 250 ml Erlenmeyer flask containing 100 ml of BG-11 medium and the flask were placed in incubator shaker at various temperatures viz. 22ºC, 32ºC and 42ºC under illumination (2600 lux) with 16 hr light period for 15 days. After 15 days growth the biomass was interpreted in terms of dry weight per 100 ml BG-11 media.

2.3.2 Effect of different pH media on growth of microalgae

To study the effect of pH on algal growth, BG-11 medium was adjusted to pH 6.0, 7.4, 8.0 and 9.0 by with 0.1 N HCl or NaOH. The growth of algae was studied and results were interpreted in terms of dry weight biomass (Table 4).

2.3.3 Cultivation in Spirulina medium

All the Cyanophyceaen strains were cultivated in the BG-11 medium for isolation and cultivation. Preliminary experiments for the pH variation indicated that the growth of A.1.7 and A.3.1 was favored at alkaline pH. Therefore these cultures were cultivated in media with higher pH i.e. Spirulina medium (pH 9.0) [19].

2.3.4 Cultivation in Modified BG-11 medium

Growth of A.1.7 was tested in BG-11 medium modified for higher pH (8-9) by adding 1.0 g/L $NaHCO_3$ and 0.5 g/L of $MgSO_4$ was added. Growth of culture A.1.7 was screened in modified BG-11 medium for 15 days. Biomass was compared with normal BG-11 medium.

2.4 Cultivation of microalgae in the photobioreactor

2.4.1 Assembly of 2-L airlift photobioreactor

CO_2 sequestration experiment was carried out in the airlift photobioreactor made of Pyrex glass. The media holding capacity of the reactor was 2 L. The reactor has 10.16 cm inner diameter and 30 cm height. The vessel contained 8.32 cm high concentric draft tube, which has bottom clearance of 2.54 cm. Agitation and aeration was achieved by injecting mixture of CO_2 and air with sintered glass sparger located at the bottom of the draft tube. The flow of the gases was regulated using flow meter. The reactor was provided with the media outlet for sample collection and air outlet for degassing of the media.

The photobioreactor was operated in the semicontinuous mode. The culture system was maintained at 30-35ºC. Photobioreactor was externally illuminated using two daylight tube lights with total light intensity of 2775 lux placed around the vessel of the reactor vessel.

2.4.2 Provision for CO_2 feeding

Microalgal CO_2 tolerance was checked at seven different concentration of CO_2. Required concentration of CO_2 was achieved by mixing compressed air with pure CO_2 gas. The percentage of the CO_2 in air was calculated by formula,

$$\% CO_2 \text{ in gas mixture} = \frac{CO_2 \text{ flow rate}}{\text{Total Gas mixture flow rate}} \times 100$$

Table 1 gives the flow rates of CO_2 L/min and airflow rate to get desired percentage of the CO_2. In order to provide continuous pure CO_2 supply, CO_2 cylinder (Chemtron Science Laboratory, India) was used. The supply of CO_2 was regulated using regulator (Hind medico products, India) and flow meters (Napro, India). Compressed air was used to obtain desired CO_2 and air concentration.

Table 1. Mathematical relation between flow rate and percentage of CO_2.

Sr. no	CO_2 flow rate L/min	Air flow rate L/min	% CO_2
1	0	0.1	0.03
2	0.005	0.1	4.76
3	0.01	0.1	9.09
4	0.02	0.1	16.67
5	0.03	0.1	23.08
6	0.035	0.1	25.92
7	0.04	0.1	28.57

2.5 CO_2 sequestration experiment

The algal cultures were subjected to the semicontinuous culture system. In the beginning only compressed air, which comprised of ambient CO_2 concentration was supplied to the culture. The airflow was maintained at the rate of 0.1 L/min. Initially 10% inoculum was added to the reactor i.e. 200 ml of actively growing algal culture was added to 1800 ml medium. Algal cultures were cultivated at the ambient CO_2 concentration for a week before inoculation. Culture conditions were identical to the above mentioned conditions. The CO_2 air mixture was provided at 0.1-0.14 L/min flow rate. The pH changes occurring during the growth were recorded after every 24 hrs. After 7 days about 1600 ml of the algal suspension was removed and it was replaced by fresh BG-11 medium. The growth of the algal culture was interpreted in terms of the dry biomass. At this stage inlet air supply was switched over to 0.005 L/min CO_2 and 0.1 L/min compressed air i.e. the algal culture was supplemented with 4.09% CO_2. Each experiment was carried out semicontinuously for 5 days and CO_2 concentration was increased after completion of previous one.

