Journal of Environmental Science and Technology

ISSN 1994-7887

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Environmental Science and Technology

ISSN 1994-7887
DOI: 10.3923/jest.2019.186.196

Research Article
Evaluation of CO_2 Removal Efficiency of *Pseudanabaena limnetica* (Lemm.) *Komárek* Grown in Na_2CO_3 Enriched Seawater Medium in 60 L Airlift Flat Panel Photobioreactor

[1]Chaitanya Magar, [1]Sagar Rambhiya and [2]Manjushri Deodhar

[1]Department of Biotechnology, KET's Vinayak Ganesh Vaze College of Arts, Science and Commerce, Mithagar Road, Mulund East, 400081 Mumbai, Maharashtra, India
[2]Botany Research Laboratory, Department of Botany, KET's Vinayak Ganesh Vaze College of Arts, Science and Commerce, Mithagar Road, Mulund East, 400081 Mumbai, Maharashtra, India

Abstract

Background and Objective: Oceans are major sinks for the anthropogenic CO_2 and microalgae play a characteristic role in mitigating it. Due to the heavily released anthropogenic CO_2 into the environment, the natural phenomena those control the CO_2 concentration in atmosphere are now not sufficient enough to normalise it. This has imparted on a need of novel environmental friendly option of CO_2 sequestration. Cultivating microalgae for biofuel can directly consume the industrially released CO_2 and help to reduce the release of anthropogenic CO_2, but making this CO_2 available into the liquid medium by artificial mean is the challenge. Buffering ability of seawater accelerates the mass transfer of CO_2 in an aqueous phase and can hasten up the removal of anthropogenic CO_2. **Materials and Methods:** In present communication salt tolerating strain *Pseudanabaena limnetica* (Lemm.) *Komárek* was grown in the operationally optimized 60L flat panel photobioreactor containing Na_2CO_3 rich Modified Seawater BG11 medium (MSWBG11). Initially, CO_2 dissolution capacity of only seawater and MSWBG11 medium was determined. Then the effect of 2 different CO_2 flow rates viz. $\leq$0.001LPM (set I) and $\leq$0.005LPM (set II) on CO_2 dissolution (DCO_2), associated pH change and its effect on biomass production in MSWBG11 were studied. **Results:** In the control experiment the DCO_2 was reduced from 2.08-1.19 mg L^{-1} and the amount of dry biomass produced was 1.15 g L^{-1}. In set I the DCO_2 concentration was maintained in the range of 8-15 mg L^{-1} and the biomass produced was 1 g L^{-1}. In set II the DCO_2 remained in the range of 3.59-4.00 mg L^{-1} and the biomass produced was 1.20 g L^{-1}. **Conclusion:** Thus, feeding of CO_2 at a lower flow rate was found to be favorable for algal growth. Though the mass transfer coefficient (KLa min^{-1}) increased at the higher CO_2 flow rate, the actual percentage of CO_2 removal was not increased.

Key words: CO_2, seawater, salt tolerating, flat panel photobioreactor, mass transfer coefficient (KLa min^{-1}), percentage CO_2 removal

Citation: Chaitanya Magar, Sagar Rambhiya and Manjushri Deodhar, 2019. Evaluation of CO_2 Removal efficiency of *Pseudanabaena limnetica* (Lemm.) *Komárek* grown in Na_2CO_3 enriched seawater medium in 60 L airlift flat panel photobioreactor. J. Environ. Sci. Technol., 12: 186-196.

Corresponding Author: Manjushri Deodhar, Botany Research Laboratory, Department of Botany, KET's Vinayak Ganesh Vaze College of Arts, Science and Commerce, Mithagar Road, Mulund East, 400081 Mumbai, Maharashtra, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

It is well known that algae fix CO_2 and must be doing so by extracting it from the aqueous medium in which it is dissolved. Typically this CO_2 is transferred from the atmospheric CO_2 concentration of 0.04%. Microalgae produce approximately 50% of the atmospheric oxygen on the earth while consuming vast amounts of Carbon dioxide[1]. The 1 kg of dry algal biomass utilizes about 1.83 kg CO_2. Therefore, in order to take advantage of high CO_2 content (~20%) in exhaust flue gases, it is conventional wisdom that the chosen strain of microalgae must be found that tolerates the high concentration of dissolved CO_2.

Higher CO_2 concentration generally favors the algal growth but the barrier is a stability of the dissolved CO_2 in aqueous solution. There has been a focus on improving the rates of CO_2 mass transfer within a microalgal growth medium. The most common method is to sparge culture with bubbles of flue gases containing CO_2. In order to achieve better CO_2 mass transfer the unique design of fluid oscillator had also been proposed by some scientists to significantly reduce the bubble size[2]. Alternatively, CO_2 from fossil fuel stations shall be absorbed into solvents containing inorganic salts such as potassium carbonate and CO_2 loaded solvent which is then pumped directly into raceway ponds or photobioreactors (PBR)[3].

It has been reported that the microalgal cultures in PBRs are usually sparged with CO_2-enriched air (generally 5%)[4], but this practice is not fruitful as most of the free CO_2 escapes out into the atmosphere and sparging excess of CO_2 doesn't mean that it will not cause CO_2 limitation. To resolve this issue, maintaining dissolved CO_2 concentration above the critical level would be mandatory. The critical CO_2 level is the level of dissolved CO_2 just enough for the growth of particular algal strain, which is determined by experimenting the different concentration of dissolved CO_2 in the growth medium and checking their effects on algal growth. When large scale cultivation is the target, the CO_2 demand would be higher with the conventional ways of CO_2 feeding which will not be economical. Hence maintaining CO_2 concentration at a critical level would reduce the cost of CO_2 feeding significantly by curtailing down it to the actual requirement of CO_2 for algae culturing[5].

CO_2 has some significant limitations in mass transfer. When it is passed into aqueous phase it reduces pH due to the formation of H^+ and HCO_3^- ions. Basically, microalgal cells preferentially uptake HCO_3^- over CO_2 despite the fact that HCO_3^- is a poor source of carbon than the CO_2[6]. And therefore this hastens up the escape of unreacted, free CO_2 into the atmosphere resulting in a significant CO_2 loss. pH is the major determinant of maintaining relative CO_2 concentration in the aqueous system. This can be done by dissolving carbonate or bicarbonate salt in seawater, making it a best buffering system. This system does not allow a sudden drop in a pH of seawater medium with CO_2 feeding and therefore, accelerates the uptake of anthropogenic CO_2. For scavenging maximum CO_2 using microalgae, process parameters such as sodium bicarbonate concentration, pH, aeration and agitation rates and rate of CO_2 sparging along with bubble size are need to be optimized.

The salt tolerant algal strain *Pseudanabaena limnetica* (Lemm.) *Komárek* isolated from salt pans of the eastern suburban region of Mumbai, India is capable of tolerating Na_2CO_3 rich alkaline seawater medium (pH reaches up to 9)[7]. The strain is also withstand to natural conditions of high light intensity up to 1,296 μmol m^{-2} sec^{-1} and temperature up to 38°C. The operationally standardized 60L flat panel PBR system for cultivation of *P. limnetica* was used here in CO_2 sequestration experiments[8].

