Using seawater-based Na_2CO_3 medium for scrubbing the CO_2 released from Bio-CNG plant for enhanced biomass production of *Pseudanabaena limnetica*

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Abstract

The concentration of CO_2 , one of the most important greenhouse gases (GHG), has reached to 409.8 ± 0.1 ppm in 2019. Although there are many carbon capture and storage (CCS) methods, they are very costly and their long term use raises concern about environmental safety. Alternatively, bio-sequestration of CO_2 using microalgal cell factories has emerged as a promising way of recycling CO_2 into biomass via photosynthesis. In the present study, Indigenous algal strain *Pseudanabaena limnetica* was cultivated in pneumatically agitated 60-L flat-panel photobioreactor system. The gas was released from Bio-CNG plant as by-product into Na_2CO_3 -rich medium and cultivated in semicontinuous mode of operation. It was observed that when CO_2 was sparged in seawater-based 0.02 M Na_2CO_3 solution, maximum CO_2 was dissolved in the system and was used for algal cultivation. Control system produced 0.64 ± 0.035 g/L of biomass at the end of 15 days, whereas CO_2 sparged Na_2CO_3 medium produced 0.81 ± 0.046 g/L of biomass. When CO_2 from Bio-CNG station was fed, it resulted in biomass production of 1.62 ± 0.070 g/L at the end of 18 days compared to 1.46 ± 0.066 g/L of biomass produced in control system which was not fed with gas released from Bio-CNG plant as by-product. Thus, feeding CO_2 directly into Na_2CO_3 medium and operating the system semicontinuously would be efficient for scrubbing CO_2 from commercial Bio-CNG plant. This study proves that feeding CO_2 gas from Bio-CNG plant into Na_2CO_3 -rich alkaline system can be used to feed algae for enhanced biomass production.

Keywords CO_2 · Flat-panel photobioreactor · Semicontinuous mode · Bio-CNG

1 Introduction

Global warming is being caused due to emission and increase in three greenhouse gases (GHGs), namely CO_2 , methane and N_2O . Atmospheric CO_2 concentration in 2018 was 407.3 ppm which increased by 2.5 ± 0.1 ppm in 2019 [1]. The Kyoto Protocol and the Paris Agreement (2015) have asked the participating countries to curb climate change impact by setting up policies out of which

crucial are CO_2 emissions by reducing fossil fuel usage and increasing carbon capture and sequestration [2, 3].

Most of chemical or physical means of capture of CO_2 from smoke stack emission involve three major steps which are carbon capture, separation and storage which enormously increase the cost of project. [4]. Microalgae possess inbuilt mechanisms to capture CO_2 from the atmosphere even in smallest concentrations. These mechanisms are referred to as carbon capture mechanisms

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(CCM) [5]. Also, microalgae can withstand toxic waste gasses such as CO_2 and NO_x , SO_x coming from industrial and sewage wastewater sources. It is a known fact that microalgal cultures show enhanced growth when they are provided with CO_2 -enriched air. Hence, microalgae are used as biological scrubbers which utilize CO_2 from the industrial gas released as by-product resulting into enhanced biomass production. Biomass can be utilized for applications such as food, animal feed, fuel and other value-added by products which could be incorporated into cosmetics and nutraceutical industry [4].

Microalgae captures CO_2 in three ways like conversion of bicarbonates into CO_2 which diffuses into cell directly, assimilation of CO_2 directly via plasma membrane and direct uptake of bicarbonates through cell membrane transporters [6]. One of the challenges in providing CO_2 into algal systems is its efficient transport to algal cells. The most common way of feeding CO_2 is through fine-pore spargers but the barrier is the solubility of CO_2 in aqueous medium. Sparging excess CO_2 does not mean that it will not cause CO_2 limitation because most of the sparged CO_2 escapes into the atmosphere. This conventional way of CO_2 feeding does not become economical, especially when large-scale algal cultivation is a target [7]. Alternative way of capturing CO_2 present in gas released from thermal power plant or Bio-CNG plant is to absorb CO_2 using solvents such as NaOH , K_2CO_3 , etc., and this CO_2 -loaded solvent is then pumped directly into raceway ponds or photobioreactors and used for algal cultivation [8].

Present communication deals with the use of two different solvents NaOH and Na_2CO_3 for absorption of CO_2 . The CO_2 released from CNG station was sparged in 0.01 M NaOH solution. After feeding CO_2 , the NaOH was converted to NaHCO_3 and then Na_2CO_3 . The conversion of NaOH to NaHCO_3 and Na_2CO_3 was checked using pH double indicators. The amount of Na_2CO_3 solution formed was also calculated. This CO_2 -enriched Na_2CO_3 solution was used as carbon source while preparing the culture medium for growth of our own algal strain *Pseudanabaena limnetica* (Lemm.) *komárek* isolated from salt pans of eastern suburban regions of Mumbai capable of sustaining elevated concentration of Na_2CO_3 [9]. It will serve dual purpose, sequestration of CO_2 from Bio-CNG station and enhancement of algal biomass which can be utilized for several commercial applications.

2 Material and methods

2.1 Strain selected

Microalgal strain selected was *Pseudanabaena limnetica* (Lemm.) *komárek*, which is an indigenous halophilic strain isolated from salt pans of Mumbai suburban region.

2.2 Culture medium

BG-11 medium used for growth of cyanobacterial strains was prepared as mentioned by Rippka et al. 1979 [10]. Modified seawater BG11 medium (MSWBG11) was formulated from the BG11 medium. MSWBG11 medium was modified BG11 seawater-based medium containing ten times higher carbonate (Na_2CO_3) compared to normal BG11 composition [11].

2.3 Design and culture conditions

To conduct the experiments for CO_2 sequestration, the operationally optimized 60-L photobioreactor was utilized. The photobioreactor was made up of glass having dimensions of 20 cm $\times$ 50 cm $\times$ 70 cm (breadth $\times$ length $\times$ height). Construction and operational standardization were carried out in our previous study by Magar and Deodhar 2018 [11]. The culture was supplied with light intensity of 185–222 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 12 h every day using white fluorescent light. The agitation was provided using spargers at the rate of 4LPM using acrylic rotameters (Napro Scientific, Pune). Perforated horizontal tubes were placed at the base of the photobioreactor through which air was sparged into the system [11].

2.4 CO_2 feeding

The CO_2 supply was fed from the commercially available 100% compressed CO_2 cylinders (Super Industrial Gases, Thane). The flow rate of CO_2 was controlled using a commercial pressure regulator. Further for accurate flow rate, rotameter of 0.001–10 LPM capacity was procured from Napro Scientific, Pune. The gaseous CO_2 was fed into the medium at a flow rate of 0.001 LPM through the borosilicate sintered glass sparger (Deepali enterprises, Mumbai). For experiment purpose, flow rate of ≤ 0.001 LPM was maintained using the same rotameter and sparger system.

2.5 pH, Temperature, dissolved carbon dioxide (DCO_2) detection and biomass estimation

The detection and monitoring of pH, temperature and DCO_2 were carried out using electronic sensors, which were immersed in the working liquid system of the photobioreactor. Each of the sensors, i.e. pH and DCO_2 , along with inbuilt temperature sensor in each of them was obtained from Mettler-Toledo India Pvt. Ltd. The dry weight of the biomass (DWB) was estimated gravimetrically.