2.6 Biomass harvesting

The biomass production in above mentioned semicontinuous CO_2 sequestration experiment was interpreted in terms of dry biomass produced per 100 ml suspension. For dry weight measurement a 100 ml sample was filtered through pre weighed Whatman No. 1 filter paper. The biomass was washed twice with distilled water in order to remove salts adhered to the algal cells. The resulting biomass was dried at 70ºC in a hot air oven for 24 hr and dry weight was calculated. Weight of the dried biomass was taken (Analytical balance Denver SI 234) until the constant weight was achieved.

2.7 Statistics

Three separate experimental sets were performed. Difference within groups in experiment was analyzed for statistical significance by ANOVA ($p<0.05$). Statistical analysis was done using the Microsoft Excel, Analyse-It software. Results were expressed as the mean ± SD.

3. Results and Discussion

3.1 Standardization of the culture conditions

3.1.1 Growth at different Temperatures

The growth of algal cells at three different temperatures was interpreted in terms of dry biomass as mentioned in Table 2. For cultures isolated from Aravali area (*Scenedesmus* sp. and *L. redekei)* optimum growth was observed at 22ºC. *Scenedesmus* sp. showed 75 mg/100 ml biomass production while *L. redekei* showed 102 mg/100 ml biomass concentration. At increased temperature 32ºC, growth declined significantly for both the cultures. *Scenedesmus* sp. fails to grow at 42ºC. *L. redekei* isolated from same location showed the similar response. At 22ºC, biomass produced was 102.5 mg/10 ml as the temperature was increased to 32ºC there was decline in a biomass to 87.50 mg/100 ml and at 42ºC the biomass produced by *L. redekei* was only 18.82 mg/100 ml on 6th day.

Three of the species isolated from hot springs (Ganeshpuri and Golavali) had significantly higher cell weight at harvest when cultured at increased temperature, 32ºC, (ANOVA $p<0.05$ Table 2). *Chrococcum* sp. was isolated from Golawali, where the temperature of the hot spring was 60ºC. This alga showed optimum growth at 32ºC with 120 mg/100 ml dry biomass. With increase in temperature, 42ºC, growth was observed with reduced biomass production. The biomass produced was 34.76 mg/100 ml, which indicates that at this temperature strain survived with less productivity. With adaptation to this temperature in second subculture this culture showed increased biomass productivity of up to 56.41 mg/100 ml after 15 days incubation.

Table 2. Effect of temperature on biomass production after 15 days of cultivation (-) culture did not survive.

Microalgal strains	Biomass mg/100 ml		
	22ºC	32ºC	42ºC
Scenedesmus sp. (Control)	75.00±1.41	42.56*±0.97	-
Chrococcum sp.	118.75±2.061	120±3.185	54.67*±4.306
L. redekei	102.5±1.047	87.50*±1.589	18.82*±1.417
G. sulphureum	24.55±3.258	40.00±2.964	34.29±4.088
P. crassa	39.21±3.307	66.21±2.044	45.34±3.718

[Each value is expressed as Mean ± SD of three replicate determinations. Means followed by * are significantly different at $p<0.05$ according to ANOVA test (n=3)]

The *G. sulphureum* isolated from Ganeshpuri where the temperature of the water was 70ºC and *P. crassa* isolated from Baragaon mandir (temperature of the spring- 68ºC), optimum biomass was produced at 32ºC. *G. sulphureum* also showed higher biomas, 52.94 mg/100ml, at 32ºC while decrease in the biomass was observed at 22ºC and 42ºC after 15 days cultivation. *P. crassa* showed higher biomass at 32ºC with decrease in growth at 22ºC and 42ºC.