In the present communication, using pneumatically agitated 60 L flat panel culture system CO_2 was sparged at different flow rates in Na_2CO_3 rich modified seawater BG11 medium. The amount of CO_2 dissolved in the medium was measured by the CO_2 sensor. Through this, an optimum suitable concentration of CO_2 feeding which favors the algal growth has been determined.

MATERIALS AND METHODS

Strain selected: Microalgal strain selected was *Pseudanabaena limnetica* (Lemm.) *Komárek*, which is an indigenous halophilic strain isolated from salt pans of Mumbai suburban region[7].

Culture medium: Modified Seawater BG11 medium (MSWBG11) was formulated from the BG11 medium as tabulated[9] in Table 1. The chemicals used were obtained from Loba Chemie Pvt. Ltd.

Photobioreactor design and culture conditions: To conduct the experiments for CO_2 sequestration, the culture was scaled up from 8-60 L photobioreactor in above-mentioned culture medium and then maintained in the 60 L photobioreactor. The photobioreactor was made up of glass and dimensions were 20 cm (breadth) × 50 cm (length) ×70 cm (height)[8]. The initial culture density of *P. limnetica* was maintained at 0.25± 0.05 g L^{-1} in the MSWBG11 medium.

Table 1: Composition of the modified seawater BG11 medium (MSWBG11)

Compounds	(g L^{-1})
$NaNO_3$	0.7
$K_2HPO_4.3H_2O$	0.04
$MgSO_4.7H_2O$	0.075
$CaCl_2.2H_2O$	0.036
Citric Acid	0.006
Ferric Ammonium Citrate	0.006
$Na_2EDTA.2H_2O$	0.001
Na_2CO_3	0.2
H_3BO_3	0.00286
$MnCl_2.4H_2O$	0.00181
$ZnSO_4.7H_2O$	0.000222
$Na_2MoO_4.2H_2O$	0.000390
$CuSO_4.5H_2O$	0.000079
$Co(NO_3)_2.6H_2O$	0.0000494

The culture was exposed to the white fluorescent light of 185-222 μmol m^{-2} sec^{-1} intensity for 12 h every day. The agitation was achieved through spargers situated as horizontal tubes at the base of the photobioreactor. The compressed air supplied at 4LPM of air flow rate which was controlled with the help of acrylic flow meters i.e., rotameters (Napro Scientific, Pune).

CO_2 feeding: The CO_2 supply was obtained from the CO_2 cylinders commercially prepared by Super Industrial Gases, Thane. The cylinder contains 100% pure compressed CO_2. The flow rate of CO_2 was controlled with the help of pressure regulator. Further, for accurate flow rate, rotameter of 0.001LPM to 0.05LPM air flow rate measurement capacity was used. The gaseous CO_2 was fed into the medium at a flow rate of 0.01LPM through the sintered glass sparger made up of borosilicate glass material (Deepali enterprises, Mumbai). For experiment purpose, two different flow rates were achieved viz. $\leq$0.005LPM and $\leq$0.001LPM using same rotameter and sparger system.

pH, temperature, dissolved oxygen (DO_2) and dissolved carbon dioxide (DCO_2)

Detection and biomass estimation: The detection and monitoring of pH, Temperature, DO_2 and DCO_2 were carried out using electronic sensors, which were immersed in the working liquid system of the photobioreactor. All the individual sensors i.e. pH, DO_2 and DCO_2 along with inbuilt temperature sensor in each of them were obtained from Mettler-Toledo India Pvt. Ltd. The readings from pH, Temperature and DO_2 sensors were noted at every minute throughout the experiment. For proper representation purpose, the three (n = 3) continuous readings of respective three consecutive min were considered. These readings were purposefully derived from the same time period every day. In case of DCO_2 sensor the readings of each and every second was recorded by the data logging system but as explained for other sensors, same was followed for obtaining three consecutive readings (n = 3) for dissolved CO_2 concentration also. The dry weight of the biomass (DWB) was estimated by gravimetric analysis. Gravimetric analysis was performed by withdrawing three culture samples (n = 3) from the photobioreactor on every 3rd day after inoculation till the end of the experiment. Each type of experiment was performed in triplicates and selective readings were noted as mentioned before. Further, f or these shortlisted readings, statistical mean value was calculated along with their respective standard deviation (SD). The final cumulative data obtained containing Mean±SD was tabulated in Table 2, 3 and 4 into the respective category of the experiments.

Comparison of CO_2 dissolution ability of tap water, seawater and MSWBG11 medium: To study CO_2 dissolution ability three different aqueous samples were selected viz. normal tap water, normal seawater and seawater-based modified BG11 medium (MSWBG11). The experiments were carried out in a 60L PBR system. During experiments, these aqueous samples were pneumatically agitated at 4LPM. CO_2 was sparged through sintered glass sparger at 0.01LPM. The rate of dissolved was noted for each water source until the pH drops down from alkaline to neutral or slightly acidic. Dissolved O_2 and associated pH change were noted with the help of dissolved O_2 sensing sensor and pH sensor, respectively. The

Table 2: CO_2 dissolution rate with pneumatic agitation in tap water, seawater and modified seawater BG11 (MSWBG11) medium and its effect on DCO_2 value and pH

	Tap water		Seawater		MSWBG11 Medium	
Time (h)	pH (Mean±SD)	DCO_2 (Mean±SD) mg L^{-1}	pH (Mean±SD)	DCO_2 (Mean±SD) mg L^{-1}	pH (Mean±SD)	DCO_2 (Mean±SD) mg L^{-1}
0	7.33±0.12	2.00±0.23	7.95±0.07	2.17±0.35	8.36±0.16	3.04±0.56
1	6.92±0.09	3.03±0.11	7.46±0.30	5.60±0.15	8.04±0.12	3.77±0.92
2	6.47±0.09	5.10±0.16	7.22±0.06	9.58±0.71	7.63±0.10	7.66±0.40
3	7.13±0.18	3.46±0.08	7.35±0.23	12.05±0.98	7.38±0.10	18.30±1.17
4	7.60±0.16	2.52±0.26	7.58±0.31	4.36±0.39	7.51±0.18	12.18±0.72
5	7.77±0.07	1.89±0.10	7.87±0.15	3.02±0.27	7.59±0.11	7.37±0.60
6	-	-	7.82±0.22	2.59±0.46	8.02±0.14	4.70±0.28
7	-	-	-	-	8.29±0.18	3.34±0.37

Table 3: Effect of growth of *P. limnetica* on DCO_2, DO_2 level and pH of the MSWBG11 medium along with the percentage of CO_2 consumption/day