2.6 Experimental setup for dissolution of CO₂ in different solvent systems

Experiment was performed by adding two different salts like NaOH and Na₂CO₃ having concentration of 0.01 M and 0.02 M, respectively, in tap water as well as seawater. Both alkaline solutions were separately prepared, each one having total volume of 5L. CO₂ gas was sparged at the rate of 0.001 LPM in the system using sintered glass sparger as mentioned above. Sampling from each of the sets was done every 10 min, and molar concentration of NaOH, Na₂CO₃ and NaHCO₃ was estimated and calculated by volumetric titration method using double indicators, namely phenolphthalein and methyl orange [15]. Change in pH was also measured every 10 min using pH sensor. Solution capable of capturing maximum CO₂ was further used to study its effect on growth of algae.

2.7 Effect of CO₂-sparged alkaline solutions on the growth of algae

In control experiment, the microalgae *P. limnetica* were grown in 60-L photobioreactor in MSWBG11 medium without any artificial CO₂ supplementation. The initial inoculum was added in the reactor which was aerated using spargers at rate of 4 LPM, and the algal growth was continued for 15 days. Culture was exposed to white fluorescent light of 10,000–12,000 lx intensity for 12 h every day. Dissolved CO₂ (henceforth mentioned as DCO₂) concentration was noted and recorded continuously using data logger system. The associated pH change was also noted with pH sensor. The growth of dried algal biomass (g/L) was weighed after every 3-day interval in order to get measureable change in biomass weight [11].

In Test 1 and test 2, the culture of *P. limnetica* was inoculated in a MSWBG11 medium in the 60-L-capacity flat-panel photobioreactor. For test 1 and test 2, the carbonate content in MSWBG11 medium was replaced by CO₂-enriched NaOH and Na₂CO₃ solutions, respectively. The culture was exposed to the white fluorescent light of 10,000–12,000 lx intensity for 12 h every day. The effect of CO₂ sparged NaOH /Na₂CO₃ solution on algal growth was interpreted in terms of biomass produced which was measured at the intervals of 3 days. The DCO₂ and pH change was also reported at every hour.

2.8 Semicontinuous mode of operation for maximum biomass yield

Algal culture *P. limnetica* was cultivated in three different 60-L flat-panel photobioreactors set A, set B and set C. The culture conditions were same as described above in Sect. 2.7. Algal culture was initially allowed to grow for

10 days till log phase was achieved and then semicontinuous mode of operation was adopted. From three sets, set A, set B and set C, three different volumes of culture media (1 L, 2 L and 3 L) were removed every day and it was replaced by the same amount of fresh MSWBG11 medium. The experiment was continued for 60 days. Everyday increase in the algal dried biomass (g/L) was measured gravimetrically and noted.

2.9 Effect of CO₂ from Bio-CNG plant on growth of Algae *P. limnetica* in semicontinuous mode of operation

Experiment was conducted in the premises of Primove Engineering Pvt. Ltd., Pune. Composition of Bio-CNG coming from plant contains 50% CH₄, 49% CO₂ and 1% other gases. CO₂ gas coming from Bio-CNG plant had 99% purity with less than 1% of other trace gases such as H₂S, N₂, etc. CO₂ gas was directly sparged in MSWBG11 medium. This CO₂-sparged MSWBG11 medium was used for replacing the medium in a semicontinuous mode of operation. Experiment was conducted in 60-L photobioreactor that was inoculated with 0.3 g/L of culture, and operating conditions were the same as mentioned above. DCO₂ concentration (mg/L), % CO₂ consumption, pH and dried biomass yield (g/L) were analysed and calculated daily.

2.9.1 Statistical analysis

All the data were statistically analysed using one-way ANOVA and Tukey's test ($\alpha = 0.05$ and 0.01) wherever applicable.

3 Results and discussions

Emission of CO₂ is the major contributor to global climate change. About one-third of global CO₂ fixation occurs due to algae. This is because algal cells have special cell organelles pyrenoids to accelerate the process of CO₂ assimilation. In algal cell, HCO₃³⁻ from medium passes through plasma membrane to chloroplasts and then to thylakoids where it is catalysed to CO₂. There it is fixed by RuBisCO enzyme to produce many sugars by Calvin's cycle. Amount of HCO₃³⁻ thus enhances the rate of photosynthesis, biomass formation and growth rate of algae. When CO₂ gas is directly bubbled into microalgal medium, most of it is wasted due to short resident time and low utilization efficiency of raceway pond. There should be a mechanism to improve efficiency of converting CO₂ to HCO₃³⁻. CO₂ bicarbonate absorbers are generally adapted to efficiently convert CO₂ to NaHCO₃ which dissolves easily in culture medium and promotes algal growth [12].

Numbers of promising materials are used to capture CO₂. Best ones are aqueous amines. But the major disadvantages in its use are amine evaporation, corrosion of equipment and requirement of heat energy to regenerate the solvent [13]. As alternative alkaline sorbents, K₂CO₃, Na₂CO₃ have received a wide attention. Recently, hydrated Na₂CO₃ was used to absorb CO₂ [14]. Also NaOH was used to capture CO₂ from coal-fired power plant [15]. CO₂ reacts with aqueous NaOH to form Na₂CO₃ and NaHCO₃. In bench-scale reactor, the operational conditions were standardized in such a way that 97% NaHCO₃ was produced which has several industrial applications as baking soda.

In the present study, CO₂ released from Bio-CNG plant was sparged in NaOH or Na₂CO₃ solutions and resulting mixture was used for preparation of culture medium for growing algae.

The algal strain *Pseudanabaena limnetica* (Lemm.) *komàrek* indigenous algal strain isolated from salt pan capable of withstanding Na₂CO₃-enriched alkaline seawater medium. The normal concentration of Na₂CO₃ in the BG11 medium is 0.02 g/L, whereas the modified seawater BG 11 medium (MSWBG11) contains ten times greater amount of Na₂CO₃ (0.2 g/L). The 60-litre flat-panel photobioreactor was used for cultivation. At the end of 18 days, the algae produces 30% enhanced biomass than normal BG11 medium [11].

3.1 Use of different alkaline sorbents for feeding CO₂

When gaseous CO₂ is fed into the NaOH solution, CO₂ is first dissolved in water to form aqueous CO₂ (Eq. 1). Subsequently, aqueous CO₂ reacts with OH⁻ to generate HCO₃⁻ and CO₃²⁻ (Eqs. 2 and 3):

When gaseous CO₂ is fed into the NaOH solution, CO₂ reacts with aqueous NaOH and forms sodium carbonate (Na₂CO₃) and later forms sodium bicarbonate (NaHCO₃) in turn. These CO₂ absorption reactions are expressed by Eqs. (4) and (5)

The reaction rates and pH for above reactions 4 and 5 are different [16]. The mole fraction of each carbonate species could be expressed as function of pH which is described in Fig. 1 which is called Bjerrum plot. As seen in Fig. 1, carbonates are prominently produced at alkaline pH 14 to 10 [15]. As more CO₂ is sparged, pH becomes less alkaline and bicarbonates are maximally produced at pH 8. At lower pH 4–6, HCO₃⁻ is produced.