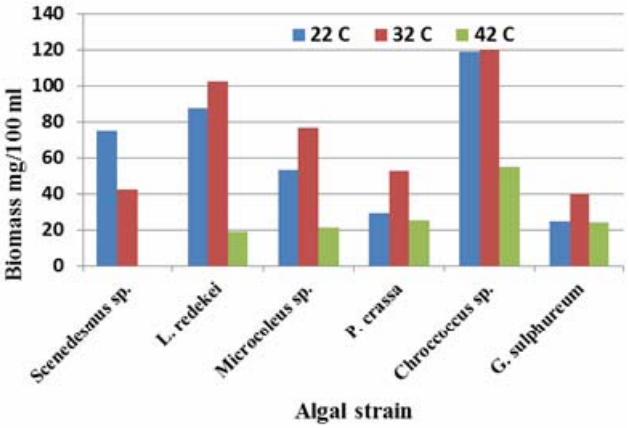

Figure 1. Effect of different temperatures on growth of isolated strains. Experiments were carried out in triplicate.

The majority of previous studies on microalgal CO_2 mitigation were focused on high-CO_2-tolerant mesophilic species, which grows at temperature range 25-30ºC. *Chroccoccus* sp., *G. sulphureum* and *P. crassa* showed temperature tolerance up to 42ºC though the optimum growth was observed at temperature 32ºC. We also found that the thermo tolerance of the culture can be increased by increasing inoculum size and acclimation of these species to this temperature for longer period through subsequent subcultures. These cultures therefore can grow well in outdoor cultivation with temperature variation found in tropical countries. Suryata et al. (2010) isolated a coccoid *cyanobacterium* from Blue Lagoon situated near the geothermal power plant, Grindavik, Iceland. Temperature of the lagoon water was approximately 50ºC [20]. This *cyanobacterium* showed maximum growth at 45ºC temperature. This strain was further utilized for mitigation of CO_2 emitted from Geothermal Power plant. Earlier Ono and Cuello (2007) studied the growth of the *Chlorogleopsis* sp. (SC2), a thermophilic cyanobacterium originally isolated from the Yellowstone National Park [10]. The organism was able to sustain temperature upto 50ºC with 5% CO_2 and the biomass produced by the organism was 1.4 g/L which was comparable to the mesophilic strains like *Chlorella*. Further this strain was selected by Green shift technology to mitigate CO_2.

3.1.2 Growth at different pH

During algal growth there is steady decrease in nutrients that is accompanied by change in pH of surrounding medium. The major components of the medium deciding pH of the medium are HCO_3^- and CO_3^{-2}. In natural buffer system it is described by the following equation,

$$H_2O + CO_2 \rightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{-2}$$

This system is major determinant of the pH. When algae perform photosynthesis, CO_2 fixation shifts equilibrium to the left and pH increases. During algal respiration CO_2 is produced and shifts the equilibrium right with decreasing pH.

To study the effect of the pH on the growth of isolated strains they were subjected to the range of pH 6-9. Growth was analyzed for the period of 15 days. Initial inoculum was 10mg/100 ml dry weight. The results are tabulated in Table 3. For *Scenedesmus* sp. optimum dry biomass (42.50 mg/100 ml) was observed at pH 7.4. As pH increased there was significant decrease in the biomass production. At pH 8.0, biomass decreased to 32.01 mg/100 ml and at still higher pH (pH 9.0). A.1.1 did not show any growth due to chlorosis (yellowing) of the cells. Culture *Chrococcum* sp. showed optimum growth at pH 7.4 (117.50 mg/100 ml) and it was decreased at lower and higher pH. Biomass produced at 6.0 was 95.47 mg/100 ml and at pH 9.0 it was 85.16 mg/100 ml. Similar results were obtained for strains *L. redekei*. At pH 6 the dry biomass produced was only 48.26 mg/100 ml. As pH was increased to 7.4 it substantially increased to 87.50 mg/100 ml. At pH 8 and pH 9.0 there was little decrease observed viz. 68.26 mg/100 ml and 65.26 mg/100 ml respectively. Difference in biomass concentration is represented in Figure 3.