Days	pH (Mean±SD)	Temperature (Mean±SD)°C	DCO_2 (Mean±SD) mg L^{-1}	DO_2 (Mean±SD) mg L^{-1}	CO_2 consumption (%)	Biomass produced (Mean±SD) g L^{-1}
1	08.73±0.14	30.70±0.45	1.97±0.07	09.66±0.28	4.37	0.33±0.025
2	08.84±0.12	30.30±0.24	1.91±0.07	09.71±0.32	5.73	-
3	08.94±0.13	30.10±0.21	1.83±0.01	09.81±0.30	7.07	0.37±0.016
4	09.05±0.09	30.33±0.74	1.70±0.03	10.03±0.27	2.91	-
5	09.28±0.25	30.30±0.49	1.70±0.02	10.43±0.40	7.02	-
6	09.44±0.40	29.93±0.58	1.60±0.02	10.42±0.35	5.56	0.39±0.008
7	09.66±0.45	30.80±1.18	1.52±0.02	10.42±0.29	4.58	-
8	10.02±0.68	30.56±0.55	1.45±0.02	10.46±0.22	4.03	-
9	10.22±0.80	29.76±0.90	1.44±0.03	10.73±0.40	4.83	0.75±0.008
10	10.09±0.14	29.86±0.86	1.39±0.03	10.76±0.36	4.23	-
11	09.96±0.65	30.56±0.94	1.33±0.03	10.60±0.32	3.70	-
12	09.89±0.63	31.03±0.86	1.32±0.02	10.51±0.25	3.01	0.80±0.041
13	09.65±0.39	30.96±0.60	1.32±0.02	10.41±0.38	3.01	-
14	09.70±0.48	31.66±0.82	1.29±0.03	10.15±0.24	5.34	-
15	09.58±0.37	31.56±0.82	1.26±0.03	10.13±0.37	5.43	0.91±0.014
16	09.54±0.37	32.00±0.62	1.18±0.14	09.95±0.25	5.69	-
17	09.53±0.40	31.33±0.50	1.18±0.14	10.10±0.39	2.50	-
18	09.43±0.35	31.63±0.74	1.18±0.14	09.79±0.26	3.33	1.11±0.017
19	09.55±0.41	32.26±0.90	1.15±0.14	09.64±0.29	3.39	-
20	09.35±0.32	31.83±0.48	1.17±0.14	09.68±0.25	2.54	-
21	09.30±0.29	31.80±0.51	1.17±0.14	09.42±0.20	3.39	1.15±0.041

Table 4: Effect of continuous feeding of CO_2 at $\leq$0.005 LPM flow rate on the growth of *P. limnetica*, DCO_2, DO_2 level and pH of the MSWBG11 medium along with percentage CO_2 consumption/day

Days	pH (Mean±SD)	Temperature (Mean±SD)°C	DCO_2 (Mean±SD) mg L^{-1}	DO_2 (Mean±SD) mg L^{-1}	CO_2 consumption (%)	Biomass produced (Mean±SD) g L^{-1}
1	7.92±0.60	30.03±1.07	08.22±0.53	8.45±0.74	23.40	0.30±0.065
2	7.63±0.12	30.93±1.00	06.55±1.03	9.06±0.50	30.55	-
3	7.63±0.11	30.43±0.70	06.82±0.83	9.23±0.41	21.52	0.36±0.005
4	7.55±0.11	29.83±1.23	07.52±0.64	9.53±0.62	27.55	-
5	7.55±0.11	30.47±1.07	08.50±0.53	9.40±0.70	17.87	-
6	7.75±0.03	30.73±1.10	09.12±0.55	9.53±0.70	32.39	0.44±0.001
7	7.63±0.05	30.60±1.10	11.97±1.19	9.73±0.57	33.83	-
8	7.61±0.11	30.73±1.06	16.56±2.19	9.68±0.59	18.42	-
9	7.71±0.07	30.63±1.06	12.52±0.10	9.63±0.61	15.41	0.60±0.009
10	7.78±0.03	30.40±1.10	11.22±0.65	9.80±0.53	31.21	-
11	7.95±0.30	30.47±1.07	10.11±4.37	9.70±0.39	37.78	-
12	7.68±0.11	30.00±0.82	13.60±3.00	9.69±0.55	19.42	0.75±0.004
13	7.76±0.04	29.50±0.57	12.29±1.05	9.67±0.47	18.41	-
14	7.83±0.03	29.80±1.02	10.34±0.73	9.69±0.35	18.33	-
15	7.86±0.07	29.23±0.79	10.90±0.85	9.76±0.50	26.75	0.84±0.009
16	7.98±0.08	29.20±1.00	12.31±0.93	9.48±0.57	09.62	-
17	7.80±0.03	29.33±1.03	13.21±0.72	9.66±0.55	10.21	-
18	7.85±0.06	29.23±1.00	13.18±2.08	9.15±0.49	21.81	0.90±0.024
19	7.83±0.11	29.40±0.78	12.77±1.75	9.86±0.60	20.15	-
20	7.85±0.09	29.60±0.65	10.45±1.38	9.67±0.36	25.25	-
21	7.91±0.08	29.63±0.58	09.79±0.72	9.68±0.58	29.34	1.00±0.023

experiments were repeated three times. The mean of all the observations±standard deviation (SD) has been given in Table 2.

Effect of various CO_2 flow rates on CO_2 dissolution efficiency of medium: In the control experiment, the microalgae *P. limnetica* was grown in 60L photobioreactor without any artificial CO_2 supplementation. The initial inoculum was 0.3 g L^{-1} (standard is 0.25±0.05 g). The aeration rate was maintained at 4 LPM and the algal growth was continued for 21 days with the 12 h exposure of 185-222 µmol m^{-2} sec^{-1} light intensity every day. Then the amount of DCO_2 was noted and continuously recorded with the help of a data logger system. The associated pH change

was also noted with the pH sensor. The growth was measured in terms of the dry weight of biomass after every 3 days (Table 3).

In test experiments, the culture of *P. limnetica* was inoculated (0.25±0.05 g L^{-1}) in an MSWBG11 medium in the 60 L capacity flat panel photobioreactor. The culture was exposed to the white fluorescent light of 185-222 µmol m^{-2} sec^{-1} intensity for 12 h every day. The gaseous CO_2 fed into the medium in two different flow rates viz. ≤0.005 LPM (Set-I) and ≤0.001LPM (Set-II) continuously for a period of 21 days. The effect of CO_2 sparged on algal growth was interpreted in terms of biomass produced which was measured at the intervals of 3 days. The DCO_2, DO_2 associated pH change was also reported at every minute. All the experiments were repeated 3 times and their mean values have been tabulated in Table 4, 5.

In the end, CO_2 mass transfer coefficient per minute (KLa min^{-1}) was calculated for all the water or medium sources and also for the experiments of CO_2 sequestration studies. Then the average of percentage CO_2 consumption in all the three sets of experiments was calculated.