3.1.1 Dissolution of CO₂ in tap water- and seawater-based 0.01 M NaOH solution

In the present study, 0.01 M NaOH solution was used for sparging of CO₂ gas. CO₂ gas was purged through fine-pore sintered glass sparger at flow rates of 0.005 – 0.01 LPM. Initially, when CO₂ was sparged in the NaOH solution, the pH of solvent is very alkaline (pH 11) At this pH, most of the fed CO₂ reacted with NaOH to form Na₂CO₃ which was analysed by acid base titration method using double indicators (phenolphthalein and methyl orange). Every 10-min interval, sample was harvested and titrated against 0.2 M HCl. Change in pH was also measured. Phenolphthalein end point from purple to orange is an indicator of consumption of all NaOH and Na₂CO₃. And only bicarbonate is present in the solution that is further determined by methyl orange indicator. Methyl orange end point gives the measure of Na₂CO₃ that is converted to NaHCO₃ after titrating with HCl. The amount of OH⁻, CO₃²⁻ and HCO₃⁻ formed during the course of CO₂ sparging was calculated as described in the literature [17].

In this experiment, capacity of tap water and seawater to capture CO₂ was studied. When NaOH was added to pure water, it dissociates to form Na⁺ and OH⁻ almost completely making the solution highly alkaline. NaOH,

Fig. 1 pH versus the mole fraction of carbonate species (Bjerrum plot). Extracted from research paper by Shim et al. (2016)

Na_2CO_3 and NaHCO_3 species are generated as explained in Sect. 3.1.1. At zero time, the amount of NaOH is highest and it is responsible for highest phenolphthalein reading. The amount of NaOH is 0.054 ± 0.004 mol/L. The pH of solution is 11.72 ± 0.012 . As more and more CO_2 was sparged over time, amount of NaOH gradually started decreasing and Na_2CO_3 was formed. When the reaction mixture was titrated with HCl, the Na_2CO_3 was converted to NaHCO_3 . The amount of NaOH or Na_2CO_3 decreased

from 0.054 ± 0.004 to 0.003 ± 0.002 mol/L. The amount of NaHCO_3 increased from 0.00 to 0.062 ± 0.002 mol/L. During CO_2 sparging, pH of reaction mixture decreased from 11.72 ± 0.012 to 9.06 ± 0.038 . At this pH, NaOH is converted to NaHCO_3 that added to additional increase in methyl orange reading (Table 1).

When 0.01 M NaOH solution was prepared, seawater was used as the medium for dissolution of CO_2 (Table 2). It was observed that initial pH of the solution

Table 1 Amount of Na_2CO_3 and NaHCO_3 formed during the course of CO_2 sparging in tap water-based 0.01 M NaOH solution

Time (mins)	pH		$\text{NaOH} + \text{Na}_2\text{CO}_3$ (mol/L)		NaHCO_3 (mol/L)	
	Mean	SD	Mean	SD	Mean	SD
0	11.72	0.012	0.054	0.004	0.000	0.000
10	11.64	0.010	0.056	0.003	0.015	0.005
20	11.61	0.015	0.049	0.007	0.016	0.004
30	11.61	0.006	0.040	0.006	0.015	0.005
40	11.52	0.026	0.034	0.009	0.017	0.001
50	11.47	0.015	0.035	0.010	0.022	0.003
60	11.39	0.010	0.036	0.005	0.024	0.004
70	11.28	0.023	0.033	0.004	0.032	0.003
80	11.15	0.035	0.034	0.002	0.034	0.002
90	11.06	0.021	0.033	0.001	0.037	0.001
100	10.87	0.006	0.031	0.005	0.040	0.005
110	10.58	0.026	0.022	0.004	0.040	0.004
120	10.51	0.015	0.024	0.002	0.047	0.002
130	10.28	0.030	0.020	0.003	0.048	0.003
140	10.11	0.025	0.016	0.003	0.052	0.003
150	10.03	0.025	0.015	0.003	0.053	0.003
160	9.82	0.017	0.017	0.003	0.049	0.003
170	9.66	0.012	0.014	0.005	0.059	0.002
180	9.56	0.036	0.009	0.001	0.057	0.005
190	9.47	0.017	0.004	0.004	0.064	0.004
200	9.31	0.010	0.004	0.004	0.064	0.006
210	9.06	0.038	0.003	0.002	0.062	0.002

Table 2 Amount of Na_2CO_3 and NaHCO_3 formed during the course of CO_2 sparging in seawater-based 0.01 M NaOH solution

Time (mins)	pH		$\text{NaOH} + \text{Na}_2\text{CO}_3$ (mol/L)		NaHCO_3 (mol/L)	
	Mean	SD	Mean	SD	Mean	SD
0	10.05	0.017	0.026	0.001	0.010	0.002
10	9.87	0.021	0.023	0.002	0.012	0.004
20	9.82	0.015	0.023	0.005	0.014	0.001
30	9.69	0.032	0.021	0.004	0.021	0.003
40	9.65	0.025	0.022	0.003	0.022	0.005
50	9.55	0.040	0.015	0.001	0.021	0.001
60	9.15	0.006	0.006	0.003	0.026	0.003
70	8.84	0.021	0.005	0.001	0.032	0.002
80	8.76	0.026	0.002	0.002	0.025	0.004
90	8.64	0.032	0.002	0.001	0.022	0.002
100	8.41	0.015	0.002	0.001	0.033	0.005
110	8.24	0.006	0.001	0.000	0.034	0.007

was only 10.05 ± 0.017 , which might be because of inherent buffering nature of seawater which restricts drastic changes in pH. CO_2 was sparged in the solution, and changes in NaOH, Na_2CO_3 and NaHCO_3 were analysed. pH decreased from 10.05 ± 0.017 to 8.24 ± 0.006 in just 110 min. Concentration of NaOH decreased from 0.026 ± 0.000 to 0.001 ± 0.000 mol/L, and at the same time, amount of NaHCO_3 increased from 0.010 ± 0.001 to 0.034 ± 0.006 mol/L which was approximately three times lower than that of compared with NaHCO_3 concentration measured in tap water.

3.1.2 Dissolution of CO_2 in tap water- and sea water-based 0.02 M Na_2CO_3 solution

Similarly, 0.02 M Na_2CO_3 solution in tap water and seawater was also used to study the dissolution of CO_2 and generation of NaHCO_3 in the system. Initially, when

Na_2CO_3 was added in water, it dissociates to form NaOH and H_2CO_3 . Thus, total available Na_2CO_3 in the system reduces. When CO_2 sparging was initiated, NaOH previously formed is converted to Na_2CO_3 . And all Na_2CO_3 is converted to NaHCO_3 which was detected by methyl orange indicator. NaOH and Na_2CO_3 concentrations were measured as already mentioned in the above experiment. It was observed that when tap water was used, the initial pH of the system was 11.31 ± 0.010 and initial NaOH/ Na_2CO_3 concentration was 0.026 ± 0.000 mol/L (Table 3). As CO_2 was sparged in the system, Na_2CO_3 and NaOH started to reduce and became 0 mol/L moles, whereas NaHCO_3 increased from 0.035 ± 0.001 to 0.061 ± 0.003 mol/L at the end of 130 min.