P. crassa and *G. sulphureum* were proven to grow more efficiently at alkaline pH range. As the pH advanced there was steady increase in the biomass observed. Strain *G. sulphureum* produced 64.98 mg/100 ml biomass at pH 6. At pH 7.4 it was as high as 66.32 mg/100 ml. At pH 8.0 and pH 9.0 it still increased to 67.59 mg/100 ml and 75.18 mg/100 ml. For *P. crassa* at pH 6 biomass produced was 75.36 mg/100 ml and at pH 7 it was 76.21 mg/100 ml. Increase in biomass of 79.57 mg/100 ml was observed at pH 8 and it increased significantly to 95.12 mg/100 ml at pH 9. The capability of *P. crassa* and *G. sulphureum* to withstand high pH has significance.

Gao et al. (1993) studied the effect of the elevated CO_2 concentration on *Covallina pilulifera* growth and pH of the medium [21]. When 360 ppm of CO_2 was supplied during the 12 hrs of day period there was a gradual increase in pH from initial 8.2 to final 8.6. While in the 12 hrs of night period with the same level of CO_2 (360 ppm) pH decreased from initial 8.6 to 7.8. Increase in pH during the light phase may be attributed to photosynthetic utilization of the HCO_3^-. At night CO_2 dissolution during respiration might have exceeded CO_2 removal by photosynthesis and hence pH drops down.

3.2 CO_2 sequestration studies

3.2.1 Assembly of CO_2 sequestration experiment

CO_2 sequestration experiments were done in 2 L airlift photobioreactor which comprises of the two concentric vessels. CO_2 from CO_2 cylinders (Chemtron Science Laboratory, Mumbai) in combination with the compressed air was sparged through air diffuser present at the base of the inner vessel of bioreactor (Figure 2(A)&(B)). The flow rate of CO_2 and compressed air was adjusted with flow meter (Table 1) and various CO_2 concentrations could be fed to the algal culture.

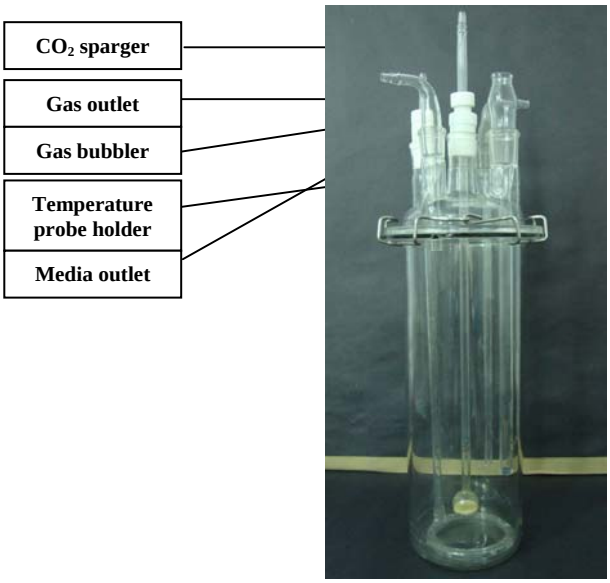

Figure 2(A)

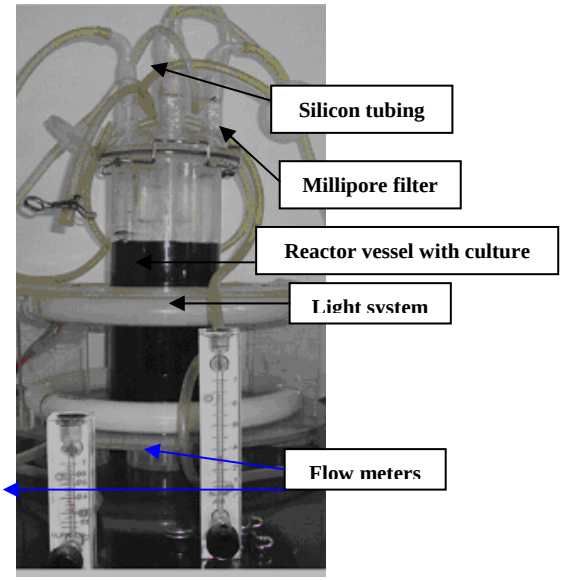

Figure 2(B)

Figure 2. (A) Assembly of the photobioreactor, showing all the ports arranged and **(B)** complete assembly of the reactor with algal growth.