RESULTS AND DISCUSSION

Microalgal strain: To make biomass cultivation cost-effective selection of water source is very crucial. The cost of a water source could be more than the half financial investment in setting up the large scale microalgae cultivation industry. Hence, any water source other than fresh water is recommended for mass cultivation of microalgae. Keeping this approach in mind scientific research communities focusing on the utilization of seawater and so we are. To work with seawater selection of appropriate microalgal strain is very much essential. Here, cyanobacterial strains, *Pseudanabaena limnetica* (Lemm.) *Komárek* was shortlisted basis on its unique properties to thrive in hypersaline conditions. This is a halophilic, filamentous cyanobacterial species isolated from salt pans of Mumbai suburban region. It possesses the following important properties that make it a potential candidate for mass cultivation[8]:

- Sustains at high salinity up to 3.5% (35000 ppm)
- Tolerates high temperature of the tropical summer season, wherein daytime water temperature goes beyond 45°C
- Produces average biomass of ~ 1.5 g L^{-1} in 18 days
- Survives in the least nutrient-containing medium

Comparison of CO_2 dissolution in tap waters, seawater and MSWBG11 medium: In this experiment, the study of CO_2 dissolution capacity of different aqueous samples viz. tap water, seawater and MSWBG11 medium were studied. The experiment was conducted in 60 L flat plate photobioreactor. The CO_2 dissolution was studied at 4 LPM pneumatic agitation rate. The 100% pure compressed CO_2 was sparged through sintered glass spargers. The flow rate of CO_2 was controlled using rotameter and was maintained at 0.01 LPM. The bubble size generated through the sintered glass was less than 500 µm in diameter. The rate of CO_2 dissolution and the associated change in pH was noted with the help of the respective sensor. The dissolved CO_2 sensor In Pro5000 (Mettler Toledo India Pvt. Ltd.) was used which makes the online measurement of dissolved CO_2. The DCO_2 sensor uses the Severinghaus electrode for the potentiometric measurement of DCO_2. The sensor employs CO_2 gas permeable silicone membrane which is tightly stretched around a special engineered flat pH meter. The CO_2 gas from the sample or process diffuses across the membrane where it dissociates to form HCO_3^- and H^+ ions: The formed H^+ ions lead to a pH change of the inner electrolyte.

Initially, at time zero before sparging CO_2 into the tap water, the DCO_2 concentration was 2.00±0.23 mg L^{-1} and pH was 7.33±0.12 (Table 2, Fig. 1) and at the end of 1 h DCO_2 was increased to 3.03±0.11 mg L^{-1} and initial pH of 7.33±0.12 was reduced to 6.92±0.09. At the end of 2 h with continuous CO_2 sparging DCO_2 was increased to 5.10±0.16 mg L^{-1} but pH drastically decreased to 6.47±0.09, which will be detrimental for the algae.

Contrary to this when CO_2 sparging experiment was conducted in seawater, the initial level of CO_2 was a little higher. The DCO_2 value measured in seawater at time zero was 2.17±0.35 mg L^{-1} at 7.95±0.07 pH value. Whereas in tap water it was 2.00±0.23 mg L^{-1}. When the seawater was sparged with gaseous CO_2 for 1 h the amount of dissolved CO_2 was increased to 5.60±0.15 mg L^{-1} and pH reduced to 7.46±0.30. After 2 h of continuous CO_2 feeding, the number of dissolved CO_2 increased to 9.58±0.71 mg L^{-1} and pH was 7.22±0.06, which would be just sufficient for the growth of algae. The dissolved carbonate species in seawater provide an efficient buffering system. For instance, the addition of 1 µmol kg^{-1} of a strong acid such as HCl in distilled water at pH 7 reduces the pH very close to 6. The same addition to seawater at pH 7 only reduces the pH to 6.99. The seawater pH buffer mainly results of the capacity of CO_3^{2-} and HCO_3^- ions to accept protons[10].

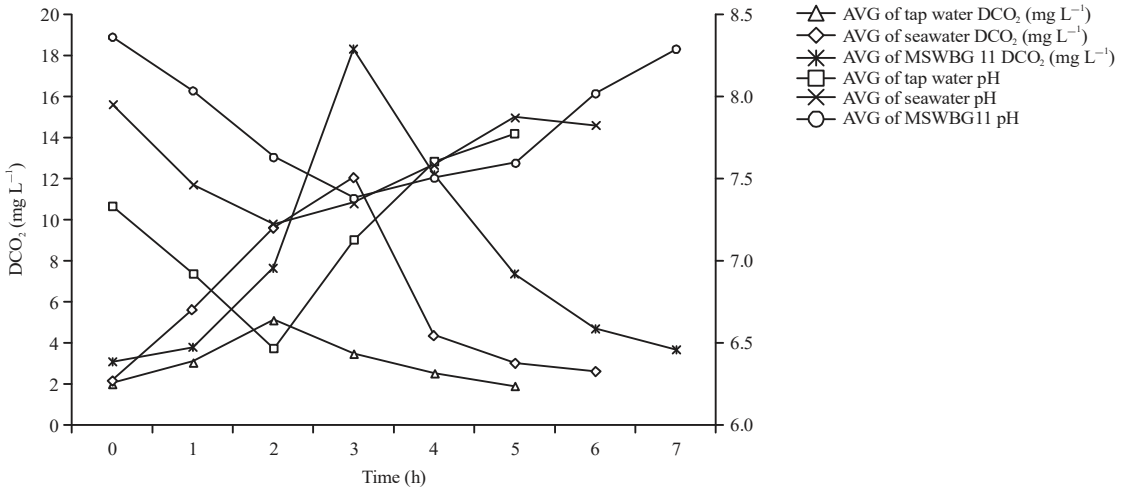

Fig. 1: Comparison of CO_2 dissolution in pneumatically agitated flat panel photobioreactor containing tap water, seawater and MSWBG11 medium
AVG: Average

The salinity of seawater is near to 3.8-4%, this always adjusted to 3.5% with the addition of the little volume of fresh water. The MSWBG11 medium was prepared by fortifying additional Na_2CO_3 in normal seawater BG11 medium (MSWBG11) (Table 1). In MSWBG11 medium amount of Na_2CO_3 added was 0.02 g L^{-1}, whereas in MSWBG11 ten times higher amount of Na_2CO_3 was added (i.e., 0.2 g L^{-1}). The optimum nitrate content in MSWBG11 is half in quantity ($NaNO_3^-$0.7 g L^{-1}) than the nitrate content of the standard medium ($NaNO_3^-$1.5 g L^{-1}). This concentration was the most suitable concentration for the growth of *P. Limnetica*[8]. Due to the addition of Na_2CO_3, the medium becomes alkaline. Such a medium has the capability of absorbing much more CO_2. owing to this cause, at the end of 3 h, the amount of DCO_2 was as high as 18.30±1.17 mg L^{-1}. Whereas in seawater when CO_2 was sparged for 3 h the DCO_2 level was reached to 12.05±0.98 mg L^{-1} only.

It has been experimentally proved that the weak alkali, Na_2CO_3, has a greater buffering capacity than the strong alkali $NaOH_3$. The optimal conditions for CO_2 absorption are obtained by maintaining a weakly alkaline aqueous pH of 9-10 by adding Na_2CO_3 as alkalinity buffering chemical at 27°C. And therefore the addition of extra Na_2CO_3 into the MSWBG11 medium, increases its ability to absorb and hold maximum CO_2 for a longer time. The major constraint of pneumatically agitated system is that the tremendous air already flows through it which might limit the CO_2 dissolution rate. But pneumatic agitation is the mandatory requirement to keep the algae freely floating in the culture system to support good growth.