Similarly, when seawater was used to sparge CO_2 , initial pH of 0.02 M Na_2CO_3 was 10.77 ± 0.031 which reduces to 7.75 ± 0.050 at the end of 100 min (Table 4). Also initial NaOH/ Na_2CO_3 concentration of 0.028 ± 0.001 decreased

Table 3 Amount of Na_2CO_3 and NaHCO_3 formed during the course of CO_2 sparging in tap water-based 0.02 M Na_2CO_3 solution

Time (mins)	pH		NaOH + Na_2CO_3 (mol/L)		NaHCO_3 (mol/L)	
	Mean	SD	Mean	SD	Mean	SD
0	11.31	0.010	0.026	0.001	0.035	0.001
10	11.22	0.026	0.027	0.001	0.034	0.004
20	10.89	0.031	0.028	0.004	0.035	0.003
30	10.63	0.012	0.030	0.004	0.034	0.001
40	10.44	0.006	0.032	0.005	0.031	0.003
50	10.30	0.020	0.026	0.006	0.038	0.003
60	10.21	0.040	0.021	0.001	0.043	0.003
70	10.07	0.021	0.021	0.001	0.041	0.005
80	9.87	0.021	0.020	0.006	0.042	0.001
90	9.71	0.031	0.008	0.002	0.050	0.003
100	9.53	0.042	0.007	0.002	0.053	0.002
110	9.04	0.032	0.004	0.001	0.053	0.003
120	8.44	0.035	0.002	0.001	0.065	0.004
130	7.82	0.076	0.000	0.000	0.061	0.004

Table 4 Amount of Na_2CO_3 and NaHCO_3 formed during the course of CO_2 sparging in seawater-based 0.02 M Na_2CO_3 solution

Time (mins)	pH		NaOH + Na_2CO_3 (mol/L)		NaHCO_3 (mol/L)	
	Mean	SD	Mean	SD	Mean	SD
0	10.77	0.031	0.028	0.001	0.030	0.002
10	10.69	0.012	0.029	0.002	0.029	0.002
20	10.06	0.015	0.023	0.004	0.036	0.004
30	9.84	0.032	0.019	0.006	0.039	0.003
40	9.74	0.010	0.016	0.002	0.044	0.003
50	9.65	0.040	0.015	0.004	0.044	0.005
60	9.41	0.031	0.016	0.005	0.043	0.000
70	9.21	0.010	0.012	0.003	0.045	0.002
80	8.95	0.045	0.006	0.001	0.051	0.003
90	8.48	0.062	0.002	0.001	0.053	0.001
100	7.75	0.050	0.000	0.000	0.058	0.004

to 0.00 and concentration of NaHCO_3 increased from 0.030 ± 0.001 to 0.058 ± 0.004 mol/L, which is comparable to tap water system.

Salmon et al. used NaOH as sorbent for capturing CO_2 [16]. When CO_2 is sparged in NaOH, it leads to formation of intermediate species like OH^- , CO_3^{2-} and HCO_3^- . The amount of intermediates was determined by titration with HCl using two colour indicators phenolphthalein and methyl orange. The effect of flow rates at which CO_2 is sparged and the initial concentration of CO_2 on gas liquid mass transfer was studied. They concluded that alkaline solutions in either pure form (NaOH or Na_2CO_3) or mixture of NaOH and Na_2CO_3 can be used effectively for CO_2 capture.

Actually, absorption of CO_2 in aqueous medium depends on gas liquid equilibrium. Henry's law constant H' quantifies the concentration that maintains this gas liquid equilibrium. Cheng and Chou studied effect of two alkaline systems NaOH and Na_2CO_3 on partition of CO_2 by calculating Henry's law constant. The study was carried out in three phases. In phase I, H' value for absorption of CO_2 in neutral tap water was determined. The study was carried out at two different temperatures 27 and 37 degrees. In phase II, alkaline-buffering agents NaOH and Na_2CO_3 were used. And in phase III, alga was used for CO_2 absorption [18]

When CO_2 was sparged in 150-L reactor, the CO_2 concentration varied from 600 to 2000 ppm. Initially, when the pH was more than 8, H' value was as low as 2.2 atm/M and at this pH much gaseous CO_2 is absorbed (1500 ppm) as the pH value ranged from 8 to 6, H' varied from 2.2 atm to 65 atm and CO_2 absorbing capacity gradually falls. When the pH decreases to pH range 4–5, the H' is in the range of 90 to 94 atm/M and it absorbs very little CO_2 .

When NaOH was used as an alkaline buffer and CO_2 was sparged for 2 h, the pH value ranged from 6.6 to 8.9. But when Na_2CO_3 was used as buffering system, the pH value ranged between 9.1 and 9.8. Adding Na_2CO_3 yielded H' value from 16.3 to 21.3 atm/M. The CO_2 concentration was 603 ppm and aqueous concentration of HCO_3^- was 4.085 mg/L. In NaOH buffering system, H' value was between 27 to 59 atm/M and there was much decline in concentration of CO_2 . Thus, weak alkali Na_2CO_3 had greater buffering capacity than stronger alkali.

Also the difference in CO_2 dissolution and holdup capacity of tap water, seawater and modified seawater BG11 medium (MSWBG11) was studied. It was observed that MSWBG11 medium that contained ten times higher Na_2CO_3 in the medium enabled maximum CO_2 dissolution with concentration of 18.30 ± 1.17 mg/L after 3 h of CO_2 sparging. Further on stopping the CO_2 sparging, system was capable of holding DCO_2 up to 4 h and was 3.34 ± 0.37 mg/L. Thus, seawater-based BG11 medium with

ten times higher Na_2CO_3 in it (MSWBG11) made the best solution for capturing and holding maximum DCO_2 in the system [9].

In our present study, when NaOH was used as alkaline system and 0.01 M NaOH was dissolved in tap water, the initial pH was as high as 11.72 ± 0.012 . After feeding CO_2 for 110 min, the pH was decreased slightly to 10.58 ± 0.026 and amount of Na_2CO_3 produced was 0.040 ± 0.004 mol/L. But when seawater was used for dissolving 0.01 M NaOH, the initial pH was comparatively low, it was 10.5 ± 0.017 . This might be due to buffering action of seawater. After feeding CO_2 for 110 min, the pH became 8.24 ± 0.006 and amount of NaHCO_3 was comparatively less 0.034 ± 0.006 mol/L.

But when Na_2CO_3 was used as alkaline system, 0.02 M was added to tap water; the pH was 11.31 ± 0.010 . When CO_2 was sparged for 110 min, it became 9.04 ± 0.032 and amount of NaHCO_3 present in system was 0.053 ± 0.0031 mol/L. When seawater was used after addition of 0.02 M Na_2CO_3 , the pH was 10.77 ± 0.031 . After feeding CO_2 for 100 minutes, it decreased to 7.75 ± 0.050 and amount of NaHCO_3 was 0.058 ± 0.004 mol/L. This concentration of NaHCO_3 using 0.02 M Na_2CO_3 was statistically proven to be best solution for CO_2 sparging and was significantly different with other solutions using one-way ANOVA and Tukey's test at level of significance of 0.05 ($\alpha = 0.05$ and 0.01) (Fig. 2).

Thus, seawater along with Na_2CO_3 was found to be efficient system for capturing CO_2 and converting it into NaHCO_3 . Hence, for further experiments of algal cultivation, Na_2CO_3 was used for CO_2 sparging.

Our further objective was to utilize these CO_2 -sparged NaOH and Na_2CO_3 solutions for algal growth.

3.2 Use of CO_2 -enriched NaOH and Na_2CO_3 medium for algal growth

3.2.1 Use of CO_2 -sparged NaOH and Na_2CO_3 solutions for preparing MSWBG11 medium

CO_2 -sparged 0.01 M NaOH tap water solution and 0.02 M Na_2CO_3 seawater-based solution were used in the preparation of MSWBG11 medium. These media were used for the growth of algae *Pseudanabaena limnetica*. MSWBG11 medium contains 0.0018 mol/L of Na_2CO_3 which is far less than Na_2CO_3 -enriched stock solutions obtained after CO_2 sparging. Hence, the stock solution was diluted accordingly to obtain required moles of Na_2CO_3 for the preparation of medium.