Table 3. Effect of pH on the biomass production of algae after 15 days cultivation at ambient temperature.

pH → / Culture ↓	Dry Biomass mg/100 ml			
	6.0	**7.4 (Normal BG-11)**	**8.0**	**9.0**
Scenedesmus sp.	35.26±1.478	42.56*±0.543	32.01* ±0.902	Yellowing
Chrococcum sp.	95.47 ±0.680	117.50*±1.020	109.80*±0.825	85.16*±0.762
L. redekei	48.26±0.665	87.50* ±0.308	68.26* ±0.370	65.26*±0.561
P. crassa	75.36± 0.254	76.21 ±0.205	79.57* ±0.852	95.12*±0.925
G. sulphureum	64.98 ±0.602	66.32 ±0.207	67.59 ±1.013	75.18 *±0.515

[Each value is expressed as Mean ± SD of three replicate determinations. Means followed by * are significantly different at $p<0.05$ according to ANOVA test (n=3)]

3.2.2 Effect of CO_2 sparged on pH of media

In the beginning only compressed air, comprising of ambient concentration of CO_2 was supplied to the culture. The airflow was maintained at the rate of 0.1 L/min. A precultured algae was inoculated in culture vessel of photobioreactor in 2000 ml culture volume at an initial biomass concentration (calculated in terms of dried weight of algal cells per L, g/L) of 0.01 g/L Initially the algal cultures were allowed to grow for 15 days to attain the stationary phase and then the reactor was operated in semicontinuous mode. After 15 days about algal suspension was removed and replaced by fresh BG-11 medium in such a way that 10% inoculum was available next operation cycle. At this stage inlet air supply was switched over to 0.005 L/min CO_2 and 0.1 L/min compressed air i.e. algal cultures were supplemented with 4.09% CO_2. The culture was allowed to grow at this concentration of CO_2 for 5 days. On the 5th day grown culture was removed and 10% volume was continued as inoculum source for the further experiment. In further experiment 0.01 L/min CO_2 and 0.1 L/min air i.e. 9.09% CO_2 was fed to the cultures. In the same fashion the bioreactor was operated in semicontinuous mode and CO_2 concentration was increased up to 25.92%.

For *L. redekei* when the culture was grown in ambient CO_2 concentration the growth of the algae was associated with the increase in pH (Table 4). The initial pH of the medium was 8.52 as the growth advanced it gradually increased to 9.56, on 3rd day it was 10.3 and on the fifth day it was as high as 10.85. The biomass produced at the end of the 5th day was 30 mg/100 ml. But when *L. redekei* was sparged with 9.09% of CO_2 increase in pH due to growth was compensated by carbonic acid (HCO_3^-) formed due to dissolution of the carbon dioxide. On 1st day pH of medium was 7.32 which increased to 8.56 on 3rd day and on 5th day it further increased to 9.16. It was associated with the production of 62.00 mg/L biomass. The culture *L. redekei* when fed with the 16.67 % CO_2 the same phenomenon was prominently observed with respect to the change in pH. In the beginning pH of the medium was 7.22 on 3rd day there was very little increase in pH to 7.26 and on 5th day it increased to 7.66. Because CO_2 dissolved in the medium was utilized by growing cultures and pH was maintained constant. The *L. redekei* showed highest growth at this concentration of CO_2 with two fold increase in biomass (that is, 68.34 mg/100 ml) than optimum CO_2 concentration. With further increase in the CO_2 concentration to 23.08% decrease in pH due to dissolution of CO_2 which was not compensated by algal growth. pH drop was 5.84 with 7.25 initial pH. And due to lowering of the pH at this CO_2 concentration the growth of algae was inhibited and biomass produced at the 5th day is only 38.50 mg/100 ml. When concentration of CO_2 was increased to the 28.92% on day one pH of media was 6.96 which decreased to 5.52 on day 5. This had a negative impact on the growth of the *L. redekei* leading to production of only 28.00 mg/100 ml dry biomass which was even less than biomass produced at ambient CO_2 concentration. Culture *Scenedesmus* sp. and *Chrococcum* sp**.** showed the similar pattern of pH variation in response to introduction of the CO_2 in culture media.