In this experiment, our objective was to study the ability of different aqueous solutions to hold dissolved CO_2 when the pneumatic agitation is continued. Hence, in all the systems gaseous CO_2 was sparged for 3 h. The pneumatic agitation was continued and amount of DCO_2 was noted at every 1 h interval until it reaches back to nearly normal (Fig. 1). The experiments were conducted three times (n = 3) and mean±SD values of DCO_2, DO_2 and pH were noted in Table 2.

As mentioned earlier in tap water at zero h dissolved CO_2 was 2.00±0.23 mg L^{-1}, at the end of 2 h of continuous CO_2 sparging it was increased to 5.10±0.16 mg L^{-1}. At this time CO_2 sparging was stopped due to a sharp decline in pH which was decreased to 6.47±0.09. After 1 h of CO_2 termination and continuous pneumatic agitation, the amount of DCO_2 was decreased to 3.46±0.08 mg L^{-1}. After 2 h DCO_2 was 2.52±0.26 mg L^{-1}, which was still higher than its original DCO_2 level in spite of continuous pneumatic agitation.

In seawater system, the original DCO_2 level was 2.17±0.35 mg L^{-1}. After 3 h of CO_2 sparging, it was increased to 12.05±0.98 mg L^{-1}. The pH was decreased to 7.35±0.23. At this point, CO_2 feeding was stopped. After one h of CO_2 termination, the DCO_2 level drastically decreased to 4.36±0.39 mg L^{-1}. After 2 h, DCO_2 decreased to 3.02 ±0.27 mg L^{-1} and after 3 h it became 2.59±0.46 mg L^{-1}.

In MSWBG11 medium the original DCO_2 level was 3.04±0.56 mg L^{-1}. After 3 h of CO_2 feeding, it reached to 18.30±1.17 mg L^{-1} and the pH was 7.38±0.10, at this point the CO_2 feeding was stopped. After 1 h of CO_2 termination, it was decreased to 12.18±0.72 mg L^{-1}. After 2 h interval, it was 7.37±0.60 mg L^{-1} and after 4 h interval, the amount of DCO_2 was 3.34±0.37 mg L^{-1}. Thus MSWBG11 medium is not only allowing the maximum CO_2 to dissolve in it, without drastic pH drop but also hold DCO_2 for a longer duration, which would be suitable for algal growth.

The ocean is a large sink for CO_2 resulted from fossil fuel burning. When the CO_2 is directly injected into mid-depth of the ocean it dissolves in water to form carbonic acid and pH decreases[11]:

$$CO_2 (g) + H_2O \leftrightarrow H_2CO_3 (aq.)$$

The carbonic acid further dissociates to form bicarbonate ions and carbonate ions as shown in the following 2 steps:

$$H_2CO_3 (aq.) \leftrightarrow H^+ + HCO_3^-$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^-$$

Increasing the alkalinity of the ocean using CaO, bicarbonates and ocean nourishment with NH_3^+ including other nutrients are some of the major approaches to accelerate an oceanic carbon uptake to lessen greenhouse gases[11].

Effect of varying CO_2 flow rate on CO_2 dissolution along with associated pH change in medium and its effect on the growth of microalgae *P. limnetica*: In present communication seawater based MSWBG11 medium, which is favorable for the growth of *P. limnetica* was used for the mitigation of CO_2.

As mentioned in material and methods the three experiments, viz. Control (without CO_2 feeding), Set-I (CO_2 feeding at $\leq$0.005 LPM) and Set-II (CO_2 feeding at $\leq$0.001 LPM) were carried out in triplicates. The readings for each triplet set was noted every day at a particular time and their mean along with standard deviation (SD) was calculated to check the significance of the data. To check the effect of CO_2 feeding on algal growth, the determination of the dry weight of the biomass was carried out on every third day by gravimetric analysis.

In the control experiment at day 1, DCO_2 was 1.97±0.07 mg L^{-1} and pH was 8.73±0.14. As the growth advanced, the CO_2 dissolved in the medium was consumed and that was associated with the increase in pH. On day 3, DCO_2 was reduced to 1.83±0.01mg L^{-1} and pH increased to 8.94±0.13. There was little increase in biomass from 0.33±0.025-0.37±0.016 g L^{-1}. On 6th day, DCO_2 was decreased to 1.60±0.02 mg L^{-1}, pH was increased to 9.44±0.40 and biomass was 0.39±0.008 g L^{-1}. On day 21st the DCO_2 was 1.17±0.14 mg L^{-1} and pH was 9.30±0.29. The biomass produced was 1.15±0.041 g L^{-1}. A gradual decrease in DCO_2 was observed as cell growth was continued. This also led to gradually increase the pH of the medium and it became alkaline when cell growth reaches to stationary phase as shown in Fig. 2. Thus, during the period of 21 days, DCO_2 consumed was almost ~0.8 mg L^{-1} with total biomass production of 1.15±0.041 g L^{-1}.

In the set I the microalgae *P. limnetica* was cultured in a 60 L photobioreactor with an initial inoculum size of 0.30±0.065 g L^{-1}. Pneumatic agitation was given at 4 LPM of air flow rate and artificial CO_2 was sparged at the flow rate of $\leq$0.005 LPM (Table 4). The biomass produced was interpreted after every 3 days up to 21 days. The amount of DCO_2 and associated pH change was noted every day. At day 1, DCO_2 was 8.22±0.53 mg L^{-1} and pH was 7.92±0.60. During 21 days period, the amount of DCO_2 was maintained between the range of 6.55±1.03-16.56±2.19 mg L^{-1} and pH was in the range of 7.55±0.03 to 7.95±0.30. The biomass produced at the end of the experiment was 1.0±0.023 g L^{-1}, which was less than the biomass obtained in the control experiment

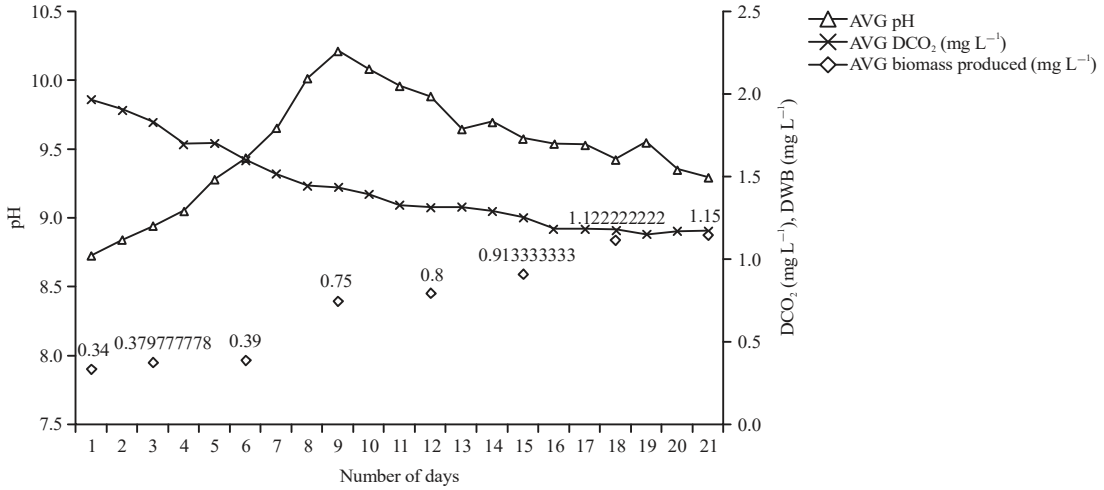

Fig. 2: Effect of growth of *P. limnetica* on DCO_2 level and pH of the modified Seawater BG11 medium
AVG: Average