However seawater-based Na_2CO_3 solution contained 0.058 ± 0.004 mol/L of NaHCO_3 . The stock was 1:5 times diluted and added to MSWBG11 medium. Both these

Fig. 2 Effect of different alkaline solutions of CO₂ dissolution. ($n=3$, mean $\pm$ SD). * signifies statistically significant difference between four different alkaline solutions for optimum CO₂ dissolution (ANOVA and Tukey's post hoc test: * $p < 0.05$, ** $p < 0.01$)

media were used to study the growth of algae *Pseudanabaena limnetica*.

3.2.2 Effect of CO₂-sparged alkaline solutions on the growth of algae

3.2.2.1 Effect of CO₂-sparged NaOH medium on growth of *P. limnetica* In case of control tank, DCO₂ concentration and pH at day 0 were 2.07 ± 0.031 mg/L and 8.53 ± 0.097 , respectively (Table 5). Initial inoculum added was 0.19 ± 0.021 g/L dried biomass weight. As days passed, algal biomass grew gradually to give dried biomass yield of 0.29 ± 0.031 g/L on day 6. Also DCO₂ was decreased to 1.50 ± 0.030 mg/L and pH became 10.16 ± 0.060 . Maximum biomass yield of algae was obtained on day 15, i.e. 0.64 ± 0.035 g/L, which was confirmed by consumption of CO₂, and DCO₂ concentration of the system decreased from 2.07 ± 0.031 to 1.15 ± 0.050 mg/L on day 15. Also pH became alkaline from 8.53 ± 0.097 to 9.86 ± 0.051 on day 15.

In test 1 tank, where CO₂-sparged NaOH solution was used for preparation of MSWBG11 medium, the initial DCO₂ concentration was 2.47 ± 0.070 mg/L (Table 6) which was little higher than control tank (2.07 ± 0.031 mg/L). In Test tank, initial inoculum added was 0.19 ± 0.012 g/L and pH 8.63 ± 0.064 , respectively. The highest biomass of algae was observed on 12th day and increase in the biomass was from 0.19 ± 0.012 to 0.58 ± 0.015 g/L which was slightly more than control tank but the increase was not significant. Growth of the algae was supported by consumption

of CO₂ and increase in the pH. On day 15, the DCO₂ decreased from 2.47 ± 0.070 mg/L to 1.39 ± 0.105 mg/L, pH got alkaline from 8.63 ± 0.064 to 9.01 ± 0.067 which resulted in significant decrease in biomass yields. These results indicate that CO₂-sparged NaOH solution can be used for algal growth.

3.2.2.2 Effect of CO₂-sparged Na₂CO₃ medium on growth of *P. limnetica* Further third set (test 2) was tested where CO₂-sparged Na₂CO₃ solution was used as the source of carbonate for preparation of MSWBG11 medium. Growth conditions provided were same as given to above two tanks. In this experiment, the initial DCO₂ of the system was 3.92 ± 0.046 mg/L which was substantially more than the other two tanks and pH was 7.82 ± 0.095 . Algal inoculum added in the system on day 0 was 0.23 ± 0.046 g/L. As days passed, DCO₂ reduced and pH started getting more alkaline indicating growth of algae in the system. As seen in Table 7 and Fig. 3, right from day 3 up to day 15, there is significant increase in biomass produced in CO₂-sparged Na₂CO₃ medium over control. On day 15, algal biomass was maximum, i.e. 0.81 ± 0.046 g/L, where as in control tank the biomass produced is significantly less, 0.64 ± 0.035 g/L. At the end of 15 days, DCO₂ was substantially decreased from 3.92 ± 0.046 mg/L to 1.23 ± 0.036 mg/L and pH was increased from 7.82 ± 0.095 to 9.54 ± 0.075 (Table 7).

Thus, the above experiments conclude that when CO₂-sparged Na₂CO₃ solution is added to MSWBG11, it is sufficiently capable of holding maximum DCO₂ as

Table 5 Control tank—No CO₂ sparged

Day	Hours	pH		DCO ₂ (mg/L)		Biomass (g/L)	
		Mean	SD	Mean	SD	Mean	SD
0	07:00	8.53	0.097	2.07	0.031	0.19	0.021
	11:00	8.75	0.100	1.93	0.080		
	19:00	8.99	0.085	1.86	0.132		
1	07:00	8.73	0.111	1.96	0.046		
	11:00	8.83	0.056	1.97	0.070		
	19:00	9.08	0.070	1.77	0.067		
2	07:00	8.81	0.085	1.84	0.045		
	11:00	8.88	0.068	1.85	0.050		
	19:00	9.25	0.061	1.68	0.075		
3	07:00	8.93	0.025	1.74	0.030	0.20	0.015
	11:00	9.04	0.133	1.70	0.031		
	19:00	9.26	0.086	1.65	0.046		
4	07:00	8.98	0.085	1.72	0.070		
	11:00	9.28	0.030	1.69	0.021		
	19:00	9.78	0.053	1.55	0.050		
5	07:00	9.36	0.055	1.60	0.103		
	11:00	9.57	0.056	1.60	0.075		
	19:00	9.67	0.047	1.56	0.065		
6	07:00	9.00	0.025	1.48	0.053	0.29	0.031
	11:00	9.66	0.040	1.56	0.060		
	19:00	10.16	0.060	1.50	0.030		
7	07:00	9.42	0.025	1.47	0.046		
	11:00	10.82	0.070	1.44	0.025		
	19:00	9.90	0.095	1.43	0.055		
8	07:00	9.29	0.085	1.39	0.091		
	11:00	9.86	0.032	1.48	0.040		
	19:00	9.88	0.053	1.45	0.040		
9	07:00	9.29	0.069	1.41	0.072	0.46	0.020
	11:00	10.01	0.117	1.37	0.050		
	19:00	9.87	0.055	1.42	0.020		
10	07:00	9.23	0.025	1.39	0.072		
	11:00	9.85	0.021	1.32	0.017		
	19:00	9.86	0.060	1.31	0.021		
11	07:00	9.18	0.070	1.33	0.057		
	11:00	9.79	0.061	1.31	0.012		
	19:00	9.85	0.087	1.25	0.044		
12	07:00	9.17	0.035	1.34	0.040	0.48	0.055
	11:00	9.76	0.060	1.33	0.015		
	19:00	9.84	0.053	1.26	0.010		
13	07:00	9.14	0.040	1.32	0.066		
	11:00	9.68	0.075	1.29	0.045		
	19:00	9.82	0.025	1.26	0.060		
14	07:00	9.07	0.062	1.21	0.015		
	11:00	9.71	0.084	1.29	0.045		
	19:00	9.85	0.044	1.20	0.100		
15	7:00	9.04	0.026	1.26	0.060	0.64	0.035
	11:00	9.60	0.020	1.15	0.050		
	19:00	9.86	0.051	1.25	0.050		