For the algal strains that supported growth at alkaline pH showed the different response. Culture *G. sulphureum* was grown in the spirulina medium with initial media pH 9.11. Initially the culture was allowed to grow with ambient CO_2 concentration. At the end of the 5th day pH of the media was increased to the 10.53 with the production of 19.45 mg/100 ml dry biomass. CO_2 concentration was then increased to 9.09%; the initial pH of the culture was 9.03. Introduction of the CO_2 caused decrease in media pH on day three to 8.13. There was no further decrease in the media pH was found. On 5th day media pH observed was 8.19.

With initial pH 8.66 of media culture was fed with 16.67% CO_2. As the growth commenced pH of the media increased to 9.81 on 3rd day of cultivation. On 5th day pH was 8.52 media pH. At this concentration of CO_2 maximum growth was observed with 53.57 mg/100 ml biomass production. The growth observed was three fold more than biomass produced at ambient CO_2 concentration. With introduction of 23.08% CO_2 growth was not hampered due to increased CO_2 concentration. Biomass produced was 52.96 mg/100 ml and with initial media pH of 8.59 it changed to 8.44 on day five.

Decrease in the biomass was observed at 28.57% CO_2 concentration. On first day pH of the media was 8.38 and decreased to the 8.21 on 3rd day of incubation. It became 7.95 on day five. Drastic decrease in the media pH indicated that the effect of the CO_2 dissolution was not compensated by the algal growth. The biomass produced at this concentration was 45.06 mg/100 ml which was less. But it was much higher than biomass at ambient concentration of the CO_2 (that is, 19.45 mg/100 ml).

In the present study also for strain *L. redekei* at ambient CO_2 concentration on day one pH of the media was 8.52. After 5 days growth (table 1.6), pH increased to 10.85. When the culture was fed with 16.67% CO_2, increased amount of the CO_2 was utilized by the photosynthetic fixation and there was no much change in the pH. On day 1 pH of the medium was 7.42, which remained unchanged on 5th day with 7.66 pH. This is also depicted in formation of the highest biomass at this concentration. At 16.67% CO_2 *L. redekei* showed highest biomass productivity with 2 fold increase in biomass. When CO_2 concentration was increased to 23.08% there was decline in pH. Initial pH 7.25 of media declined to 6.54 after five days growth. This decrease in pH did not support the photosynthesis and growth. At this concentration there was sharp decline in production of biomass. The biomass produced was 38.50 mg/100ml. Similar observations were made by Reddy (2002) [22]. They carried out CO_2 sequestration using *Scenedesmus* sp. CO_2 concentration applied was in the range of 0-50% CO_2. The initial pH of the medium was 7.85 when culture was supplemented with ambient CO_2 concentration. pH of the medium was increased to 10.45 at the end of five days. When culture of *Scenedesmus* sp. was supplemented with 5% CO_2, the pH of the medium initially drops down to 6.1 and after growth for 5 days the final pH was increased to 7.18. Growth at increased concentration of CO_2 (20%) brought about significant decrease in the initial pH of the medium up to 5.83 and after five days growth there was no significant change in pH and the final pH was 6.51.

3.2.3 Effect of various concentrations of CO_2 on algal growth

Effect of the different concentrations of the CO_2 on biomass production of algae is presented in table 1.4. For *Scenedesmus* sp., at ambient concentration of CO_2 the dry biomass produced at the end of the 5th day was 35.00 mg/100 ml. As the concentration of CO_2 was increased from ambient to 16.67% CO_2 there was steady increase in growth and the biomass produced was 51.33 mg/100 ml. 23.08% CO_2 there was decline in the growth and biomass was decreased to 34.17 mg/100 ml. Similar trend was observed for *L. redekei* and *G. sulphureum* with 16.67% CO_2 concentration as the most conducive for formation of the higher biomass.