(i.e., 1.15±0.041 g L^{-1}). It has been described in the scientific literature that microalgal cells can tolerate CO_2 up to a certain level after which it becomes detrimental for their growth[6]. High CO_2 concentration that is above the limit of cell's tolerance induces environmental stress and causes a biological reduction in the capacity of algal cells to sequester CO_2. Thus, 6.55±1.03 to 16.56±2.19 mg L^{-1} of DCO_2 concentration was found to be inhibitory to the growth of microalgae. And therefore in this case for continuous CO_2 sequestration by *P. limnetica*, the pure CO_2 should not be fed above 0.005 LPM flow rate.

The microalgal cultures in PBR are supplemented with 5-10% of CO_2 to ensure that algal cell will not be CO_2 limited. For every algal culture, there is a critical dissolved CO_2 concentration. If the concentration of DCO_2 drops down below critical level, the specific CO_2 uptake rate (SCUR) decreases. Hence, it is essential to monitor the dissolved CO_2 concentration in algal cultures. To monitor DCO_2 concentration indirectly, researchers have used dissolved O_2 to find out the value of critical CO_2 concentration and SCUR value[4] of CO_2. If a specific O_2 production rate (SOPR) and photosynthetic quotient are known, SCUR can be calculated. The SOPR increase with dissolve CO_2 concentration and come to halt at critical CO_2 concentration. Beyond which even if DCO_2 increased SOPR remains in the steady state. So, the graph of SOPR and pH as a function of dissolved CO_2 concentration is used for calculating critical CO2 concentration. For the culture of *C. vulgaris* critical CO2 concentration was achieved at about 7 mg L^{-1}.

In set II the CO_2 was sparged at the rate of ≤0.001 LPM (Table 5). The biomass, DCO_2 and pH changes were noted for 21 days. During these 21 days, the DCO_2 remained in the range of 2.71±0.35-3.78±0.80 mg L^{-1} and pH was in the range of 8.00±0.08 to 8.76±0.14. This DCO_2 concentration in the range of 2.71±0.35-3.78±0.80 mg L^{-1} was reported as the critical CO_2 level for this strain. The biomass produced at the end of 21 days was 1.2±0.038 g L^{-1} which was higher than the biomass obtained in the control experiment (i.e., 1.15±0.041 g L^{-1}). Thus, the low flow rate of CO_2 sparging i.e., ≤0.001 LPM, along with pneumatic agitation at 4 LPM was found to be the most suitable for maximum CO_2 mass transfer into the medium. This also favored better algal growth. The DCO_2 concentration maintained between 2-4 mg L^{-1} favors algal growth. Thus it is not economical to continuously sparge 10-15% of CO_2. Instead, sparging CO_2 at very low flow rate favors the mass transfer and is just sufficient for higher growth of the algae.

It has been depicted that needless high supply of CO_2 would have many negative effects on algae cultivation[12]. The lethal conditions such as severe mechanical shear, medium acidification, etc arise from higher CO_2 supplementation in a conventional way of algae cultivation. These practices consume much energy and resources which put the algae cultivation industry in a financial crunch. Therefore establishing the technology with low energy demand and with low CO_2 dosing relatively higher CO_2 mass transfer would be essential for cost-effective microalgal cultivation.

Table 5: Effect of continuous feeding of CO_2 at ≤0.001LPM flow rate on the growth of *P. limnetica*, DCO_2, DO_2 level and pH of the MSWBG11 medium along with percentage CO_2 consumption/day

Days	pH (Mean±SD)	Temperature (Mean±SD) °C	DCO_2 (Mean±SD) mg L^{-1}	DO_2 (Mean±SD) mg L^{-1}	CO_2 consumption (%)	Biomass produced (Mean±SD) g L^{-1}
1	8.67±0.18	29.80±1.10	3.04±0.39	09.75±0.30	31.37	0.25±0.007
2	8.68±0.21	30.07±0.62	3.09±0.69	09.78±0.37	39.18	-
3	8.76±0.14	30.07±0.68	2.95±0.39	09.85±0.36	27.88	0.32±0.006
4	8.46±0.08	31.00±0.94	3.36±0.96	09.79±0.35	41.10	-
5	8.64±0.05	31.13±0.90	3.08±0.31	09.96±0.44	28.13	-
6	8.27±0.04	31.13±0.57	2.78±0.21	09.83±0.41	34.63	0.38±0.018
7	8.31±0.05	31.18±0.86	3.07±0.42	09.90±0.57	34.85	-
8	8.00 ±0.08	31.67±0.90	3.22±0.58	09.89±0.41	35.63	-
9	8.46±0.04	31.57±0.74	2.86±0.39	10.06±0.47	31.08	0.44±0.022
10	8.36±0.28	31.33±0.60	3.17±0.62	09.96±0.27	35.81	-
11	8.53±0.05	30.97±0.71	2.94±0.38	10.13±0.42	34.28	-
12	8.44±0.18	30.77±0.82	3.42±0.59	10.04±0.37	31.76	0.60±0.006
13	8.53±0.20	30.80±0.73	2.71±0.35	09.94±0.33	38.00	-
14	8.63±0.06	30.03±0.57	3.16±0.61	10.11±0.31	38.67	-
15	8.43±0.18	29.87±0.82	3.40±0.65	10.07±0.36	29.27	0.74±0.003
16	8.45±0.04	30.17±0.86	3.09±0.69	09.80±0.24	39.18	-
17	8.20±0.17	30.10±0.37	2.83±0.17	09.95±0.38	33.72	-
18	8.67±0.15	29.93±1.19	3.05±0.39	10.58±0.56	31.37	0.90±0.036
19	8.65±0.28	29.90±0.70	3.09±0.69	10.43±0.36	39.18	-
20	8.50±0.22	29.97±0.78	3.37±0.59	10.27 ±0.57	31.92	-
21	8.48±0.21	30.53±1.06	3.78±0.80	10.17±0.41	25.42	1.20±0.038

A group of scientist from Institute for Biotechnology and Bioengineering, Portugal, have studied the effect of various CO_2 concentrations and aeration rates on the growth of *Chlorella* sp. in 110 mL bubble column photobioreactor[13]. The aeration rates employed were 0.1, 0.4 and 0.7 vvm (liter volume of air per liter volume of liquid medium per minute) and the CO_2 was supplied in different concentrations viz. 2%, 6 and 10%. At lower CO_2 concentration i.e., 2% as the aeration rate increased the growth was also increased. At 0.1vvm aeration rate, the biomass productivity was 0.7 g L^{-1}/day and it was increased to 1.1g L^{-1}/day at 0.7 vvm. At 6% CO_2 concentration the optimum productivity was observed at 0.4 vvm i.e., 1.3 g L^{-1}/day. But, at 0.7 vvm it was decreased to 1.2 g L^{-1}/day. At 10% CO_2 concentration the optimum biomass productivity was observed at 0.4 vvm but it was lesser than 6% CO_2 concentration.