Table 6 Test 1—CO₂-sparged NaOH solution using for algal growth

Day	Hours	pH		DCO ₂ (mg/L)		Biomass (g/L)	
		Mean	SD	Mean	SD	Mean	SD
0	7:00	8.63	0.064	2.47	0.070	0.19	0.012
	11:00	8.66	0.079	2.45	0.040		
	19:00	8.60	0.035	2.55	0.021		
1	7:00	8.49	0.095	2.59	0.091		
	11:00	8.65	0.050	2.44	0.059		
	19:00	8.52	0.046	2.42	0.082		
2	7:00	8.57	0.057	2.32	0.026		
	11:00	8.72	0.026	2.19	0.092		
	19:00	8.82	0.020	2.13	0.085		
3	7:00	8.67	0.075	2.22	0.117	0.22	0.025
	11:00	8.71	0.017	2.20	0.106		
	19:00	8.89	0.105	1.91	0.042		
4	7:00	8.67	0.070	2.05	0.062		
	11:00	8.74	0.040	2.06	0.101		
	19:00	8.90	0.100	1.90	0.025		
5	7:00	8.60	0.070	2.10	0.087		
	11:00	8.68	0.032	2.02	0.086		
	19:00	8.80	0.075	1.77	0.030		
6	7:00	8.56	0.061	2.07	0.071	0.31	0.040
	11:00	8.69	0.090	1.91	0.046		
	19:00	8.85	0.050	1.76	0.051		
7	7:00	8.24	0.040	2.13	0.006		
	11:00	8.57	0.070	1.86	0.060		
	19:00	8.78	0.026	1.56	0.108		
8	7:00	8.34	0.040	1.76	0.051		
	11:00	8.63	0.023	1.63	0.067		
	19:00	8.89	0.015	1.45	0.061		
9	7:00	8.22	0.020	1.75	0.050	0.48	0.031
	11:00	8.57	0.066	1.50	0.100		
	19:00	8.91	0.101	1.34	0.040		
10	7:00	8.17	0.075	1.77	0.031		
	11:00	8.52	0.015	1.61	0.051		
	19:00	8.91	0.035	1.34	0.070		
11	7:00	8.19	0.060	1.73	0.046		
	11:00	8.44	0.040	1.58	0.031		
	19:00	8.85	0.046	1.33	0.035		
12	7:00	8.17	0.020	1.86	0.060	0.58	0.015
	11:00	8.54	0.040	1.63	0.067		
	19:00	8.64	0.032	1.34	0.117		
13	7:00	8.16	0.056	1.85	0.055		
	11:00	8.51	0.106	1.60	0.025		
	19:00	8.68	0.076	1.28	0.068		
14	7:00	8.18	0.075	1.87	0.015		
	11:00	8.53	0.030	1.69	0.025		
	19:00	8.67	0.010	1.37	0.061		
15	7:00	8.18	0.040	1.88	0.006	0.47	0.006
	11:00	8.63	0.025	1.50	0.025		
	19:00	9.01	0.067	1.39	0.105		

Table 7 Test 2—CO₂-sparged Na₂CO₃ solution used for algal growth

Days	Hours	pH		DCO ₂ (mg/L)		Biomass (g/L)	
		Mean	SD	Mean	SD	Mean	SD
0	7:00	7.82	0.095	3.92	0.046	0.23	0.046
	11:00	8.02	0.070	3.90	0.015		
	19:00	8.53	0.112	3.84	0.127		
1	7:00	8.90	0.100	3.94	0.152		
	11:00	8.93	0.031	3.88	0.075		
	19:00	9.01	0.046	3.64	0.076		
2	7:00	9.05	0.015	3.77	0.061		
	11:00	9.05	0.123	3.76	0.021		
	19:00	9.19	0.070	3.22	0.116		
3	7:00	9.13	0.035	3.75	0.050	0.42	0.057
	11:00	9.24	0.029	3.69	0.080		
	19:00	9.24	0.006	2.95	0.050		
4	7:00	9.16	0.055	3.40	0.010		
	11:00	9.21	0.025	3.38	0.086		
	19:00	9.35	0.046	2.75	0.068		
5	7:00	9.26	0.060	3.25	0.040		
	11:00	9.30	0.095	3.14	0.040		
	19:00	9.39	0.101	2.68	0.075		
6	7:00	9.26	0.060	3.24	0.145	0.57	0.047
	11:00	9.34	0.015	3.10	0.106		
	19:00	9.44	0.036	2.66	0.067		
7	7:00	9.32	0.061	3.25	0.056		
	11:00	9.33	0.010	3.02	0.021		
	19:00	9.44	0.021	2.38	0.075		
8	7:00	9.33	0.006	2.35	0.060		
	11:00	9.57	0.061	2.32	0.021		
	19:00	9.60	0.061	1.81	0.015		
9	7:00	9.39	0.075	2.18	0.012	0.76	0.066
	11:00	9.42	0.045	2.17	0.089		
	19:00	9.55	0.060	1.73	0.072		
10	7:00	9.39	0.020	2.13	0.036		
	11:00	9.44	0.030	2.07	0.062		
	19:00	9.52	0.120	1.68	0.035		
11	7:00	9.35	0.056	1.94	0.101		
	11:00	9.38	0.010	1.91	0.105		
	19:00	9.48	0.006	1.71	0.035		
12	7:00	9.36	0.055	1.92	0.046	0.78	0.080
	11:00	9.39	0.076	1.87	0.061		
	19:00	9.48	0.050	1.63	0.023		
13	7:00	9.34	0.021	2.04	0.036		
	11:00	9.35	0.012	2.01	0.015		
	19:00	9.42	0.020	1.73	0.080		
14	7:00	9.26	0.065	1.91	0.060		
	11:00	9.26	0.116	1.86	0.056		
	19:00	9.39	0.085	1.37	0.067		
15	7:00	9.48	0.036	1.49	0.090	0.81	0.046
	11:00	9.52	0.025	1.37	0.070		
	19:00	9.54	0.075	1.23	0.036

Fig. 3 Effect of CO_2 -sparged NaOH and Na_2CO_3 solutions on algal growth and biomass yield. ($n=3$, mean $\pm$ SD). * signifies statistically significant difference between Control, Test 1 and Test 2 (ANOVA and Tukey's post hoc test: * $p < 0.05$, ** $p < 0.01$)

indicated by high DCO_2 in third set, i.e. 3.92 ± 0.046 mg/L on day 0. Also it supports well the algal growth. In control tank and Tank 1 provided with NaOH medium, the biomass yields at the end of 15 days were 0.64 ± 0.035 g/L and 0.58 ± 0.015 g/L, respectively, which were very less compared to test 2 which yielded 0.81 ± 0.046 g/L of yield, thus proving that Na_2CO_3 solution when used to sparge CO_2 suits best as a medium for maximum CO_2 holding capacity and maximum algal yield. Hence, it was continued for further work.

Statistical analysis by one-way ANOVA proved there was significant difference in three sets Control, Test 1 and Test 2 at level of significance of 0.05. Further post hoc Tukey's test proved there was significant difference in biomass yields of test 2 over Control and Test 1. ($\alpha=0.05$ and 0.01) (Fig. 3).

3.3 Semicontinuous mode of operation for high biomass yield of algae

Cultivation of algae can be done in various ways such as batch mode, fed-batch mode or continuous mode. Previous experiments were conducted in batch mode of operation where experiments were run for about 15 days to complete the growth cycle of algae. After 15 days, algal biomass started decreasing indicating stationary phase of algae.

To further increase the biomass production of algae, it was cultivated in semicontinuous mode of operation. Initially, the culture was allowed grow for 6 to 7 days till the log phase is achieved. The semicontinuous operation

is initiated by removing some amount of algal culture and replacing it with fresh medium. By doing so, the algal culture remains in log phase continuously producing biomass. In the present work, it was necessary to standardise the amount of culture to be removed in semicontinuous operation in which 1 L (set A), 3 L (set B), 5 L (set C) of culture were harvested and the same volume of fresh media was added into each of the sets every day. Increase in the biomass yield in each set was evaluated gravimetrically on a daily basis.