P. crassa has highest ability to sequester CO_2. For culture *P. crassa* and *Chrococcum* sp. the tolerance level of the CO_2 was up to 23.08%. For *P. crassa* the biomass produced at the ambient CO_2 concentration was 18.9 mg/100 ml and it increased to 35.60 mg/100 ml at 23.08% CO_2. At 28.51% CO_2 concentration biomass produced was very little less than the 23.08% and it was 31.05 mg/100ml, which was much higher than the biomass produced at the ambient CO_2 concentration.

Among all strains screened *L. redekei* and *G. sulphureum* showed highest biomass production. For *L. redekei* at ambient CO_2 concentration biomass produced was 30 mg/100 ml whereas at 16.67% CO_2 the biomass produced was 68.34 mg/100 ml that was twofold more than the biomass at ambient CO_2 concentration. At 23.08% it declined drastically to 38.5 mg/100 ml, whereas *G. sulphureum* showed the 53.57 mg/100 ml biomass at the 16.67% which was the three times greater than the biomass produced at ambient CO_2 concentration (19.45 mg/100 ml). At 23.08% there was little decline in the biomass produced, which was 52.96 mg/100 ml. Chinnasamy et al. (2009) studied the growth response of *Chlorella vulgaris* ARC-1 under narrow range of CO_2 concentration from 0.038-20% CO_2 [7]. When CO_2 concentration was raised from 1% to 6% there was a sharp increase in chlorophyll synthesis and biomass production. A maximum increase of 114% in dry weight was observed when CO_2 was raised from 2 to 3%. The growth reached a plateau between 6 to 12% and then it declined sharply. The highest biomass 200 µg/ml was observed at 6% CO_2. At 10% CO_2 biomass declined to 150 µg/ml. At 12% there was sharp decline in the biomass production but it was comparable to ambient CO_2 concentration.

3.2.4 Effect of the CO_2 concentration on the biomass productivity of algae

In the present study biomass production for each culture at various concentration of the CO_2 was carefully monitored in semicontinuous photobioreactor. For *Scenedesmus* sp. isolated from the hot spring of Aravali, when the CO_2 concentration was increased from 0.03% to the 16.67% there was increase in biomass production. The growth reached a plateau at 16.67% CO_2 concentration and declined at 23.08% CO_2 concentration (Figure 3). This shows the efficiency of the isolated *Scenedesmus* sp. to sequester CO_2 at higher concentration as compared to the earlier reports. This strain was able to sequester CO_2 up to 28.57% concentration. Among the cyanophyceaen group, for *L. redekei*, the growth showed sharp increase from 4.76% and plateau was observed at 16.67% CO_2 concentration. Growth was sharply decreased with 23.08% and 28.57% CO_2 concentration. For *G. sulphureum* similar growth changes were found. Growth was increased from ambient CO_2 concentration and at 16.67% CO_2 concentration plateau was achieved. At 28.57% CO_2 concentration the biomass was decreased to 45.06 mg/100 ml dry biomass. But this biomass was more than biomass at ambient CO_2 concentration (Table 4). This shows that increase in the CO_2 concentration upto 23.08% is acceptable for the culture *G. sulphureum.* This alga also sustains comparatively quite high temperature (42ºC) and CO_2 concentration and wide range of pH.

At 16.67% CO_2 biomass produced was 51.33 mg/100 ml and the biomass productivity was 0.228 g dw/L/day. The biomass productivity for *Scenedesmus* sp. isolated by Yoo et al. (2010) at 10% CO_2 concentration was 0.217 g dw/L/day. Here culture was grown in photobioreactor for 14 days [23].