At lower CO_2 concentration as the air flow rate increased, the mass transfer coefficient increased consequently. CO_2 dissolution and O_2 stripping enhanced and more CO_2 were available for algal growth. But, at high CO_2 concentration, the CO_2 reaches to saturation point. Beyond this point even though the aeration rate was increased it does not contribute to mass transfer. Extra CO_2 is released to the atmosphere and does not remain in the medium for algal growth. Thus, an increase in air flow rate over a valid range is not efficient in increasing the microalgal growth. This should be always considered for cost-effective CO_2 absorption for greater growth of microalgae.

In our earlier studies, a 25 L horizontal tubular photobioreactor was used for CO_2 sequestration[14]. An indigenous thermotolerant strain Geitlerinema sulphureum was used for this purpose. The media was circulated through photobioreactor with the help of peristaltic pump at 100 rpm which facilitate the liquid medium to flow at 1 LPM speed. Compressed air was supplied at the rate of 0.1 LPM when no artificial CO_2 was sparged. The CO_2 content found at the outlet was 450 ppm. When the air was sparged at 0.1 LPM and artificial CO_2 was sparged at the rate of 0.01 LPM (9.09% CO_2 concentration), the amount of CO_2 detected at the inlet was more than 5000 ppm. The concentration of CO_2 at the outlet was 540 ppm. When the air was sparged at 0.1 LPM and CO_2 was sparged at a rate of 0.02 LPM (16% CO_2 concentration), the average CO_2 at the outlet was increased to 675 ppm. But when the air was sparged at 0.03 LPM (23% CO_2 concentration), the amount of CO_2 detected at the outlet was substantially increased to 1400 ppm. Thus, the low flow rate of CO_2 (0.02 LPM) was the most suitable for optimum mass transfer. In this experiments the CO_2 concentration at inlet and outlet air were measure but the actual amount of CO_2 dissolved in the medium was not known. The following equation which utilizes pH values is used for calculating the dissolved CO_2 concentration in the medium[12]:

$$[CO_2] = \frac{\left[10^{-pH} - 10^{pH-14} + \Delta\left(Na^+\right)\right]10^{-2pH}}{10^{-6.381-pH} + 2\times10^{-16.758}} \left(\text{mol } L^{-1}\right)$$

The titrimetric method is an another option to mathematically calculate DCO_2 and carbon consumption during biomass production of some microalgal species such as *Nannochloropsis oculate*[15]. Many researchers have used pH values for the determination of dissolved CO_2 concentration before and after dosing aqueous CO_2 into the culture medium. Whereas, some have used total organic carbon analyzer (TOC-VCSH, SHIMADZU) to detect total inorganic carbon in the medium[3].

In the present communication, a dissolved CO_2 sensor is used which makes the online measurement of dissolved CO_2 at various time intervals, through 21 days experimental period. The optimized 60 L PBR system assembled with sparger of 2 mm pore size and 4 LPM air flow rate was found to be ideal for optimum biomass production. At 4 LPM of pneumatic agitation, the CO_2 supply of very low CO_2 flow rate ($\leq$0.001 LPM) was the most suitable for better CO_2 mass transfer and algal growth.

Relation between flow rates, mass transfer rates and CO_2 capture efficiency: In the study where relation between mass transfer and growth rate was elaborately explained utilized an experimental setup comprised of 12 airlift photobioreactors (ALPs)[12]. Each of them was of 3 L capacity together arranged in 2 rows. *Dunaliella* culture were sparged with CO_2 enriched gas (5% CO_2+95% N_2) for 30 min each day with varying flow rates ranging from 0.3, 0.5, 0.7, 0.9, 1.1LPM. As the flow rate increased, CO_2 mass transfer coefficient (KLa min^{-1}) was increased. They also calculated CO_2 capture efficiency i.e., the amount of CO_2 absorbed over the amount of CO_2 fed at varying flow rates. They observed that CO_2 capture efficiency was reduced when the flow rate was increased, enhancing flow rates, KLa min^{-1} is enhanced but due to it the amount of undissolved CO_2 increases and that goes wasted. For higher dissolution of CO_2, dosing in lower flow rates or decreasing the bubble size to the nanoscale is beneficial.

The results obtained in present study showed resemblance with finding of the group of researchers from University of Sheffield, UK about the enhancement of mass

Table 6: Estimation of mass transfer coefficient (KLa min^{-1}) for tap water, seawater and MSWBG11 medium (with and without CO_2 feeding) along with average percentage CO_2 consumption

Medium	$[CO_2]^*$	$[CO_2]_0$	$[CO_2]_t$	$[CO_2]^*$-$[CO_2]_0$	$[CO_2]^*$-$[CO_2]_t$	$([CO_2]^*-[CO_2]_0)/([CO_2]^*-[CO_2]_t)$	KLa $min^{-1} = \ln([CO_2]^*-[CO_2]_0)/([CO_2]^*-[CO_2]_t)$	Average CO_2 consumption (%)
Tap water	5.03	1.75	3.15	3.28	1.88	1.745	0.557	-
Seawater	12.79	2.00	8.17	10.79	4.62	2.335	0.848	-
MSWBG11	25.36	2.30	18.19	23.06	7.17	3.216	1.168	-
Control	25.36	2.08	1.19	23.28	24.17	0.963	-0.038	4.36
CO_2 at ≤0.005 LPM	25.36	1.63	10.80	23.73	14.56	1.629	0.488	23.30
CO_2 at ≤0.001 LPM	25.36	3.09	4.90	22.27	20.46	1.088	0.084	34.00

transfer of CO_2 in microbubble driven airlift bioreactor[12]. In our study the ambient supply of CO_2, the KLa min^{-1} is negligible(-0.038). At ≤0.001 LPM of CO_2 feeding, there is little increase in KLa min^{-1} (0.084). At ≤0.005 LPM of CO_2 flow rate the KLa min^{-1} substantially improves to 0.488 min^{-1}. But when the percentage of CO_2 consumption was seen, the CO_2 feeding rate at ≤0.001 LPM was found to be far better. Before this, tap water, seawater and MSWBG11 medium were fed with CO_2 at 0.010 LPM flow rate with pneumatic agitation at 4 LPM to check the KLa min^{-1}. The value of KLa min^{-1} for tap water, seawater and MSWBG11 medium were 0.557, 0.848 and 1.168, respectively (Table 6). This proves that due to fortified carbonate in MSWBG11 the value of volumetric mass transfer coefficient is higher than any other water source. Details of percentage CO_2 consumption per day at different flow rates are indicated in Table 3-5. Table 6 shows the comparison between different types of water for KLa min^{-1} and the average percentage CO_2 consumption.