In set A, initial inoculum was 0.30 ± 0.058 g/L which was allowed to grow for 6 days till the biomass increased to 0.71 ± 0.023 g/L which was considered as day 0 for starting semicontinuous mode of operation. Daily 1 L of culture was harvested and analysed for biomass yield. Same amount of fresh medium was added into the tank to compensate for the lost harvested culture. It was found that in initial 10 days biomass of 0.71 ± 0.023 g/L increased to 1.18 ± 0.049 g/L and further it went on increasing from 10th till 60th day till which the experiment continued. The final biomass that was obtained at the end of 60 days was 2.62 ± 0.032 g/L.

However, in set B, the initial biomass of 0.82 ± 0.021 g/L was gradually increased to 1.43 ± 0.36 g/L at the end of 30 days. But the biomass produced at the end of 30 days in set B was significantly lesser than biomass in set A (Fig. 4). The same trend was found to be continued, on 60th day the biomass produced in set A (2.61 ± 0.032 g/L) which was significantly higher than set B (1.60 ± 0.025 g/L).

Fig. 4 Effect of different volumes in semicontinuous mode of cultivation on biomass yield of algae. ($n=3$, mean $\pm$ SD). * signifies statistically significant difference in biomass yields of set A (1 L), set B (3 L) and set C (5 L) (ANOVA and Tukey's post hoc test: * $p < 0.05$, ** $p < 0.01$)

Similarly, in set C where 5 L of media was removed every day, the initial biomass was 0.81 ± 0.040 g/L, and at the end of 30 days, it negligibly increased to 1.08 ± 0.015 g/L which was significantly less than biomass produced in set A. The condition was still worst on day 60, and the biomass produced was still reduced to 0.76 ± 0.012 g/L (Table 8, Fig. 4).

These results indicated that replacing 1 L of culture with fresh media is the suitable way to replenish the lost nutrient into media and also the biomass harvested in 1 L culture can be overcome by exponentially growing culture. On the other hand in set B and C where 3 L and 5 L of culture were harvested, biomass was not able to grow at that fast rate to cope up the loss of culture. (Table 8). This

was statistically proven by performing one-way ANOVA and Tukey's test at $\alpha=0.05$ and 0.01 (Fig. 4).

In one of the studies, it was reported that *A. braunii* when cultivated in batch cultures, the maximum biomass concentration (X_m) was 1588 ± 11 mg L⁻¹ using 20 mM of NaNO₃. A fed-batch process with the addition of 20 mM NaNO₃ each 48 h from the first to the sixth cultivation day reached $X_m = 2753 \pm 7$ mg L⁻¹. The semi-continuous process was effective in eradicating the lag phase of algae allowing it to grow to maximum biomass of $X_m = 2399 \pm 5$ mg L⁻¹. As seen in our experiment semi-continuous mode of operation increased the biomass gradually beyond 18 days till 60th day and reached maximum biomass of 2.62 ± 0.032 g/L [19]. It could be concluded from the data that semicontinuous mode of operation is not only optimum for mass scale production

Table 8 Effect of amount of daily removal of culture medium in semicontinuous mode on biomass formation

No. of days	Biomass (g/L)					
	1 L		3 L		5 L	
	Mean	SD	Mean	SD	Mean	SD
0	0.71	0.023	0.82	0.021	0.81	0.040
10	1.18	0.049	1.03	0.038	1.01	0.031
20	1.56	0.015	1.20	0.015	0.81	0.010
30	1.97	0.020	1.43	0.036	1.08	0.015
40	2.02	0.025	1.40	0.030	0.97	0.020
50	2.54	0.035	1.31	0.036	0.81	0.021
60	2.61	0.032	1.60	0.025	0.76	0.012

of algae but can also be used to feed CO₂-rich medium in system every day to compensate for the loss of CO₂ in the environment due to high temperatures in tropical environments.

3.4 Using Na₂CO₃-sparged MSWBG11 medium in semicontinuous mode of operation

In this experiment, CO₂ gas coming from Bio-CNG plant was directly sparged in MSWBG11 (Test1). This CO₂-sparged MSWBG11 medium was used for replacing the medium in a semicontinuous mode of operation. Experiment was conducted in 60-L photobioreactor that was inoculated with approximately 0.35 g/L of culture and agitated with 4LPM of ambient air. Set-up was kept in partial shade near Bio-CNG vicinity of CNG station plant in the premises of Primove Engineering Pvt. Ltd., Pune. DCO₂ concentration (mg/L), % CO₂ consumption, pH and dried biomass yield (g/L) were analysed and calculated daily.

In one set, only MSWBG11 medium with no sparged CO₂ was used as the source of medium for semicontinuous

mode and this was considered as control tank. In both sets, i.e. test and control, algal biomass was initially cultivated for 10 days till biomass attains log phase. In control set, initial inoculum added was 0.35 ± 0.072 g/L which increased to 0.77 ± 0.052 g/L at the end of 10 days. DCO₂ at day 0 was 1.82 ± 0.012 mg/L which reduced to 1.68 ± 0.070 mg/L at the end of 10 days. Initial pH of 8.75 ± 0.050 increased to 8.91 ± 0.036 after 10 days. However, in Test tank, initial inoculum of 0.34 ± 0.096 g/L increased to 0.77 ± 0.066 g/L at the end of 10 days. Initial DCO₂ and pH of the system were 2.08 ± 0.060 mg/L which reduced to 1.28 ± 0.085 mg/L after 10 days indicating algal culture grew consuming DCO₂ of the system.

After 10 days, the semicontinuous operation was initiated in both control and test tanks, i.e. every day, 1L of culture from both tanks was replaced with same volume of media. In control tank (Table 9), 1L of culture was replaced with same volume of fresh MSWBG11 medium (with no CO₂ sparged in it), whereas in test tank 1L of culture was replaced with same volume of CO₂-sparged

Table 9 Control: MSWBG11 medium was used in semicontinuous mode of operation

Day	Hours	pH		DCO ₂ (mg/L)		Biomass (g/L)	
		Mean	SD	Mean	SD	Mean	SD
0	7:00	8.91	0.036	1.68	0.070	0.77	0.052
	11:00	8.72	0.045	1.70	0.015		
	19:00	8.98	0.040	1.61	0.012		
1	7:00	8.73	0.072	1.60	0.095	0.85	0.036
	11:00	8.74	0.050	1.74	0.130		
	19:00	8.95	0.068	1.65	0.092		
2	7:00	8.69	0.090	1.73	0.056	1.03	0.050
	11:00	8.83	0.080	1.71	0.106		
	19:00	9.00	0.076	1.61	0.120		
3	7:00	8.77	0.112	1.63	0.092	1.10	0.080
	11:00	8.83	0.031	1.73	0.075		
	19:00	9.08	0.075	1.57	0.078		
4	7:00	8.76	0.065	1.72	0.140	1.13	0.035
	11:00	8.86	0.036	1.72	0.111		
	19:00	9.07	0.070	1.68	0.097		
5	7:00	8.78	0.031	1.74	0.140	1.18	0.057
	11:00	8.94	0.020	1.74	0.081		
	19:00	9.10	0.100	1.71	0.115		
6	7:00	8.79	0.030	1.73	0.080	1.34	0.030
	11:00	8.95	0.035	1.75	0.080		
	19:00	9.10	0.020	1.69	0.120		
7	7:00	8.78	0.017	1.72	0.111	1.41	0.091
	11:00	9.01	0.015	1.85	0.050		
	19:00	9.11	0.070	1.72	0.065		
8	7:00	9.02	0.025	1.73	0.101	1.46	0.066
	11:00	9.42	0.081	2.02	0.042		
	19:00	9.21	0.017	1.73	0.042		

fresh MSWBG11 medium containing DCO_2 concentration of 7–8 mg/L.