However very few references are available on CO_2 sequestration and biomass studies using filamentous blue green algae. The most frequently used *Cyanophyceaen* filamentous algae is *Spirulina* sp. The study on filamentous cyanobacterium, *Spirulina* sp., was carried out by Morais and Costa (2007) [24]. The strain was grown at 6% and 12% CO_2 concentration in three-stage tubular photobioreactor. Maximum biomass productivity found was 0.22 g/L/day with 6% CO_2 concentration and at 12% CO_2 there was no much change in the biomass productivity. Ho et al. (2010) studied the growth of *S. obliquus* CNW-N with the 20% CO_2 concentration and showed biomass productivity of 0.201 g dw/L/day [25]. The cultivation of the *Spirulina* was carried out in photobioreactor for 14 days with artificial CO_2 gas.

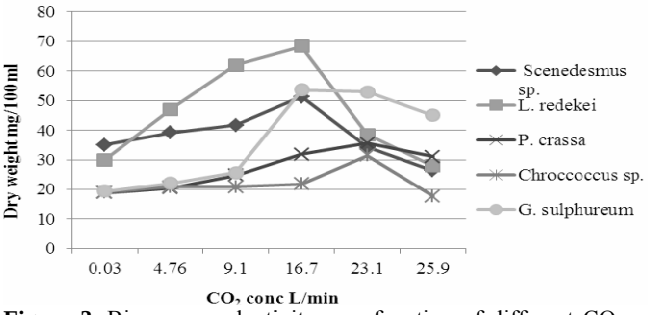

Figure 3. Biomass productivity as a function of different CO_2 concentrations. Cultures were cultivated at 32ºC with 0.1 L/min air and CO_2 mixture.

4. Conclusion

In the present study maximum biomass production was found for culture *L. redekei* with biomass productivity of 0.304 g/L/day. *G. sulphureum* at 16.67% CO_2 concentration biomass productivity was 0.242 g dw/L/day. While *P. crassa* showed maximum CO_2 tolerance with biomass productivity of 0.163 g/L/day. Thus High temperature adaptability, high CO_2 tolerance and reasonably high biomass production makes *G. sulphureum* a promising candidate for the CO_2 sequestration in the Tropical climatic conditions. Again culture is easy to harvest, on standing media for half hour settle down the culture, which can be separated by decanting clear media and filtering the algal

Table 4. Effect of CO_2 on algal biomass production and pH of the media, experiments carried out at ambient temperature.

CO_2 conc. %	*Scenedesmus* sp.			*L. redekei*		
	Algal biomass mg/100 ml	Initial pH	Final pH	Algal biomass mg/100 ml	Initial pH	Final pH
0.03	35.00	7.20	7.89	30.00	8.52	10.85
4.76	39.20	7.20	7.36	47.00	10.00	10.69
9.09	41.67	7.40	6.35	62.00	7.32	9.16
16.67	**51.33**	7.40	6.12	→ **68.34**	7.22	7.66
23.08	34.17	7.15	5.88	38.50	7.25	6.54
28.57	26.30	7.01	5.48	28.00	6.96	6.12

CO_2 conc. %	*P. crassa*			*Chrococcum* sp.			*G. sulphureum*		
	Algal biomass mg/100 ml	Initial pH	Final pH	Algal biomass mg/100 ml	Initial pH	Final pH	Algal biomass mg/100 ml	Initial pH	Final pH
0.03	18.90	9.26	9.81	18.98	7.50	10.52	19.45	9.11	10.53
4.76	20.45	7.86	9.56	20.91	8.70	6.31	22.13	9.26	8.64
9.09	24.80	7.82	8.27	20.91	7.37	7.75	25.71	9.03	8.19
16	31.80	7.75	8.65	21.84	7.51	6.95	→ **53.57**	8.66	8.52
23.08	**35.60**	7.88	8.56	**31.46**	7.28	6.89	52.96	8.59	8.04
28.57	31.05	7.09	6.89	17.67	7.49	6.55	45.06	8.18	7.55

flocks. This reduces the harvesting cost making *G. sulphureum* a promising candidate for CO_2 sequestration and mass cultivation.

CO_2 mitigation becomes feasible when the resulting biomass is used as fuel, food or feed. Attempts are being made to screen the isolated cyanophyceaen genera for lipid and fatty acid production.

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