When no artificial CO_2 was supplied (Control experiment) the average CO_2 consumption was 4.36%. When CO_2 supplied at ≤0.001 LPM, the average value of CO_2 consumption was optimum i.e., 34.00%. But, when the flow rate was increased to ≤0.005 LPM the average CO_2 consumption decreased to 23.30%.

Effect of different CO_2 flow rates on O_2 removal: An additional supply of CO_2 contributes many benefits to algal cultures. It enhances algal metabolism, it acts as a buffering system to neutralize increased pH caused by algal growth. Furthermore, along with the microalgal growth, accumulation of generated O_2 in the culture medium is toxic to microalgal cells and it is one of the major limiting factors for scaling up of the bioreactor[16]. Introducing CO_2 in culture helps to strip off accumulated O_2 and prevents algal cells from toxicity. By increasing the concentration of dissolved CO_2, reducing the accumulation of O_2 level improves productivity[12]. Performance of bioreactor depends on high gas mass transfer for both CO_2 dissolution and O_2 removal.

In present communication in the control experiment when cultures were grown at ambient CO_2 concentration the amount of DO_2 determined was in the range of 9.42±0.20 to 10.76±0.36 mg L^{-1} (Table 3). When the cultures were sparged with ≤0.001LPM of CO_2, the DO_2 concentration ranged from 9.75±0.30 to 10.58±0.56 mg L^{-1} (Table 5) and when CO_2 was sparged at the rate of ≤0.005 LPM, the DO_2 concentration was in the range of 8.45±0.74 to 9.86±0.60 mg L^{-1} (Table 4).

CONCLUSION

In the current research, the direct measurement of dissolved CO_2 concentration was carried out while sequestering it with growing microalgae. The chosen indigenous halophilic strain *Pseudanabaena limnetica* (Lemm.) *Komárek* was found to be the suitable candidate for CO_2 sequestration studies along with mass cultivation. This strain thrives at high temperature and light intensity of the summer season and it was grown in actual seawater which will make the large-scale biomass production cost effective.

Previously designed, constructed and operationally optimized 60 L flat panel photobioreactor system for *P. limnetica* growth was successfully operated for CO_2 sequestration studies. MSWBG11 medium which containing additional carbonate was found to be a good source of the medium in which maximum CO_2 can be fortified and made available for algal growth.

To improve the biomass productivity CO_2 from the artificial source was provided in different concentrations. The continuous CO_2 supplementation at ≤0.001 LPM of flow rate showed enhanced biomass production. Volumetric mass transfer coefficient (KLa min^{-1}) was found to be increasing with an increase in CO_2 feeding rate. But, percentage CO_2 consumption by P. limnetica was found to be highest at ≤0.001 LPM CO_2 flow rate, which is making it an ideal flow rate for CO_2 mitigation and mass cultivation of this microalgal strain.

ACKNOWLEDGMENT

The authors acknowledge the Department of Biotechnology (DBT), New Delhi for its financial support to this research project under the call 'Mass Cultivation of Algae for Cost-Effective Biofuel Production'. The grant agreement number was BT/PR12347/PBD/26/476/2015.

REFERENCES

1. Gaikwad, R.W., M. Gudadhe and S. Bhagat, 2016. Carbon dioxide capture, tolerance and sequestration using microalgae-a review. Int. J. Pharm. Chem. Biol. Sci., 6: 345-349.
2. Zimmerman, W.B., M. Zandi, H.C.H. Bandulasena, V. Tesař, D.J., Gilmour and K. Ying, 2011. Design of an airlift loop bioreactor and pilot scales studies with fluidic oscillator induced microbubbles for growth of a microalgae *Dunaliella salina*. Applied Energy, 88: 3357-3369.
3. Zheng, Q., G.J. Martin and S.E. Kentish, 2016. Energy efficient transfer of carbon dioxide from flue gases to microalgal systems. Energy Environ. Sci., 9: 1074-1082.
4. Padmanabhan, P., 2017. Technical insight on the requirements for CO_2-saturated growth of microalgae in photobioreactors. 3 Biotech, Vol. 7, No. 2. 10.1007/s13205-017-0778-6.
5. Chisti, Y., 2013. Constraints to commercialization of algal fuels. J. Biotechnol., 167: 201-214.
6. Kumar, K., C.N. Dasgupta, B. Nayak, P. Lindblad and D. Das, 2011. Development of suitable photobioreactors for CO_2 sequestration addressing Global warming using green algae and cyanobacteria. Bioresour. Technol., 102: 4945-4953.
7. Mhaskar, K. and M. Deodhar, 2016. Isolation of halophilic cyanobacteria from saltpans of eastern suburbs of Mumbai. Int. J. Sci. Res. Methodol., 4: 37-48.
8. Magar, C.S. and M.A. Deodhar, 2018. Operational strategies for cost effective mass cultivation of halophilic microalgal strain *Pseudanabaena limnetica* in 1000 L flat panel photobioreactor. J. Pet. Environ. Biotechnol., Vol. 9. 10.4172/2157-7463.1000380.
9. Rippka, R., J. Deruelles, J.B. Waterbury, M. Herdman and R.Y. Stanier, 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol., 111: 1-61.
10. Zeebe, R.E. and D.A. Wolf-Gladrow, 2001. CO_2 in Seawater: Equilibrium, Kinetics, Isotopes. Elsevier Science, Amsterdam, The Netherlands, ISBN-13: 9780080529226, Pages: 360.
11. Jones, I.S.F. and C.H. Lu, 2003. Engineering carbon sequestration in the Ocean. Proceedings of the 2nd Annual Conference on Carbon Sequestration, May 6, 2003, Washington, DC.
12. Ying, K., D.J. Gilmour, Y. Shi and W.B. Zimmerman, 2013. Growth enhancement of *Dunaliella salina* by microbubble induced Airlift Loop Bioreactor (ALB)-the relation between mass transfer and growth rate. J. Biomater. Nanobiotechnol., 4: 1-9.
13. Anjos, M., B.D. Fernandes, A.A. Vicente, J.A. Teixeira and G. Dragone, 2013. Optimization of CO bio-mitigation by *Chlorella vulgaris*. Bioresour. Technol., 139: 149-154.
14. Kenekar, A.A. and M.A. Deodhar, 2014. Operational strategies for lab scale horizontal tubular photobioreactorfor mitigation of CO_2 using an indigenous thermophilic microalgal strain *Geitlerinema sulphureum*. J. Pet. Environ. Biotechnol., Vol. 5. 10.4172/2157-7463.1000176.
15. Budiono, R., H. Juahir, M. Mamat, Sukono and M. Nurzaman, 2018. Modelling interaction of CO_2 concentration and the biomass algae due to reduction of anthropogenic carbon based on predator-prey model. Int. J. Applied Environ. Sci., 13: 27-38.
16. Richmond, A., 2008. Handbook of Microalgal Culture: Biotechnology and Applied Phycology. John Wiley & Sons, New York, USA., ISBN-13: 9781405172493, Pages: 584.