In control tank, it was observed that as semicontinuous mode of operation started, initial DCO_2 of 1.68 ± 0.070 mg/L observed at 7:00 h increased to 1.70 ± 0.015 mg/L at 11:00 h after addition of fresh medium. As culture grew throughout the day, DCO_2 was consumed and its concentration reduced to 1.61 ± 0.012 mg/L till evening at 19:00 h. Growth of the culture was confirmed by increase in dried weight biomass from 0.77 ± 0.052 g/L to 0.85 ± 0.036 g/L at end of day 1. As DCO_2 was consumed by algae, there was decrease in the pH from 8.91 ± 0.036 on day 0 to 8.95 ± 0.068 on day 1. Slowly as days passed and fresh medium was added, DCO_2 gradually increased from 1.74 ± 0.130 mg/L on day 1 to 2.02 ± 0.042 mg/L at the end of 8 days and pH of the system became more alkaline from 8.95 ± 0.068 to 9.42 ± 0.081 after 8 days. Biomass yield gradually increased from 0.85 ± 0.036 g/L on day 1 to maximum on day 8, i.e., 1.46 ± 0.066 g/L.

In test tank (Table 10), initial DCO_2 of 1.28 ± 0.085 mg/L increased to 1.42 ± 0.140 mg/L on addition of CO_2 -enriched

fresh MSWBG11 medium to the system after 1 day. On addition to DCO_2 , pH got alkaline from 9.14 ± 0.040 on day 0 at 7:00am to 9.58 ± 0.075 at 7:00 pm on day 1 and culture grew from 0.77 ± 0.066 g/L to 0.80 ± 0.070 g/L in a day. As days passed, DCO_2 concentration gradually increased from day 1 to day 8, DCO_2 on addition of media at day 1 was 1.42 ± 0.140 mg/L which slightly increased to 1.55 ± 0.120 mg/L on day 8, DCO_2 was continuously available for the growth of algae and algal biomass increased from 0.80 ± 0.070 g/L on day 1 to 1.62 ± 0.070 g/L after 8 days of semicontinuous mode of operation. Significant difference in the biomass yields between Control and Test was proven by using one-way ANOVA ($\alpha = 0.05$) (Fig. 5).

Also Yadav et al. set up a small-scale pilot reactor at Oil and Natural Gas Corporation, Hazira, Surat, India, to capture CO_2 from vent gas. It consisted of a cylindrical CO_2 absorber of volume 0.25 m^3 . The pH of fresh medium was adjusted to 11 by using 0.1 g/L of NaOH and was stored in storage tank. The medium was transferred to column using peristaltic pump. CO_2 containing vent gas was tapped from exhaust stack at Hazira plant and was purged

Table 10 Test: MSWBG11 sparged with CO_2 from CNG station was used for semicontinuous operation

Days	Hours	pH		DCO_2 (mg/L)		Biomass (g/L)	
		Mean	SD	Mean	SD	Mean	SD
0	7:00	9.14	0.040	1.28	0.085	0.77	0.066
	11:00	10.13	0.121	1.21	0.121		
	19:00	9.68	0.075	1.04	0.168		
1	7:00	9.11	0.090	1.42	0.140	0.80	0.070
	11:00	10.01	0.115	1.52	0.075		
	19:00	9.58	0.075	1.26	0.065		
2	7:00	9.05	0.050	1.33	0.091	0.90	0.125
	11:00	9.83	0.058	1.39	0.120		
	19:00	9.76	0.056	1.20	0.130		
3	7:00	9.10	0.095	1.30	0.130	0.98	0.085
	11:00	9.97	0.076	1.53	0.125		
	19:00	9.66	0.146	1.25	0.056		
4	7:00	9.09	0.115	1.35	0.055	1.20	0.105
	11:00	9.82	0.070	1.51	0.111		
	19:00	9.55	0.055	1.27	0.070		
5	7:00	9.08	0.085	1.34	0.035	1.25	0.120
	11:00	10.08	0.097	1.43	0.030		
	19:00	9.62	0.125	1.22	0.070		
6	7:00	9.10	0.195	1.35	0.120	1.42	0.070
	11:00	10.15	0.131	1.56	0.065		
	19:00	9.64	0.051	1.30	0.090		
7	7:00	9.13	0.080	1.39	0.096	1.60	0.105
	11:00	10.23	0.100	1.66	0.067		
	19:00	9.69	0.140	1.28	0.085		
8	7:00	9.21	0.115	1.38	0.085	1.62	0.070
	11:00	10.20	0.175	1.55	0.120		
	19:00	9.73	0.075	1.31	0.111

Fig. 5 Effect of CO₂ from Bio-CNG plant on growth and biomass yield of algae. ($n = 3$, mean $\pm$ SD). * signifies statistically significant difference in Biomass yields of Control and Test (ANOVA: * $p < 0.05$)

into absorption column at a flow rate 5.1 L/min. The gas was bubbled from bottom to ensure a long contact time between alkaline solution and gas. The pilot carbonation column was able to bring down the initial CO₂ concentration of 30 vol% to 15 vol%, and the pH of medium was decreased from 11 to 6.8 or 7. This CO₂-sparged medium was then allowed to flow into algal raceway pond of capacity 0.2m³. The growth of microalgae consortium was measured as increase in OD from 0.001 to 0.402. Due to algal growth, the pH of pond rose from 8 to 10. This high pH medium was reused for absorbing CO₂ in absorption column [20].

4 Conclusions

In the current research, indigenous halophilic strain *Pseudanabaena limnetica* (Lemm.) Komárek was used for CO₂ sequestration studies along with mass cultivation. CO₂ sequestration studies were carried out in previously designed, constructed and operationally optimized 60-L flat-panel photobioreactor system for growth of *P. limnetica*. MSWBG11 medium was the nutrient medium for the growth of the algae.

To enhance maximum CO₂ dissolution and holdup, different alkaline systems were studied in seawater and tap water out of which 0.02 M Na₂CO₃ in seawater was the best solutions which could hold maximum CO₂. To improve the algal biomass productivity, these solutions were used as source of carbonate. To further enhance the biomass, the

system was operated in a semicontinuous mode where 1 L of culture replaced with 1 L of fresh medium everyday showed maximum algal biomass yield even after 60 days operation. Further to sequester CO₂ continuously so that it could be used for industrial application, semicontinuous system was set up at premises of Bio-CNG plant. Here, the gas released from Bio-CNG plant as by-product containing CO₂ was sparged in Na₂CO₃-containing medium and 1 L of old culture was replaced with this CO₂-enriched medium every day and proved to be helpful in enhancing the biomass production.

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Compliance with ethical standards

Conflict of interest The author(s) declare that they have no competing interests.

Consent to participate All authors have contributed equally and voluntarily in the conduct of study and preparation of the manuscript.

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